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KINETIC CHARACTERISTICS OF UREASE ISOLATED FROM SELECTED BEANS SPECIES AS AFFECTED BY GERMINATION

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ABSTRACT

In this research four different beans (*Glycine max, Mucuna pruriens, Kerstings' geocarpa and Vigna mungo*) were germinated in order to evaluate the implication of germination on the kinetic parameters (*Vmax* and *Km*) of urease isolated from the various beans and compared to the non-germinated (control). The results showed that, *Vmax* and *Km* of urease from germinated beans increased significantly when compared ($p<0.05$) to urease from the control. This simply means that, urease from germinated beans demonstrated higher activity with lower substrate sensitivity when compared ($p<0.05$) to the control.

Keywords: Kinetics, Germination, Urease.

INTRODUCTION

Urea is efficiently hydrolyzed by urease (E.C 3.5.15) a nickel dependent enzyme, to form ammonia and carbon (IV) oxide. The enzyme urease was first isolated and crystallized by James Summer in 1926 form jack beans (*canavalia ensiformis*) as a pure crystalline enzyme (James, 1926). Crystalline form of urease was the first obtained for a known enzyme.

Urea fertilizer is the predominant solid nitrogen (N) fertilizer in Chinese agriculture, and constitutes over 50% national nitrogenous fertilizer consumption (Zhu and Chen, 2002). However, the reaction catalyzed by urease is essential to make urea nitrogen available for plants growth and development (Gerenda´s et al., 1999). This is as aresult of the fact that urea is rich in nitrogen and urease gets the N available for plants.

Besides, urease is a very efficient way of determining urea level in biological fluid as well as in drinking and industrial water. Additionally, it is also used as reducing agent in alcoholic beverages (Fujinawa and Dela, 1990; Fujinawa and Ouch, 1991). Urease is also found useful since urea has commercial uses for the manufacture of hair conditioner, glues, plastic and animal feed as a browning agent in factory produced pretzels and also drug uses of urea in topical dermatological products, for nonsurgical debridement of nails and as a diuretic (Wikipedia, 2006). In the assessment of environmental pollution with mercury (II), array based enzyme biosensors have been reported for screening various environmental pollutants, mainly heavy metals and pesticides. Urease has been extensively used as a model enzyme to elucidate the applicability of inhibitory assay for mercury (II) (Tsai and Doong, 2005; Preinmger, 1999 and Mahnaod, 2001).
This work was designed to investigate the kinetic characteristics of urease from germinated beans when compared to the non-germinated.

Materials and Mathods

Soya bean-*glycine max*, Black gram-*Vigna mungo*, Mucuna spp- *Mucuna prureins* and Kerstingella-*Kerstings geocarpa* were procured from local market. Nessler's reagent, tris buffer and other chemical used were of analytical grade.

Methods

Germination Process

The beans were allowed to germinate or to develop the beginning of a root system. This was achieved in 3 days. Beans are essentially a seed and under warm and moist condition will begin to sprout or root, just as it would if it were growing in nature. Under this humid condition, bean seed initially swelled than germinated such that the radicle appeared. The germination process was stopped by freezing the beans for 12 hours followed by drying.

Urease Isolation

Individual germinated and non-germinated beans were blended coarsely with laboratory mortar and pestle (wooden) and 50g of the same beans were soaked overnight in 100ml extraction buffer (0.025m Tris-acetate buffer, pH 6.5) at 4^0C (Refrigeration temperature). The pH range for Tris buffer (7.5-9.5), is higher than the chosen pH 6.5; this was done for purification reason and to enhance maximum extractable activity and higher percentage recovery of enzyme during purification (Kayastha and Nilanjana, 1999). The next day, the soaked beans meals were swirled for 2 minutes and then sieved with four layers of pre-washed and dried white handkerchief, and the filtrate centrifuged

at15000 rev/min for 15mins at 4^0C. The clear supernatant was collected while the sediment was discarded.

