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Activatory Effect of Germination on Catalytic Capacity of Urease Extracted from Beans Samples (Effect of Germination on the Kinetics of Urease from Beans)

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Authors' contributions

This work was carried out in collaboration between all authors. Author CEE designed the study and supervised the work. Author MOA carried out the laboratory work, statistical analysis, wrote the protocol, manage the literature searches, wrote the first draft of the manuscript and manage the analysis of the study. All authors read and approved the manuscript.

Research Article

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ABSTRACT

Aim: This research work was designed to determine the effect of germination on catalytic capacity of urease extracted from seven beans samples.
Study Design: Experimental.
Place and Duration: Department of Biochemistry, School of Science and Science Education, Federal University of Technology, Minna, Nigeria, between December, 2010 and September, 2011.
Methodology: Seven samples of beans namely; *Phaseolus lunatus, Parchyrhizus tuberrosus, Glycine max, Cajanus cajan, Mucuna pruriens, Kerstings geocarpa* and *Vigna mungo* were used by comparing urease activity in germinated to ungerminated beans samples.
Result: The protein level in germinated and ungerminated beans samples as well as, optimum pH, optimum temperature, substrate concentration and kinetic parameters, V_{max}, K_m, and V_{max}/K_m of urease were determined. The pH, temperature and substrate

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concentration ranged from 5.50-8.0, 30-80ºC and 0.1-0.6M respectively. From the results, the protein level in germinated beans samples reduced significantly ($p<0.05$) compared to ungerminated beans samples. The optimum pH and temperature ranged from 6.5-7.0 and 60-70ºC respectively for both germinated and ungerminated beans sample. Therefore, germination did not affect the pH and temperature stability of urease when compared to ungerminated beans samples. The results showed that germination significantly ($p=0.05$) increased V_{max} and there was no significant ($p=0.05$) difference in K_m of urease in germinated and ungerminated beans sample studied. However, the catalytic capacity (V_{max}/K_m) of urease in germinated beans was significantly ($p=0.05$) higher than ungerminated.
Conclusion: This implies that, with germination urease activity can be increased to meet both application of urea as fertilizer (agriculture), clinical analysis of urea in biological fluid, artificial kidney development and other applications.

Keywords: Urease; germination; protein; catalytic capacity.

1. INTRODUCTION

Urease (EC 3.5.1.5, urea amidohydrolase), a nickel-dependent metalloenzyme, catalyzes the hydrolysis of urea to form ammonia and carbon (IV) oxide. The hydrolysis of one molecule of urea results into the release of two molecules of ammonia and one molecule of carbon (IV) oxide [1]. Several different assays, based mainly on the measurement of the amounts of products released during the reaction, are available for quantifying urease activity.

From the onset of scientific discovery, urea and urease had been subjects of investigation. Urease from jack bean (*Canavalia ensiformis*) was the first enzyme ever to be crystallized [2]. Based on the available data on the biochemical and structural conformations of urease enzyme, urease from *Canavalia ensiformis* [3] and *K. aerogenes* [4] are the best-characterized. About 50 years later, it was shown that urease contains a metal (Nickel) at the active site. Hence, nickel-dependent ureases have been isolated from many bacteria, fungi [5,6] and higher plants [7]. However, despite the fact that they are isolated from different sources, their structural conformations [3] and mechanisms of catalysis [1] are quite similar.

Genetic analysis of urease expression [8,9] showed that, the process of activation requires participation of several accessory proteins that incorporate nickel into the urease forming catalytic site [7,5].

In the same vein, activation of urease is somewhat important most especially in the area of applications. Today, urease has several clinical; analysis of urea in biological fluid [10] and artificial kidney development [11], industrial; wastewater treatment [11] and environmental remediation [12] and agricultural [13] importance. Although some works had been done on urease activity, however, this work focuses on the study of the mechanism of urease catalyzed-reaction so as to determine its rate of reaction and how it (urease) changes in response to changes in concentration of urea (substrate) in seedling.

2. MATERIALS AND METHODS

The various beans samples used for this work: Lima beans (*Phaseolus lunatus*), Mexican yam beans (*Parchyrhizus tuberrosus*), Soya beans *Glycine max,* Pigeon pea (*Cajanus cajans*), Velvet beans (*Mucuna pruriens*), Kerstingiella (*Kerstings geocarpa*) and Black beans (*Vigna mungo*) were purchased from local market in Kaduna Nigeria. Acetone, Nessler's reagent, Tris buffer, and other chemical used were of analar grade.

2.1 Germination

The various beans samples were layered on woolen material and also covered with the same material, and water was applied to moisturize the beans. Under the humid and warm condition, the bean seeds initially swelled, then began to germinate (develop radicles) after 72 hours. The germination process was stopped by freezing the beans for 12 hours.

2.2 Extraction of Urease from Germinated and Ungerminated Beans

Urease was extracted from different beans samples according to the method described by [14]. The beans samples were blended coarsely with laboratory mortar and pestle and 50g of the beans meal was soaked overnight in 100ml extraction buffer (0.025m Tris-acetate buffer, pH 6.5) at 4ºC (Refrigeration temperature). The soaked beans meals was swirled for 2 minutes and then sieved with four layers of pre-washed and dried white muslin cloth, the filtrate was centrifuged at 15000rev/min for 15mins under refrigeration condition 4ºC. The clear supernatant was collected while the sediment was washed and discarded. The filtrate from the protein was added to the general pool.

2.3 Uraese Enzyme Assay

Urease assay was done according to the method described by [14]. To determine the urease enzyme activity in both germinated and ungerminated beans samples studied, 0.9ml of assay buffer (0.05M Tris-acetate, pH 7.0) was added to 0.1ml of appropriately diluted enzyme (20-fold dilution in same buffer) and incubated at 60ºC in 1ml of 0.2M urea. After 30mins, the reaction was stopped by reducing temperature to 10ºC. An aliquot (1ml) of the reaction mixture was transferred to a 50ml beaker and 1ml Nesslers' reagent was added with constant swirling. The volume was made up with distilled water after 1 min of reaction [12]. The yellow colour produced was measured at 405nm with UV/VIS spectrophotometer (Biochrom UV 2800 Double beam; UV/VIS scanning spectrophotometer). A blank was run without the enzyme and urea. An enzyme Unit (U) in this present work is defined as the amount of enzyme required to liberate 1mmol of ammonia in 1 sec under the reaction conditions (0.2M urea, 0.05M Tris-acetate buffer, pH 7 at 60ºC). The experiments were conducted in triplicate.

2.3.1 Protein assay

The protein was calculated according to method described by [15], after precipitation with acetone. This method for protein determination is based on the ability of the aromatic amino acid (Tyrosine, Tryptophan and phenylalanine) to absorb light at ultraviolet region of the light spectrum, 235-280nm.

2.3.2 Optimum pH

To determine the optimum pH for urease activity from germinated and ungerminated beans samples, enzyme activity were assayed in pH ranging from 5.5 to 8.0 at temperature 60ºC and 0.2M urea for 30mins.

2.3.3 Optimum temperature

To determine the optimal temperature for urease activity from both germinated and ungerminated beans samples, urease enzyme was suspended in Tris-acetate buffer (0.05M) at optimum pH above, and incubated at different temperatures (30 to 80ºC) for 30 min before the activity was measured.

2.3.4 Determination of the kinetic parameters (V_{max} and K_m)

To determine the kinetic parameters (K_m and V_{max}) the substrate concentration was varied (0.1-0.6M), at optimum pH and temperature of urease from germinated and ungerminated beans. The values of *K*m and *V*max were determined using Lineweaver–Burke plots. The catalytic capacity (V_{max}/K_m) was determined for urease from germinated and ungerminated beans samples according to the method of [16].

