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RESEARCH ARTICLE

ASSESSMENT OF UREASE ACTIVITY IN SELECTED VARIETIES OF LEGUMINOUS SEEDS

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ABSTRACT

Urease, a seed protein found in plants, catalyzes the breakdown of urea into ammonia and carbon dioxide, making urea a nitrogen source for plants. The study quantified urease activity and protein content of eight leguminous seed varieties (Soybean, Bambara nut, Iron beans, Kaduna Brown beans, Bakin Ido beans, Sokoto White beans, Mung beans and Oloyin beans) to optimize urea fertilizer use and identify a valuable urease source for clinical and industrial applications. Crude protein concentration was determined using the Micro-Kjeldahl method, while total protein concentration and urease activity were determined using Biuret method and pH increment method respectively. Soya beans has the highest crude protein of 27.7% and urease activity of 1.82 U/ml content, with Sokoto White beans having the least crude protein of 13.8% and urease activity of 0.45 U/ml. This study guides cultivation of locally available legumes, focusing on efficient fertilizer and pesticide application, enhancing yield, food security, employment opportunities, and innovation in line with Sustainable Development Goals.

KEYWORDS

Urease, Legumes, Leguminous seeds, Enzyme activity, Sustainable Development Goals (SDGs)

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Introduction

Urease (urea amidohydrolase, EC 3.5.1.5) is an important nickel-dependent enzyme that catalyses the hydrolysis of urea to carbon dioxide and ammonia. It is produced by bacteria, fungi and plants such as legumes (Joseph et al., 2022). Urease has a unique place in the history of biochemistry and enzyme studies. In 1926, J.B. Sumner demonstrated the proteinaceous nature of enzymes through his work on *Canavalia ensiformis* (jack bean) seeds urease, making urease the first enzyme ever to be isolated and crystallized (Real-Guerra, Staniscuaski, & Carlini, 2013). This important research milestone won him the Nobel Prize in Chemistry in 1946. Also, N.E. Dixon and his coworkers in 1975 elucidated the biological importance of nickel (Ni^{2+}) and showed its indispensable role in urease' catalytic activity following their studies of the active site of jack bean urease (Joseph et al., 2022). More recently, Follmer et al. (2001) identified urease as a toxin in some plants, where it plays a role against their pests.

In plants, ureases have been shown to possess insecticidal and antifungal activities (Becker-Ritt et al., 2007). Industrially, they are used for pet stain removal (Javadi et al., 2021), as well as enzyme-induced carbonate precipitation (EICP), which is a promising technique used for soil biocementation and repair of concrete cracks (Kavazanjian and Hamdan, 2015). Urease can also be used as a diagnostic tool for the determination of urea in human blood, and as a urea-reducing agent in the preparation of alcoholic beverages (Mirbod et al., 2002).

In plants, nitrogen is one of the primary nutrients needed for optimum growth and development (Iqbal et al., 2021). These nutrients can be naturally present in the soil or added directly in form of fertilizer (Riaz et al. 2020). Whether internally derived or externally applied, urea can only serve as a useful nitrogen source to plants through the action of urease (Joseph et al., 2022). This makes urease an enzyme of agricultural importance, as urea is one of the most commonly used fertilizers worldwide (Follmer, 2008). However, in the process of urea fertilization, unhealthy amount of ammonia can find its way into the atmosphere as a result of elevated urease activity, thereby causing pollution and economic loss (Sigurdarson et al., 2000). Hence, it is important to pay extra attention in this regard when cultivating plants such as legumes, which contain varying amount of urease.

Legumes belong to the Leguminosae (also known as Fabaceae), a plant family with over 18,000 species (Joseph et al., 2022). They are widely cultivated and consumed all over the world due to the high amount of nutrients they contain (Rajan and Ankur, 2017). Legumes contribute immensely to food security, as they are important dietary staples in middle and low-income countries. They serve as relatively cheap alternatives to animal proteins, which are usually out of reach for many households. In Nigeria, they are cultivated over a wide range of agro-environments, ranging from the forest in the South to the Sahel savanna of the North. However, most of the production takes place in the North. Common legumes include beans (*Phaseolus vulgaris*), Bambara groundnut (*Vigna subtarranea*), cowpea (*Vigna unguiculata*) peanut (*Arachis hypogaea*), lentils (*Lens culinaris*), soybeans (*Glycine max*).