Protein Determination

The protein content of the beans was determined spectrophotometrically after precipitation with acetone (Whitaker and Granum, 1980).

Urease assay

To measure the urease activity, the ammonia liberated on incubating the enzyme with urea for 30mins was determined using Nessler's reagent (Kulshrestha and Husain, 2006). One unit of urease activity liberates 1.0 mol of ammonia per min from 0.10 M urea under standard assay conditions.

The urease from both germinated and the control beans were characterized for the following parameter under standard condition of assay;

- pH.
- Temperature.
- Substrate concentration and enzyme kinetics (*Km* and *Vmax*).

pH Stability

In order to determine the effect of pH on activity of urease from germinated and control beans, pH values of 0.05M Tris-acetate buffer ranged between 5.5 and 8.0 and incubated at constant temperature 37^0C and 0.2M urea.

Thermal Stability

To determine the optimal temperature up to which the urease enzyme from both germinated and the control beans can withstand thermal stress, free urease enzyme was suspended in Tris-acetate buffer (0.05 M) and optimum pH above, and incubated at different temperatures (30 to 80 °C) for 30 min before the activity was measured.

Determination of the Kinetic Parameters

The kinetic parameters Km and Vmax were calculated by Lineweaver-Burk equation chosen according to the properties of substrate concentration, the linear transformation of Michaelis- Menten equation (Segel, 1975):

$$\frac{1}{V} = \frac{Km}{V_{max}[S]} \cdot \frac{1}{} + \frac{1}{V_{max}}$$

Where V is the enzyme reaction velocity, [S] is the concentration of substrate (mmol L-1), Km is the Michaelis constant (mmol L-1). V*max* is the maximum enzyme reaction velocity (µmol/s/mg protein) K*m* indicates the affinity of urease to its specific substrate urea, and gives the substrate concentration at which the reaction rate reaches half of its maximum value (V*max*/2). The rate of the reaction was measured as umoles of ammonia produced / s / mg enzyme.

Result and Discussion

Experimental results about urease activity from both germinated and non-germinated were reported. The results showed that germination had significant influence on urease kinetic parameters when compared ($P<0.05$) to the control, under standard condition of assay (Table 2).

The table 1.0 below shows the result of optimum pH, temperature and substrate concentration for maximum hydrolysis of urea by urease isolated from germinated and control (non-germinated) of the various beans species. Results showed that, urease possessed optimum pH and temperature between 6.5 and 7.0 and 60 and 70^0 respectively (Krajewska, 2009; Ya Qing FENG, 2005), where the urease relative enzyme activity was higher in germinated beans than the control (mean value).