3. RESULTS AND DISCUSSION

3.1 Protein Level in Germinated and Ungerminated Beans Samples

The results of protein level in germinated and ungerminated beans are presented in Table 1. From the result, germination significantly (*P*=.05) reduced the protein level in the beans when compared to the ungerminated beans samples. However, the effect of germination on protein level of *Cajanus cajan* was more significant (*P*=.05) than other beans samples, with 71.36% and 3.49fold decrease in protein level, whereas, germination effect on *Parchyrhizus tuberrosus* was least significant (*P*=.05) with 2.89% and 1.03fold decrease in protein level when compared to others. Generally, it was observed that germination significantly (*P*=.05) reduced the level of protein in beans samples studied. [17], had shown that, urease in conjunction with arginase utilizes seed protein reserve during germination. Additionally, according to [3], proteins, also act as urease-specific chaperones in the process of nickel fixation into urease active site, by playing an activatory role during germination [17,18,19], thereby making free protein unavailable.

Table 1. Protein level in germinated and ungerminated beans samples

Beans samples	Germinated	Ungerminated	%Decrease	Fold decrease
Glycine max	60.13±8.89^{b}	90.17±2.11^{a}	33.32	1.50
P. tuberrosus	43.73±0.47^{a***}	455.03±7.65^{a***}	2.89	1.03
P. lunatus	46.93±9.25 ^{a***}	51.00±4.16^{a}	7.98	1.09
Cajanus cajan	15.13±2.14^{b}	52.83±2.02^{a}	71.36	3.49
M. pruriens	91.13±5.54^{b***}	95.57±7.68^{a**}	4.65	1.05
K. geocarpa	29.30±2.08^{b***}	67.17±2.14^{a}	56.38	2.29
Vigna mungo	22.37±6.04^{b***}	72.90±2.08^{a}	69.31	3.26

Down columns, values with ns, *, ** and * are statistically different from Glycine max at P< .05, <.01 and <.001 level of significance respectively. Across rows, values with different letters (superscripts) are statistically different at P=0.05. Each data is mean±SD of three replicates.*

From the foregoing, it became obvious that, naturally as plants grow, the protein levels of the seedlings reduce as a result of biological handling of nickel and nickel trafficking in the urease system. However, the information about the role of proteins in nickel incorporation during urease activation is not limited to plants only. Bacteria such as *K. aerogenes, Helicobacter spp.* etc and any other urea metabolizing organism make use of their accessory protein for urease maturation [4,5]. Based on these observations, there was reduction in protein levels in germinated beans when compared to the ungerminated, as shown in this study.

In the same vein, the pattern of decrease (folds and %) in protein level differs in the beans samples studied. These variations could be attributed to the fact that, each of the beans samples had different protein level.

3.2 Optimum pH and Temperature

The result of optimum pH for urease extracted from germinated and ungerminated beans samples are presented in Figs. 1.1 (a-c) to 1.2 (a-b) respectively. The result showed that, the optimum pH of urease from both germinated and ungerminated beans samples ranged between 6.5 and 7.0. The results of optimum temperature for urease from germinated and ungerminated beans are shown in Figs. 2.1 (a-c) to 2.2 (a-b) respectively. From the results, the optimum temperature for urease from both germinated and ungerminated beans samples ranged between 60 and 70ºC.

The pH optimum for crude urease in both germinated and ungerminated beans samples shifted towards neutral and was stable within the pH range of 6.5-7.0 as shown in the result. This result is supported by works from other researchers. For instance, optimum pH for urease activity in Jack beans [20,21], *Rhyzopus oryzea* [22] and surface black soil [23] were found to be 7.0, 7.0 and 6.45 respectively. Moreover, Urea is said to be a very stable organic compound in an aqueous medium (pH). This stability also contributes to its rate of hydrolysis by the action of urease enzyme [24].

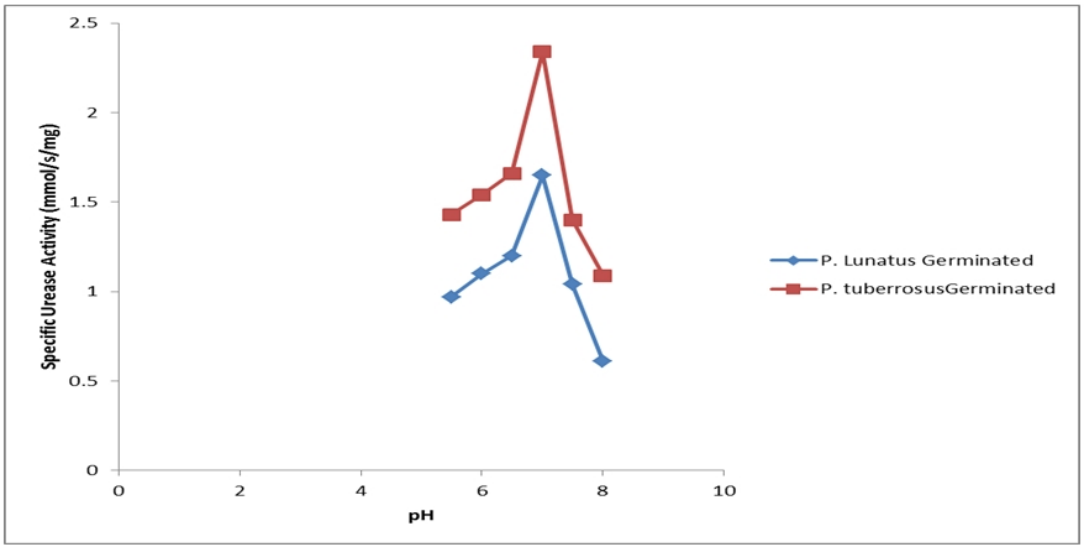

Fig. 1.1a Effect of pH on urease activity (germinated beans)

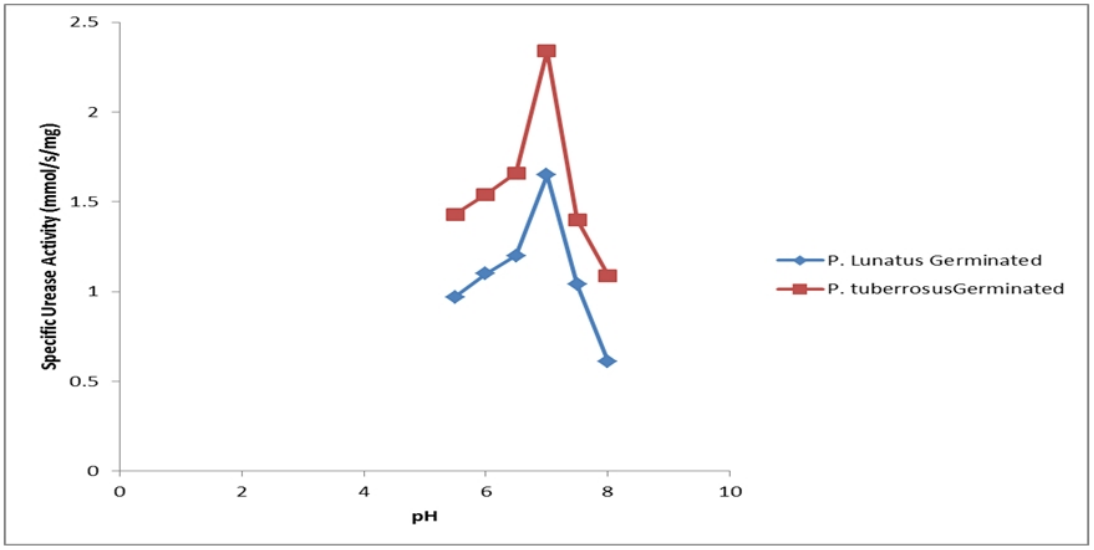

Fig. 1.1b Effect of pH on urease activity (germinated beans)