The high cost of urease is a hindrance for its use and application for clinical and industrial purposes (Kavazanjian and Hamdan, 2015). Therefore, it is imperative to find low-cost sources of this enzyme. Moreover, it is possible to cheaply extract urease from leguminous seeds such as beans (Bedan, 2020). This would significantly enhance its use for various industrial activities, including construction and environmental protection where the binding together of granular soil particles is essential (Javadi et al., 2021). Studies have shown that plants such as legumes are rich in urease, and could therefore serve as good sources for its extraction (Tirkolaei et al., 2020).

Having an understanding of urease activity in locally available leguminous seeds would provide local farmers and agricultural extension workers a better understanding in the management of urea fertilizer and pesticide application, which will increase crop yield and ultimately lead to zero hunger in line with Sustainable Development Goal 2 (SDG2). Also, the understanding of urease activity in locally available leguminous seeds would enhance their exploitation in the production of urease for diagnostic, industrial and research purposes.

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This will enhance industry, innovation and infrastructure in line with Sustainable Development Goal 9 (SDG9). Hence, this study assessed the urease activity of some locally available leguminous seeds.

Materials and Methods

Materials

Soybeans (*Glycine max*), Bambara nut (*Vigna subtarranea*), Mung beans (*Vigna radiata*), Iron beans (*Phaseolus vulgaris*), Kaduna Brown beans (*Phaseolus vulgaris*), Bakin Ido beans (*Phaseolus vulgaris*), Sokoto White beans (*Phaseolus vulgaris*), Oloyin beans (*Phaseolus vulgaris*) were obtained from Sokoto Fish and Vegetable Market. Urea, Nessler's reagent, boric acid, $H_2SO_{4(aq)}$, $HCl_{(aq)}$ and $Na_2SO_{4(aq)}$ were obtained from BDH Chemicals Ltd. Poole England. Ammonia solution and $NaOH_{(aq)}$ were obtained from Fisons Plc England. KH_2PO_4 and K_2HPO_4 were obtained from Hopkin and Williams England. Kjeldahl catalyst was obtained from BDIT England. Total protein reagent kit was obtained from RANDOX Laboratories Ltd. United Kingdom.

Determination of Crude Protein Concentration

The crude protein concentration was determined using the Micro-Kjeldahl method. Two grams (2g) of the grounded sample was collected and put in a clean dry 100ml Kjeldahl flask. One tablet of the mixed catalyst and 20ml concentrated $H_2SO_{4(aq)}$ were added. Little amount of distilled water was also added into the flask to digest the organic matter present, which was then heated in a fume cupboard until a clear solution was obtained. The contents were cooled and transferred into a volumetric flask. 10mls of aliquot was put into Kjeldahl flask to make the volume up to 50ml with distilled water and 20mls of 40% $NaOH_{(aq)}$ was also added to extract the ammonia out of the sample which will be evaporated into the boric acid indicator (20ml of boric acid indicator) was used as a receiver of the nitrogen extracted. The ammonia produced was trapped in the acid (boric acid) until the volume was made up to 40mls in the conical flask. The colour changed from pink to green. The collected sample with ammonia was then titrated against 0.01N $HCl_{(aq)}$ to end point (the colour changed from green to pink). The end point and titre value were recorded, and percentage nitrogen and protein was calculated from the titre value using the following formula:

$$\% \text{ Nitrogen} = \frac{\text{TV} \times 0.01 \times 0.014 \times 50}{weight\ of\ sample \times ml\ of\ aliquot} \times 100$$

$$\% \text{ crude protein} = \% \text{ N} \times 6.25$$

Determination of Total Protein Concentration

Total protein concentration was determined using Biuret method as modified by Liu and Pan (2017). 1ml of the sample was added into a test tube and 4ml of biuret reagent was added. The mixture was mixed properly by shaking and then incubated at room temperature for 10-15mins. The resultant mixture was read spectrophotometer at 540nm. The total protein concentration was calculated using the formula:

$$\text{Total protein concentration} = \frac{Asample}{Astandard} \times Concentration\ of\ standard\ (6.0g/dl)$$