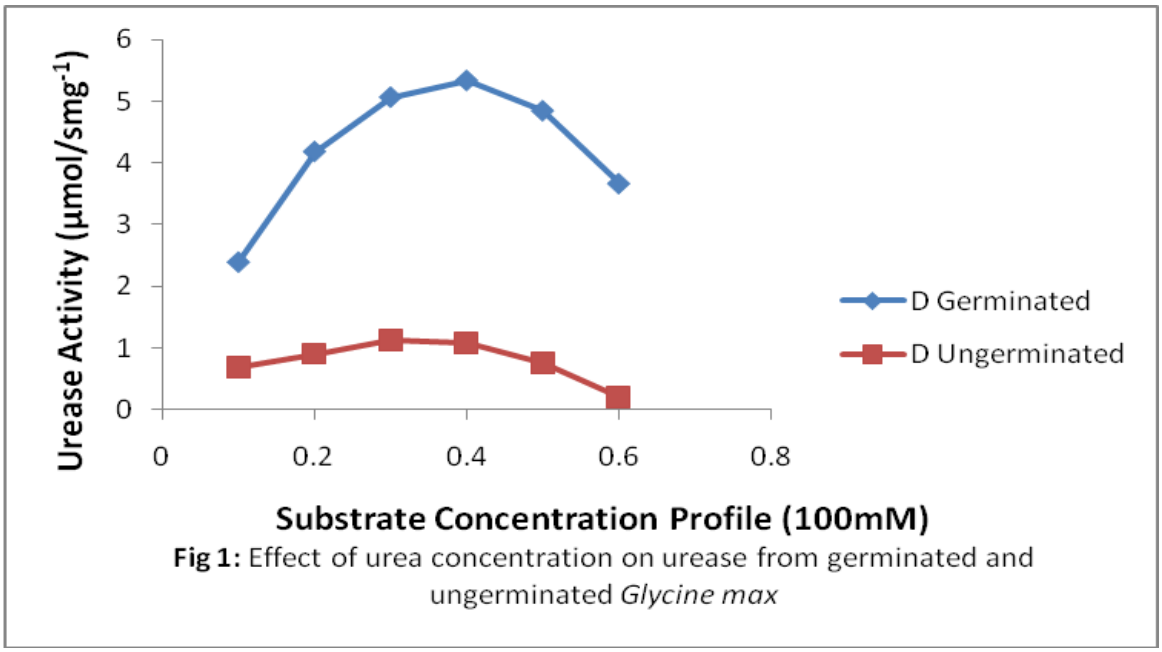

Fig 1: Effect of urea concentration on urease from germinated and ungerminated *Glycine max*

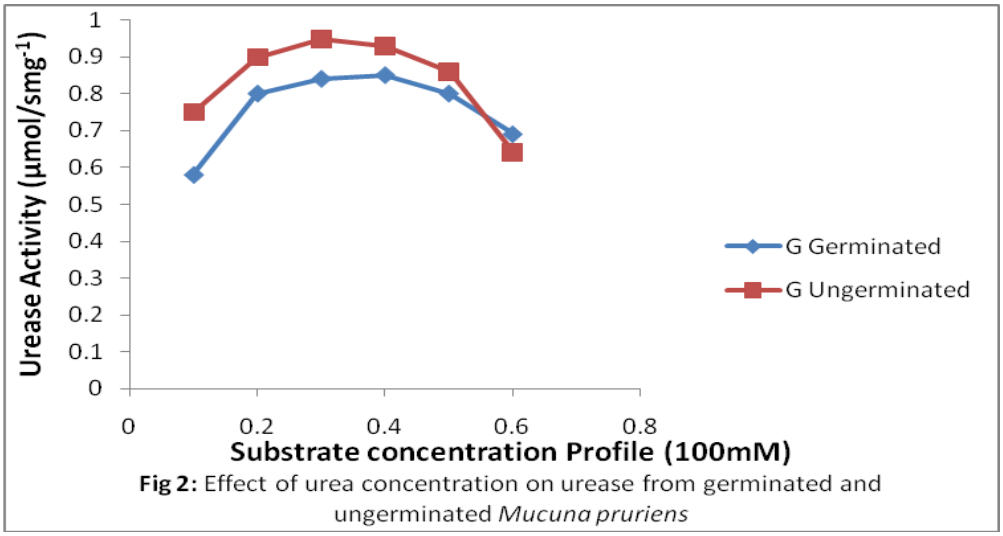

Fig 2: Effect of urea concentration on urease from germinated and ungerminated *Mucuna pruriens*