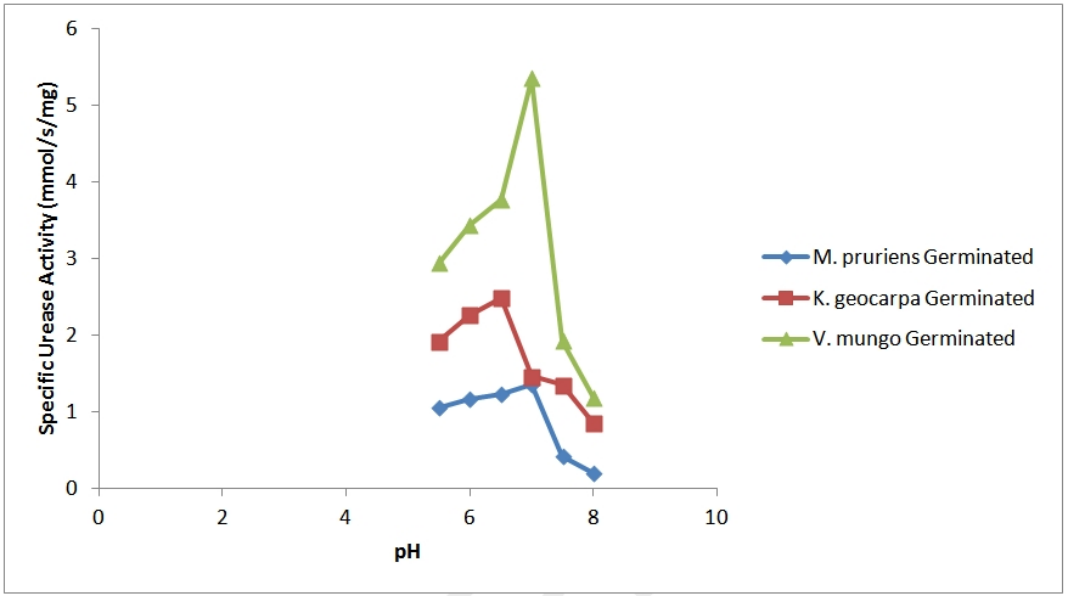

Fig. 1.1c Effect of pH on urease activity (germinated beans)

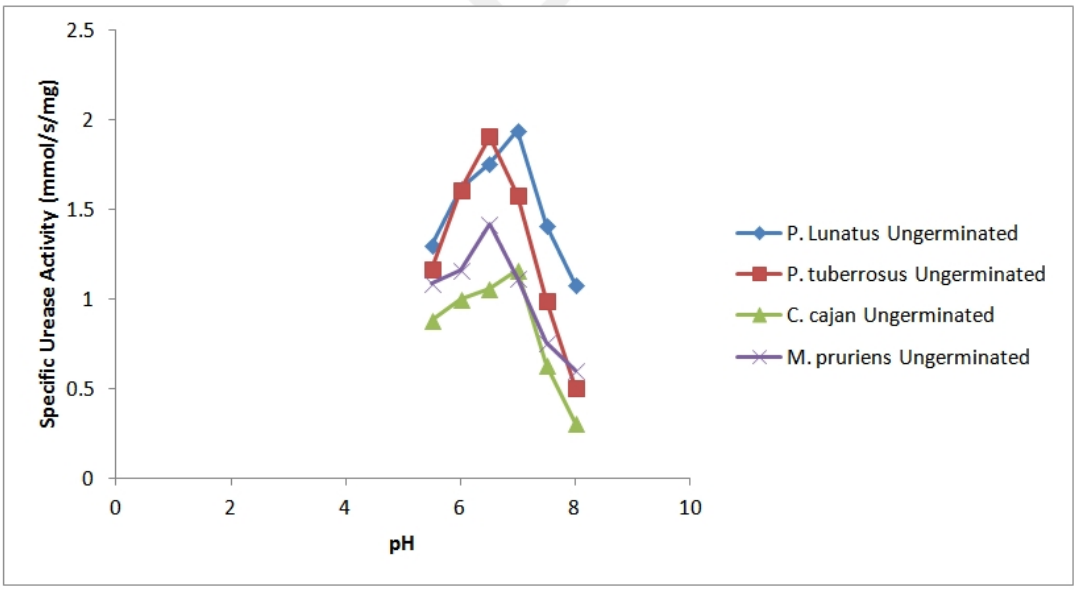

Fig. 1.2a Effect of pH on urease activity (ungerminated beans)

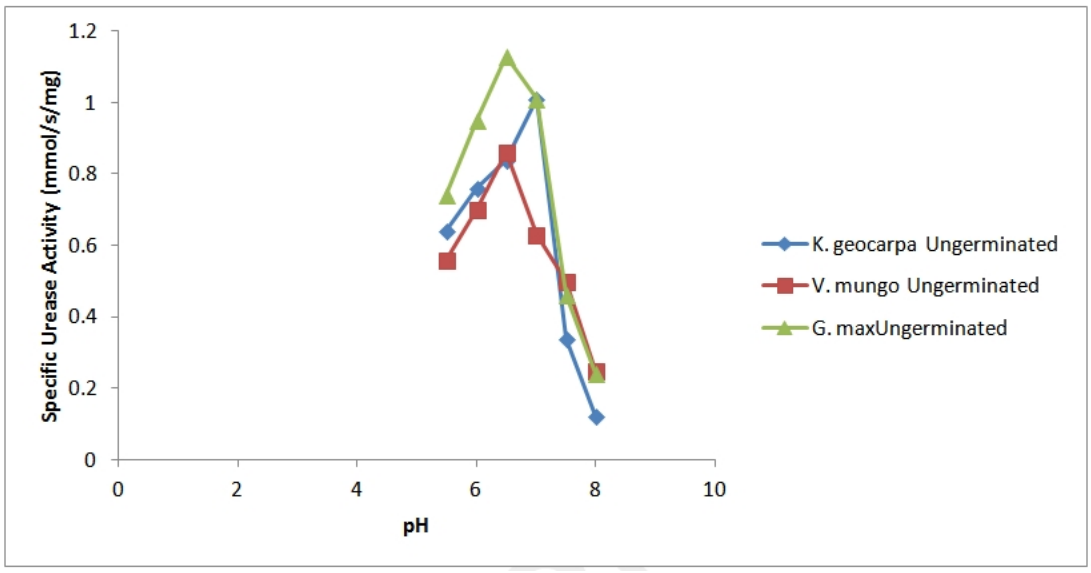

Fig. 1.2b Effect of pH on urease activity (ungerminated beans)

The stability of urease activity around neutral pH values may be explained by the structural changes occurring in enzyme proteins caused by pH variation. The tertiary structure of a protein depends on interactions such as hydrogen bonding between enzyme amino acids functional groups. A change in pH can alter the ionization of these amino acid side chains and disrupt the native conformation and in some cases denature the enzyme. Hence, each enzyme has an optimal pH range that helps the maintenance of its native conformation in an environment, where it operates. The degree of ionization of the surface functional groups of

amino acid residues is a function of the medium's pH. The main factor involved here is the titration of the ionizable group which maintains surface charge, in the active site, or stabilizes the enzyme resulting in an optimum pH for the enzyme. Observations from this work indicated that, germination did not affect optimum pH for urease activity. This is because; it has been shown that, the incorporation of nickel in the active site of urease during the process of germination does not alter the conformational state of the urease enzyme [18]. Since the optimum pH of enzyme only depends on tertiary structure of protein, therefore, germination did not confer any special trait on urease that could influence the optimum pH, owing to the fact that, there was no meaningful difference in optimum pH of urease activity in germinated and ungerminated beans samples.