Determination of Urease Activity

Urease activity was determined using the pH increment method as described in the report of Liu et al. (2021) with some modifications. Approximately 0.200g (± 0.001g) of sample was placed into a test tube, and 10ml of buffered urea solution was added – this was called the ***test sample.*** The content was mixed gently and placed in a water bath at 30^0C, noting the time put into the bath. Approximately 0.200g (± 0.001g) of sample was placed into another test tube, and 10ml of phosphate buffer was added – this was called the ***blank sample.*** The content was mixed gently and placed in a water bath at 30^0C noting the time put into the bath. A five minute interval was allowed between the preparation of the test and blank samples. The contents of tubes in both test and blank samples were mixed approximately every five minutes during the required time in the water bath. At end of 30 minutes, the tubes were removed and the contents were mixed one last time (each tube had a different removal time from the water bath depending on time put in). Each tube was allowed to stand for a few minutes after which approximately 5ml of the supernatant liquid was transferred into separate beakers (beaker must provide for electrode of pH meter to fit into it and to be covered with the liquid). The pH of

the supernatant liquid was determined approximately 5 minutes after removal from the water bath. The difference between pH of test and pH of blank was calculated as an index of ammonia production.

$$\text{Urease activity} = \frac{Concentration\ of\ ammonia\ produced}{30\ min}$$

2.5 Statistical Analysis

All data obtained from this study were expressed as mean ± standard deviation of 3 independent variables (SD). Data were analysed by one-way analysis of variance (ANOVA) using InStat 3 software (San Diego, USA). Difference in mean (± SD) were considered to be significant at $p<0.05$.

Results

Table 1: Crude Protein Concentrations of the Selected Leguminous Seeds

SAMPLES	% CRUDE PROTEIN
Soybean (*Glycine max*)	27.7 ± 0.35
Bambara nut (*Vigna subtarranea*)	25.0 ± 0.60
Iron beans (*Phaseolus vulgaris*)	22.1 ± 0.35
Kaduna Brown beans (*Phaseolus vulgaris*)	21.9 ± 0.60
Mung beans (*Vigna radiata*)	21.3 ± 0.65
Bakin Ido beans (*Phaseolus vulgaris*)	15.4 ± 0.35
Oloyin beans (*Phaseolus vulgaris*)	14.4 ± 0.60
Sokoto White beans (*Phaseolus vulgaris*)	13.8 ± 0.65

Values in the table are mean ± standard deviation from triplicate determination.

Table 2: Urease Activity and Total Protein Concentration of the Selected Leguminous Seeds

SAMPLES	ACTIVITY (U/ml)	TOTAL PROTEIN (mg/ml)	SPECIFIC ACTIVITY (U/mg)
Soybean (*Glycine max*)	1.82 ± 0.004	59.6 ± 2.76	0.0305
Mung beans (*Vigna radiata*)	1.71 ± 0.05	60.6 ± 0.93	0.0282
Oloyin beans (*Phaseolus vulgaris*)	1.65 ± 0.02	126.5 ± 0.60	0.0130
Bambara nut (*Vigna subtarranea*)	1.09 ± 0.07	41.7 ± 0.5353	0.0261
Iron beans (*Phaseolus vulgaris*)	0.47 ± 0.13	49.5 ± 0.35	0.0095
Kaduna Brown beans (*Phaseolus vulgaris*)	0.47 ± 0.07	54.2 ± 0.40	0.0087
Bakin Ido beans (*Phaseolus vulgaris*)	0.47 ± 0.09	64.3 ± 2.74	0.0073
Sokoto White beans (*Phaseolus vulgaris*)	0.45 ± 0.05	49.3 ± 1.23	0.0091

The values of enzyme activity and total protein are expressed as mean ± standard deviation from triplicate determination.