The optimum temperature ranged between 60-70^{o}C for both germinated and ungerminated beans samples as indicated in the result. From the result, germination did not have effect on the optimum temperature of urease when compared to the ungerminated (Figs. 2.1 and 2.2). [24], observed that, urease is a thermostable enzyme. Published works on optimum temperature for urease activities in Jack beans [18], *Rhizopus oryzae* [22], *Yersinia enterocolitica* [25], purified *Chenopodium album* leaves [26] are 60, 55, 65, 60 and 40^{o}C respectively.

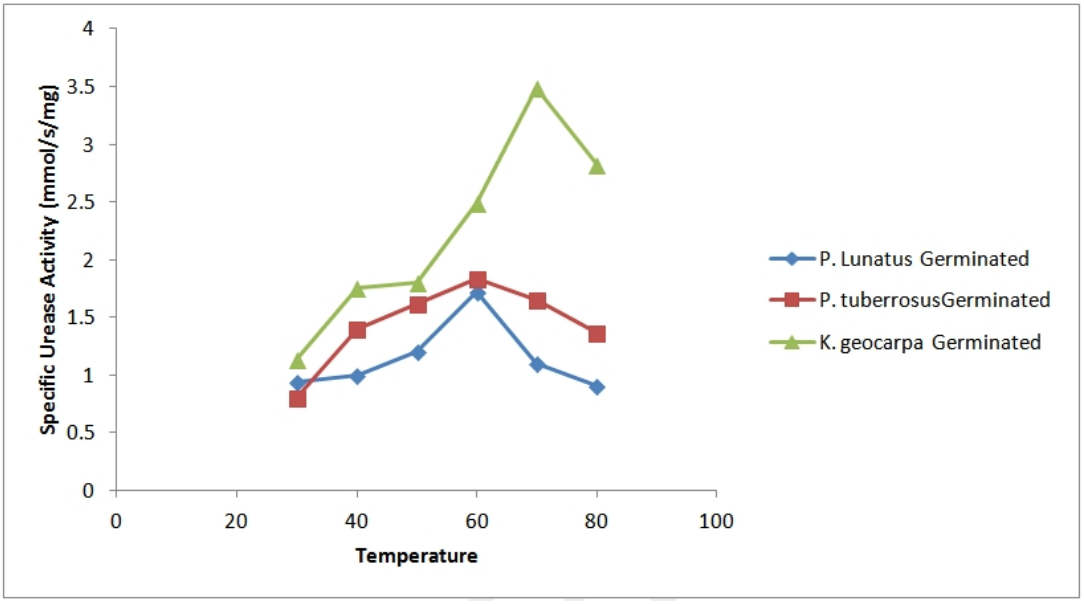

Fig. 2.1a Effect of temperature on urease activity (germinated beans)

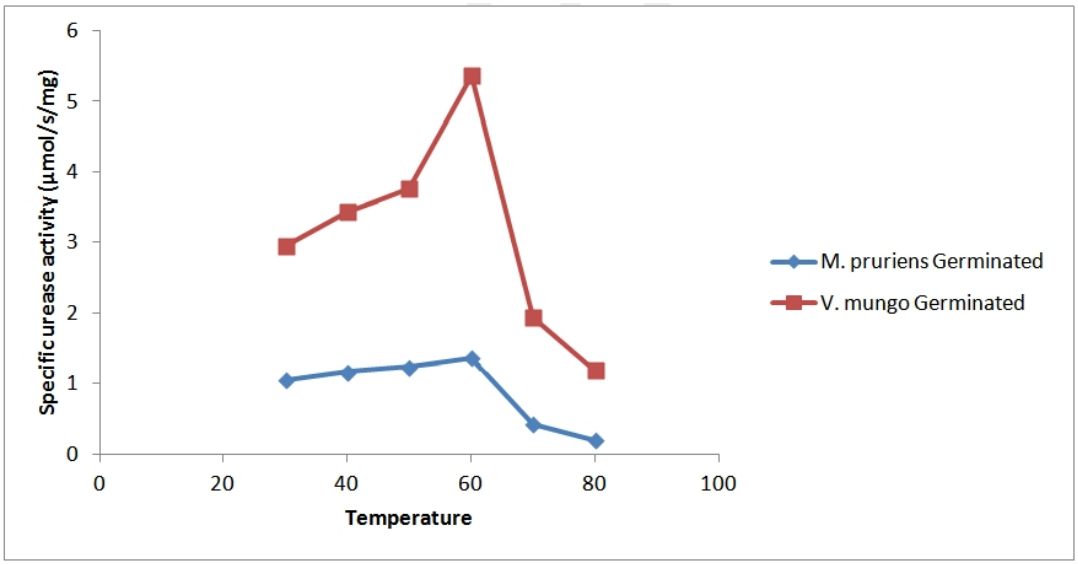

Fig. 2.1b Effect of temperature on urease activity (germinated beans)

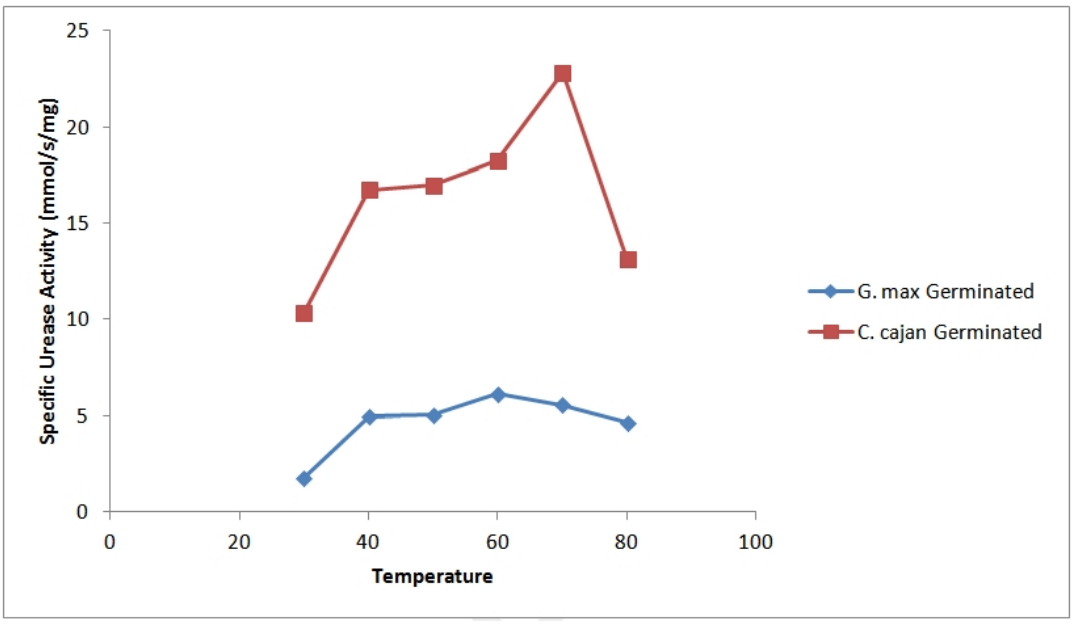

Fig. 2.1c Effect of temperature on urease activity (germinated beans)

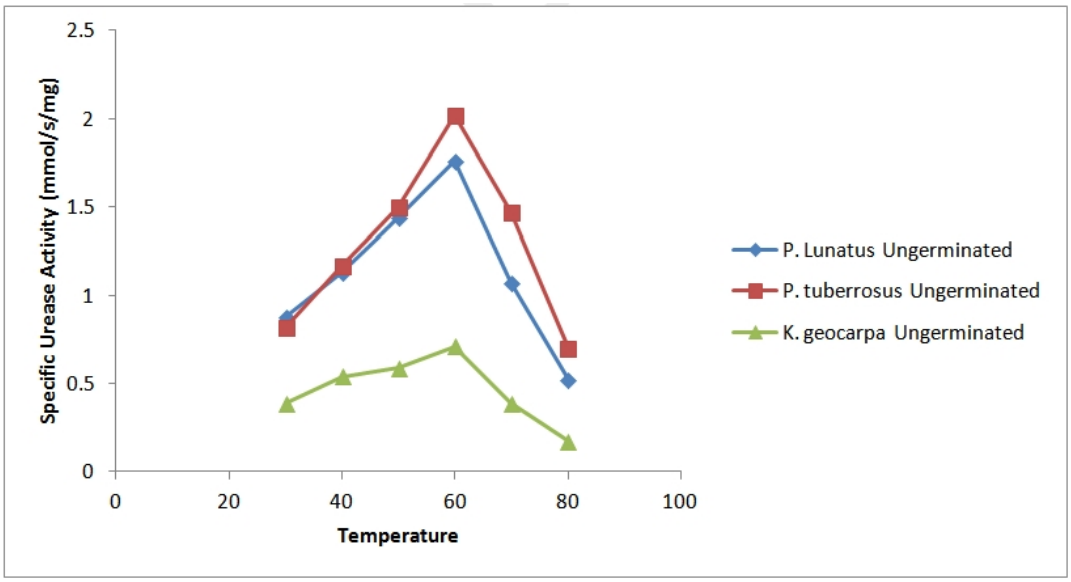

Fig. 2.2a Effect of temperature on urease activity (ungerminated beans)

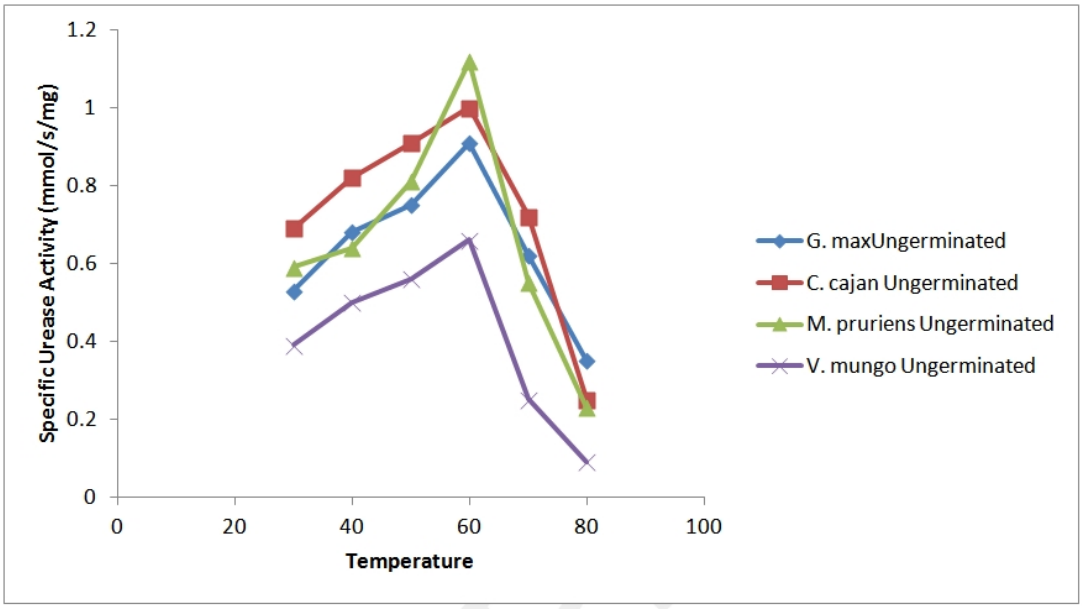

Fig. 2.2b Effect of temperature on urease activity (ungerminated beans)

When molecules collide (e.g enzyme and its substrate), the kinetic energy of the molecules can be converted into chemical and potential energy of the molecules. If the chemical and potential energy of the molecules become great enough, the activation energy of an exergonic reaction can be achieved and a change in chemical state will result. Thus, the greater the kinetic energy of the molecules in a system, the greater is the resulting chemical

and potential energy when two molecules collide. As the temperature of a system is increased, it is possible that, more molecules per unit time will reach the activation energy. Thus the rate of the reaction may increase.

In order to convert substrate into product, enzymes must collide with and bind to the substrate at the active site. Increasing the temperature of a system will increase the number of collisions of enzyme and substrate per unit time. Thus, within limits, the rate of the reaction will increase.

As the temperature of the system is increased, the internal energy of the molecules in the system will increase. The internal energy of the molecules may include the translational energy, vibrational energy and rotational energy of the molecules, the energy involved in chemical bonding of the molecules as well as the energy involved in nonbonding interactions. Some of this heat may be converted into chemical and potential energy. If this chemical potential energy increase is great enough some of the weak bonds that determine the three dimensional structure of the active enzyme may be broken. This could lead to a thermodynamic increase of reaction rate followed by a steep drop caused by thermal denaturation of the enzyme. Thus, too much heat can cause the rate of an enzyme-catalyzed reaction to decrease because the enzyme or substrate becomes denatured [27]. Therefore, since nickel fixation into the active site of urease during the process of germination does not alter the structural conformation of the urease enzyme [18], and given the above considerations, the temperature dependence of urease-catalyzed reaction exhibits an optimum at these various points (Figs. 2.1 and 2.2).

3.3 Substrate Effect and Enzyme Kinetics (V_{max} and K_m)

The results of substrate effect on urease enzyme activity are presented in Figs. 3.1 (a-b) and 3.2 (a-b) respectively. From the results, urease activity follows the expected Mechelis-Menten curve. The results of reaction velocity (V_{max}) and the Michelis-Menten's constant (K_m) are presented in Figs. 4.1 and 4.2 and the summary of the kinetic parameters in Table 2 respectively. The V_{max} of urease from germinated beans samples was significantly (P=.05) higher than the ungerminated beans, while the K_m showed a corresponding Michelis-Menten pattern. It has been shown earlier that, germination influences proper assemblage of Ni^{2+} at the active site of urease enzyme (enzyme activation). Result showed that, no significant (P=.05) difference in K_m values was observed in urease from germinated beans samples compared to the ungerminated except in *Glycine max*.

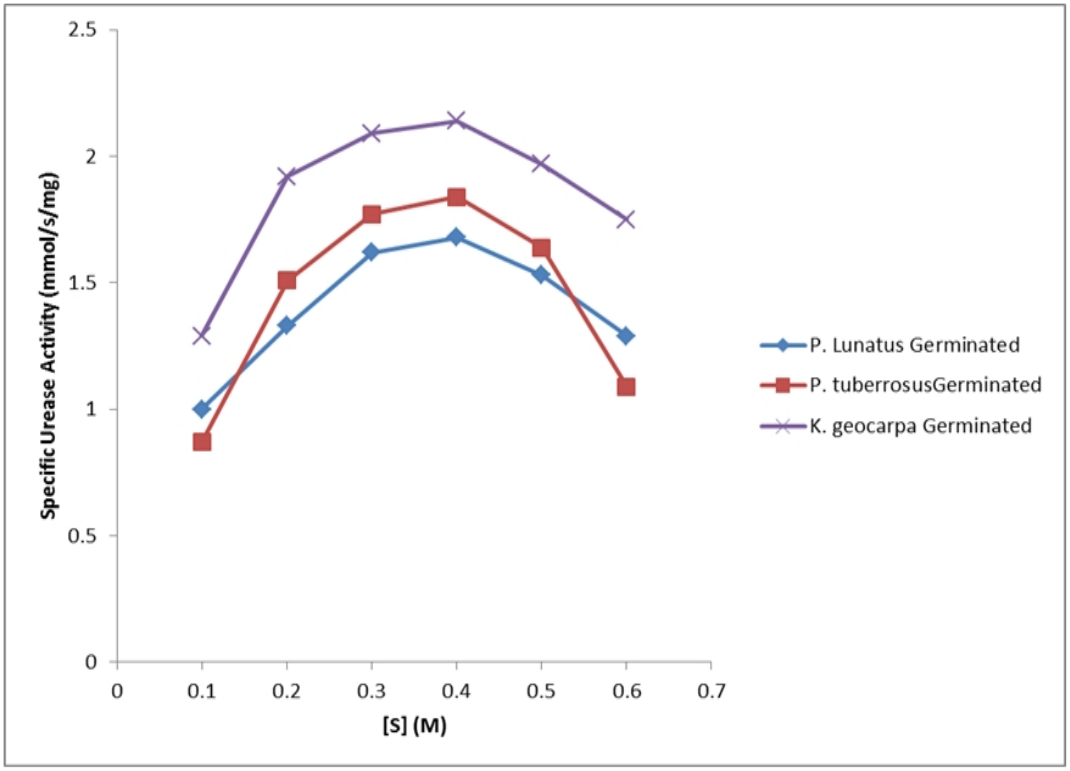

Fig. 3.1a Effect of substrate concentration on urease activity (germinated beans)