Discussion

Legumes are known to contain varying amounts of proteins like other plant types (Wadhave et al., 2014). The results in Table 1 show varying protein contents for the different leguminous seeds assessed. All the tested varieties had their percentage crude protein within the range of 11.6 – 52.6% reported by Aremu et al. (2017). In this study, soybean showed the highest percentage of crude protein amongst the varieties of legumes tested. This is consistent with a previous study on some protein-rich plants (Wadhave et al., 2014). The proteins present in these legumes are enzymes, hormones and other important compounds needed for proper growth and development. Plants are able to synthesize proteins using nitrogen from inorganic sources (Aremu et al., 2017). However, the protein content of plants reduces with increasing plant maturity (Wadhave et al., 2014).

Urease is found in all plant species with varying levels of activity (Carlini and Polacco, 2008; Witte and Medina-Escobar, 2001). This can be seen from the urease activity and specific activity of the various leguminous seeds tested (Table 2). Soybean showed the highest activity of urease (1.82U/ml). This is consistent with previous reports of high level of urease activity in soybean (Real-Guerra et al., 2013). The activity of soybean urease is higher in the seeds than other parts of the plant (Joseph, 2022). The specific activity of soybean urease from this study is equivalent to what was reported by Tirkolaei et al. (2020). The other seeds tested also showed significant urease activity, with Sokoto White beans having the least activity (0.45 U/ml).

Legumes like soybeans with higher urease activity can hydrolyze urea more effectively, converting it into nitrogen usable by plants. Such legumes could be prioritized by farmers in areas needing efficient nitrogen utilisation, thereby minimizing fertilizer wastage and boosting crop yield. This insight is useful for agricultural practices involving these seeds because urease plays an important role in the utilisation of nitrogen by leguminous plants (Cao et al., 2010). Hence, urease

activity is vital to the growth and development of legumes and other plants (Rechenmacher et al., 2017). Moreover, urea fertilizer is the most widely used fertilizer in the world (FAO, 2011). Soybeans genetically deprived of urease have been shown to have a build-up of urea in their tissues (Rechenmacher et al., 2017).

However, the high urease activity in legumes like soybeans can potentially cause the release of excess ammonia into the soil and atmosphere if not properly managed. Hence, the proper management of urea fertilizer applications based on a crop's specific urease activity could support environmentally sustainable practices. This finding highlights the need for controlled application of urea fertilizers, especially in soybean cultivation, to prevent soil degradation and excessive ammonia emissions.

Furthermore, the seeds with higher urease activity are more likely to be viable with little or no insecticide and pesticide application. This is because aside their ureolytic function, ureases also play other roles in plants (Joseph, 2022). They defend plants against microbial attack and pests (Carlini and Ligabue-Braun, 2016). Urease-inhibited plants have been shown to be more susceptible to attack by microorganisms and herbivores (Real-Guerra et al., 2013). This urease-related pest resistance could support organic farming by reducing the need for chemical pesticides. Therefore, farmers could benefit from growing legumes with high urease activity as part of an integrated pest management strategy.

Legumes are generally rich in urease (Joseph, 2022), and urease has been previously extracted from broad beans (Bedan, 2020). More so, these legumes are widely cultivated and readily available. Soybean in particular ranks as number one amongst the most produced legume (Tamimie and Goldsmith, 2019). The seeds with higher urease activity can also serve as cheap sources of urease, thereby reducing the costs associated with importing or synthesizing urease for various applications. The high urease content of soybean contributes to its agricultural importance (Real-Guerra et al., 2013).

Conclusion

Urease is of immense agricultural importance owing to its role in making nitrogen available for plants through urea. This study serves as a guide in the cultivation of locally available legumes, especially with respect to the application of urea fertilizer, as well as pesticides. The efficient use of fertilizer by these crops will increase their yield and ultimately lead to food security and employment opportunities, which are the focus of Sustainable Development Goal 2 (SDG2) – zero hunger.

Furthermore, this study shows that while soybeans had the highest urease activity, the other varieties of leguminous seeds assessed also showed significant urease activity. They can therefore be exploited as cheap sources of urease for clinical diagnosis, industrial applications and research. This will enhance more productivity and promote innovation in line with Sustainable Development Goal 9 (SDG9), which is focused on industry, innovation and infrastructure.

Further studies exploring urease activity in different environmental conditions and across more legume species is highly recommended. More attention should be paid to the urease content of both crops and soil for better utilization of urea fertilizer.

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