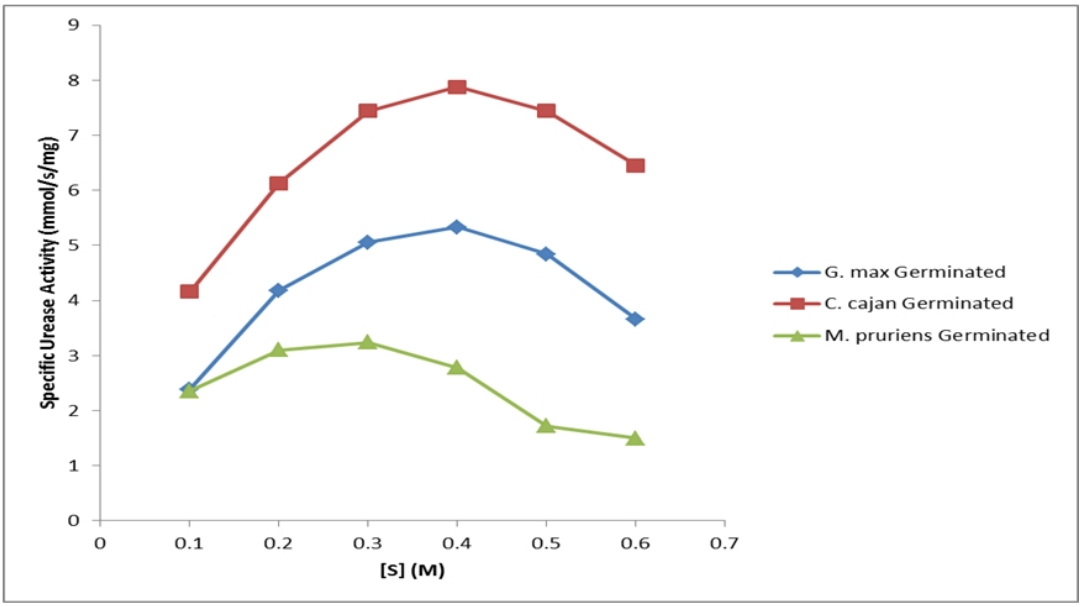

Fig. 3.1b Effect of substrate concentration on urease activity (germinated beans)

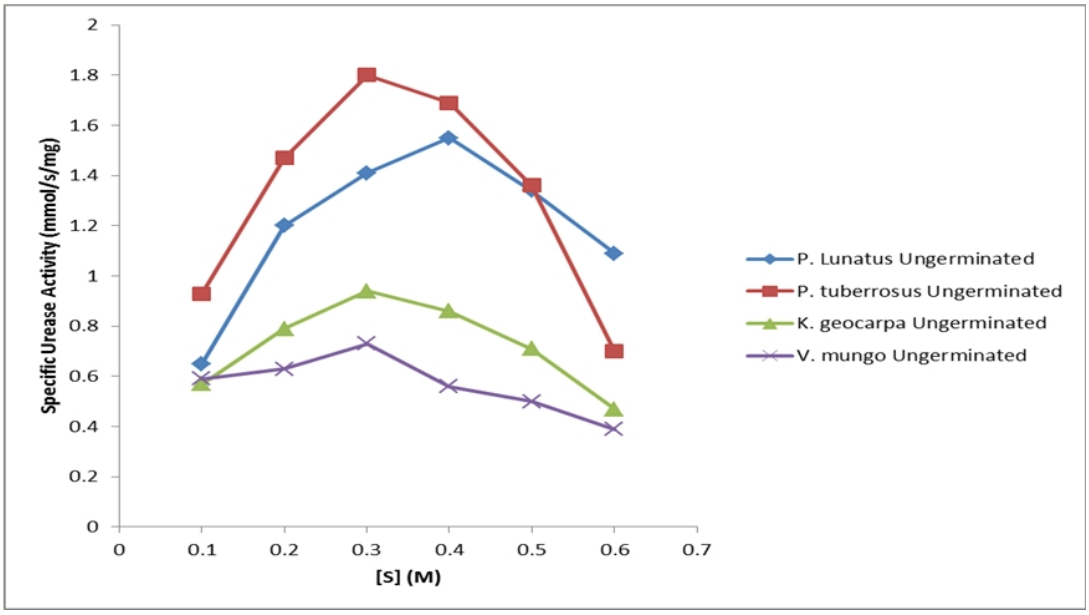

Fig. 3.2a Effect of substrate concentration on urease activity (ungerminated beans)

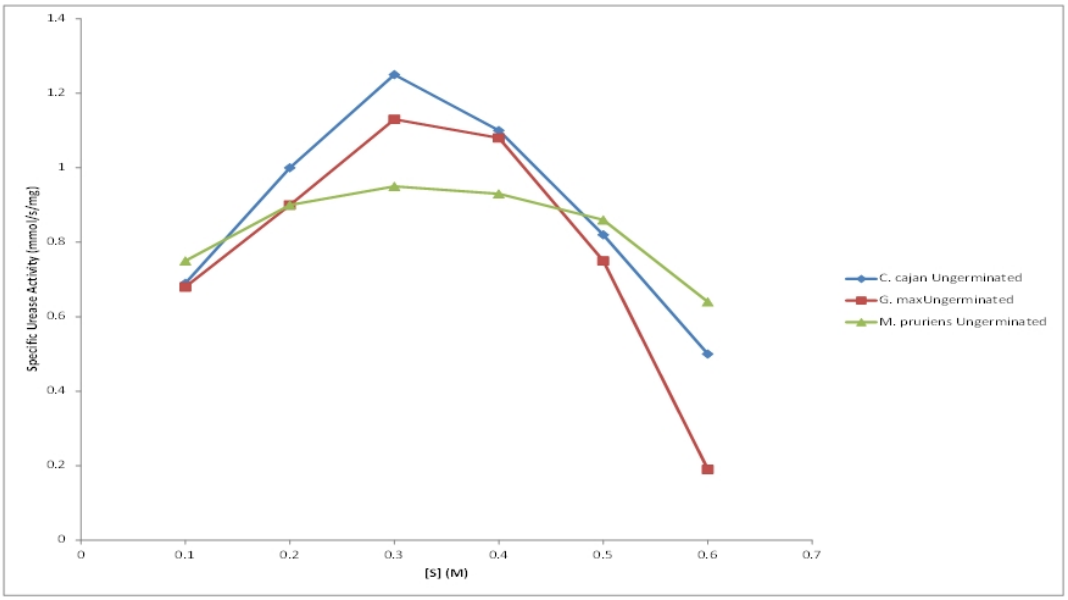

Fig. 3.2b Effect of substrate concentration on urease activity (ungerminated beans)

This showed that the process of activation by germination increased the affinity of urease for its specific substrate urea, thereby making its active sites more accessible to its substrate. However, the K_m values varied between the urease in different beans samples studied. The values of the V_{max} were also different in each of the beans samples, germinated and ungerminated respectively. The effect of germination on K_m had a corresponding relationship

with the V_{max}. That is, the activity of the enzyme depends on its affinity for urea. Therefore, the increase in maximum velocity (V_{max}) observed in urease, is due to the effect of germination on the urease active site compared to the ungerminated samples. This observation is further strengthened by the catalytic capacity (V_{max}/K_m) of urease in germinated compared to ungerminated beans samples respectively. From results presented in Table 2, it was clearly shown that, germination increased the catalytic capacity of urease. It was also deduced from the result that, with increased V_{max} and reduced K_m, the catalytic capacity of the enzyme is heightened. Therefore, the ratio V_{max}/K_m of an enzyme is an index of the catalytic capacity of enzyme through enzymatic reactions [16]. That is, the higher the affinity of the enzyme for the substrate (K_m), the higher the maximum velocity (V_{max}) and this will also increase catalytic capacity of the enzyme. Hence, the relationship of K_m and V_{max}, and the mechanism of nickel fixation into active site of urease by germination are in accordance with the principle of enzyme kinetics. [18], showed that, the K_m and V_{max} of urease from jack beans are 0.25mmol/L and 0.154mmol/mg respectively. Whereas, [25] showed that, *Yersinia erocolitica* had K_m and V_{max} of 1.7±0.4mM and 7.29±0.42µmol/min respectively. Comparing these results, urease from germinated beans samples is of better catalytic capacity than urease from these sources.

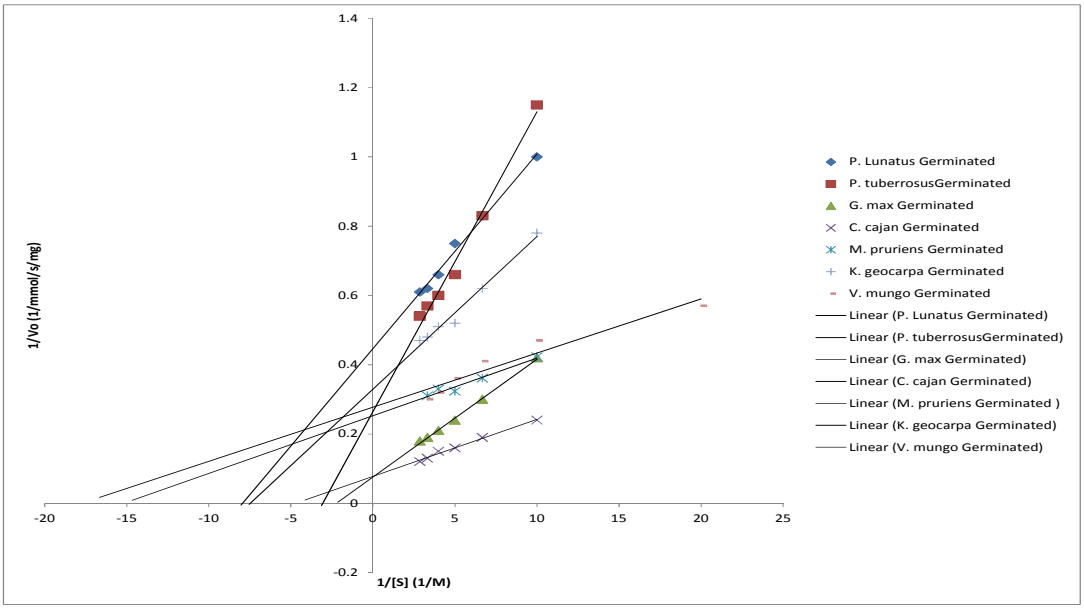

Fig. 4.1 Double reciprocal plot of rate V^1 *vs 1/[S]* for urease from germinated beans

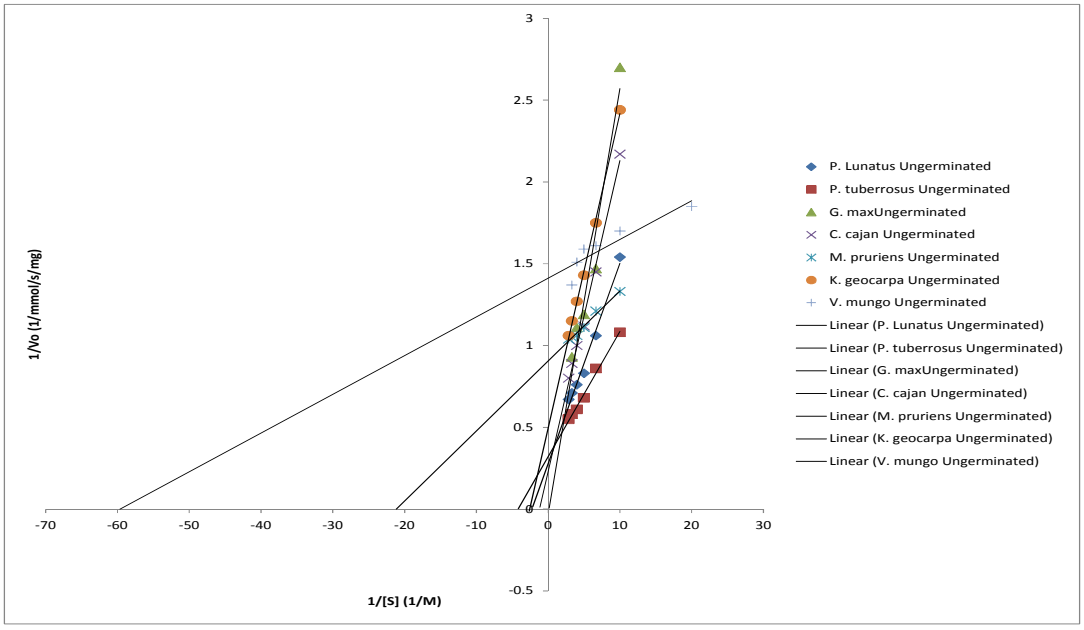

Fig. 4.2 Double reciprocal plot of rate V^{-1} *vs 1/[S]* for urease from ungerminated beans

Although, there were obvious differences in the values of V_{max} and K_m of urease from the various beans samples, the comparison of V_{max} values among the beans samples studied (Table 2) showed that, there was no significant (p=0.05) difference in V_{max} of germinated *Glycine max* and *Cajanus cajan*. However, the V_{max} of urease in germinated *Mucuna pruriens* was significantly (p=0.05) higher compared to *Vigna mungo, Kerstings geocarpa, Phaseolus lunatus and Parchyrhisus tubberrosus*. Generally, the V_{max} values were higher in germinated than the ungerminated beans samples because germination had activatory effect on urease from samples studied. Moreover, there was a consistent trend of higher catalytic capacity (V_{max}/K_m) as influenced by germination in all the germinated beans samples.

Hence, since germination increased the catalytic capacity of urease, it indicated that, plants during germination can efficiently hydrolyze both internally generated urea and the one in the soil. It therefore means that, the bulk flow of urea from hydrolysis of arginine by arginase [14] and soil into the plant increase the activity of the enzyme. That is, with an increased urea concentration, there is higher hydrolysis of urea (higher V_{max}) until the active site becomes saturated (optimum substrate concentration), thereby lowering the sensitivity of the enzyme to the substrate. Owing to this phenomenon, when the concentration of a substrate is increased, there will be lower enzyme activity on substrate at the optimum. Thus, the biochemical characteristics of urease during plant germination mean that, the V_{max} is increased so as to metabolize urea generated in the plant and the urea supplied as fertilizer (external). This observation elucidates the importance of urease and urea in agriculture, most especially in the area of soil fertility. Consequently, enzyme kinetic parameter is a function of substrate concentration and enzyme activity, and Michelis-Menten constants (K_m) for enzymes usually range between 10^{-2} and 10^{-5} M for most enzymes [27].

Table 2. Kinetic characteristics of urease from germinated and ungerminated beans

	V_{max}(mmol/s/mg)		K_m(mM)		Catalytic capacity	
Beans sample	**Germinated**	**Ungerminated**	**Germinated**	**Ungerminated**	**Germinated**	**Ungerminated**
Glycine max	11.67±0.73[b]	1.64±0.17[a]	0.38±0.04[b]	0.15±0.03[a]	**30.71**	**10.93**
P. tuberrosus	4.09±0.47[a]***	3.68±0.88[a]***	0.30±0.10[a]	0.35±0.15[a]**	**13.63**	**10.51**
P. lunatus	2.23±0.16[a]***	1.87±1.63[a]	0.15±0.05[a]***	0.29±0.04[a]**	**14.87**	**6.45**
Cajanus cajan	12.49±0.56[b]	2.04±0.65[a]	0.21±0.01[a]*	0.20±0.11[a]	**59.48**	**10.20**
M. pruriens	6.68±0.81[b]***	3.39±0.20[a]**	0.28±0.05[a]	0.29±0.22[a]*	**25.69**	**12.11**
K. geocarpa	3.06±0.26[b]***	1.32±0.08[a]	0.14±0.04[a]**	0.13±0.01[a]	**21.86**	**10.15**
Vigna mungo	4.95±0.71[b]***	1.02±.06[a]	0.18±0.06[a]**	0.046±0.004[b]*	**27.50**	**22.17**

*Down columns, values with ns, *, ** and *** are statistically different from Glycine max at P< .05, <.01 and <.001 level of significance respectively. Across rows, values with different letters (superscripts) are statistically different at P<.05. Each data is mean±SD of three replicates.*

4. CONCLUSION

In this study, it was observed that germination reduced the protein level and increased the activity of urease when compared to ungerminated beans samples. In view of what other researchers have done, seed reserved protein was used to assemble the active site of urease by incorporation of nickel. It was discovered that, the optimum pH and temperature stability of the enzyme were not affected by germination, since both germinated and ungerminated beans samples have similar optimum pH and temperature. The results of this experiment also showed that, the K_m and V_{max} were greatly influenced by germination in that, germination increased V_{max} and reduced $K_{m.}$ Therefore the catalytic capacity of the enzyme was increased. These express the significance of the application of urease in agriculture, clinical chemistry (blood urea analysis can efficiently be carried out with urease enzyme) and industry. The mechanism that activated urease activity during germination makes urease an important enzyme in agriculture and also suggests urea as a very good way of increasing soil fertility, owing to urease ability to change in response to change in urea concentration. And also, the incidence of leaf necrosis (dryness of leaf tips) which could have reduced surface area for photosynthesis due to excess urea in plant is equally masked by increase in urease activity.

The urease kinetics observed in *Mucuna pruriens* compared to *Glycine max* and *Cajanus cajan* showed that, the legume could be a very good and cheaper source of plant urease for industrial or clinical purposes most especially because of its nutritional disadvantages.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCE

1. Callahan, Yuan, Wolenden. Urease: the most efficient enzyme. J Am Chem Soc. 2005;127:10828.
2. Summer JB. The isolation and crystallization of enzyme urease, J Biol and Chem. 1926;69:435-441.
3. Hirai H, Kawai-Hirai R, Hirai T, Ueki T. Structural change of jack bean urease induced by addition of surfactants studied with synchrotron-radiation small-angle X-ray scattering. J Euro Biochem. 1993;215:55-61.
4. Jabri E, Carr MB, Hausinger RP, Karplus PA. The crystal structure of urease from *Klebsiella aerogenes*. Sci. 1995;268:998-1004.
5. Mobley HL, Island MD, Hausinger RP. Molecular biology of microbial ureases. Microbiol Rev. 1995;59:451-480.
6. Tange Y, Niwa O. Identification of the *ure1*+ gene encoding urease in fission yeast. Curr Gene. 1997;32:244-246.
7. Polacco JC, Holland MA. Genetic control of plant ureases; in genetic engineering (Setlow, J.K., ed.). Plenum Press, New York. 1994;16:33-48.

8. Stebbins N, Holland MA, Cianzio SR, Polacco JC. Genetics tests of the roles of the embryonic urease of soybean. Plant Physiol. 1991;97:1004.
9. Zonia LE, Stebbins NE, Polacco JC. Essential role of urease in germination of nitrogen limited Arabidopsis thaliana seeds. Plant physiol. 1995;107(4):1097-1103.
10. Chellapandian M, Krishnan RVM. Removal of urea from blood in the treatment of uremia. Pro Biochem. 1998;33(6):595.
11. Nose Y. Artificial kidney research, is it not really necessary? Artificial organs. 1990;14:245.
12. Tsai H, Doong R. Simultaneous determination of pH, urea, acetylcholine and heavy metals using array based enzymatic optical biosensor. Biosen Bioelect. 2005;20:1796-1804.
13. Gerendas J, Zhu Z, Sattelmacher B. Influence of N and Ni supply on nitrogen metabolism and urease activity in rice (*Oryza sativa* L.). J Experi Bot. 1998;49:1545-1554.
14. Kayastha AM, Das N. A simple experiment for teaching enzyme immobilization with urease and its application in blood urea estimation. Biochem Edu. 1999;27:114-117.
15. Whitaker JR, Granum PE. An absolute method for protein determination based on difference of absorbance at 260 and 280nm. Anal Biochem. 1980;109:156-159.
16. Juan YH, Chen LJ, Wu ZJ, Wang R. Kinetics of soil urease affected by urease inhibitors at contrasting moisture regimes. J Soil Sci Plant Nutri. 2009;9(2):125-133.
17. Hausinger RP. Urease active site assembly. J Biochem; 1997.
18. Salomone-Stagni, Zameli M, Ciurli, Protein. Biochem. 2007;68:749-761.
19. Polacco JC, Holland MA. Roles of urease in plant cells. Int Rev Cytol. Jeon, K.W. & Jarvik, J., eds., Academic Press, Inc., San Diego. 1993;145:65-103.
20. Bahadur A, Kespi P, Neelam M. Immobilization of urease in alginate, paraffin and lac J Serb Chem Soc. 2010;75(2):175–183.
21. Zu Pei LIANG, Ya Qing FENG, Shu Xian MENG, Zhi Yan LIANG. Preparation and Properties of Urease Immobilized onto Glutaraldehyde Cross-linked Chitosan Beads. Chinese Chem Letters. 2005;6(1):135-138.
22. Neveen SIG. Purification and characterization of intracellular Urease Evzyme Isolated from *Rhizopus oryzae*. Asian Net Scient Info; Biotech. 2006;5(3):358-364.
23. Lu RK. Methods of soil and agrochemistry analysis. Chinese Agric Sci Techn. Press, Beijing; 2000.
24. Krajewska B, Ureases I. Functional, catalytic and kinetic properties: A Rev. J Mol Cata B: Enz. 2009;59:9-22.
25. Jugsharan SV, Neeru B. Molecular and biochemical characterization of urease and survival of *Yersinia enterocolitica* biovar 1A in acidic pH *in vitro.* Biomed Cent Microbiol. 2009;9:262.
26. El-Shora HM. Properties and immobilization of urease from leaves of *Chenopodium album* [C3]. Bot Bull Aca Sini; 2001;42.
27. Wolfgang A. Enzyme in industry 3rd ed. 2007; ISBN: 978-3-527-31689-2.

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