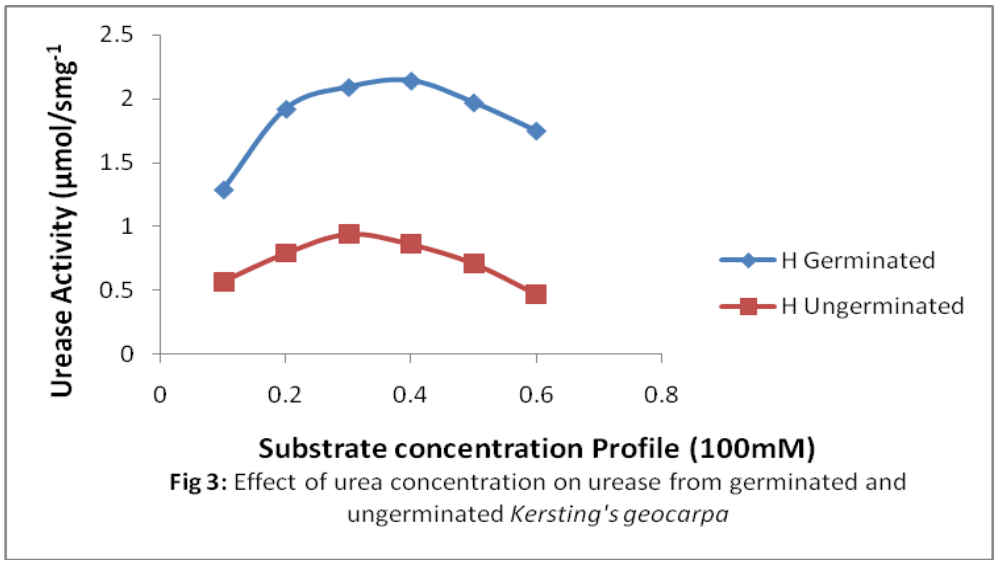

Fig 3: Effect of urea concentration on urease from germinated and ungerminated *Kersting's geocarpa*

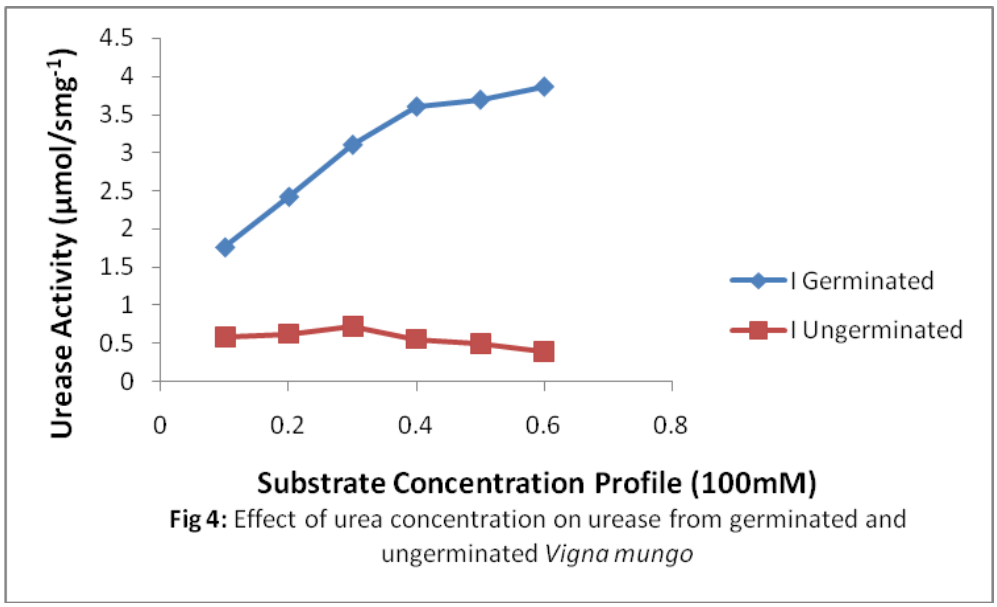

Fig 4: Effect of urea concentration on urease from germinated and ungerminated *Vigna mungo*

Table 1: Result of optimum pH temperature and substrate concentration

Sample	Germinated			Control		
	pH	T^0	[S]	pH	T^0	[S]
Glycine max	6.5	60	0.4	6.5	60	0.4
Mucuna pruriens	7	60	0.4	7	60	0.4
Kerstings geocarpa	6.5	60	0.4	7	70	0.4
Vigna mungo	7	60	0.4	6.5	60	0.4

The effect of urea concentration on the activity of urease from germinated and control of the beans was examined (Figures 1-4). The urease from germinated bean specie exhibited higher activity (V_{max}) than the control (Figures 5-8 and Table 2).

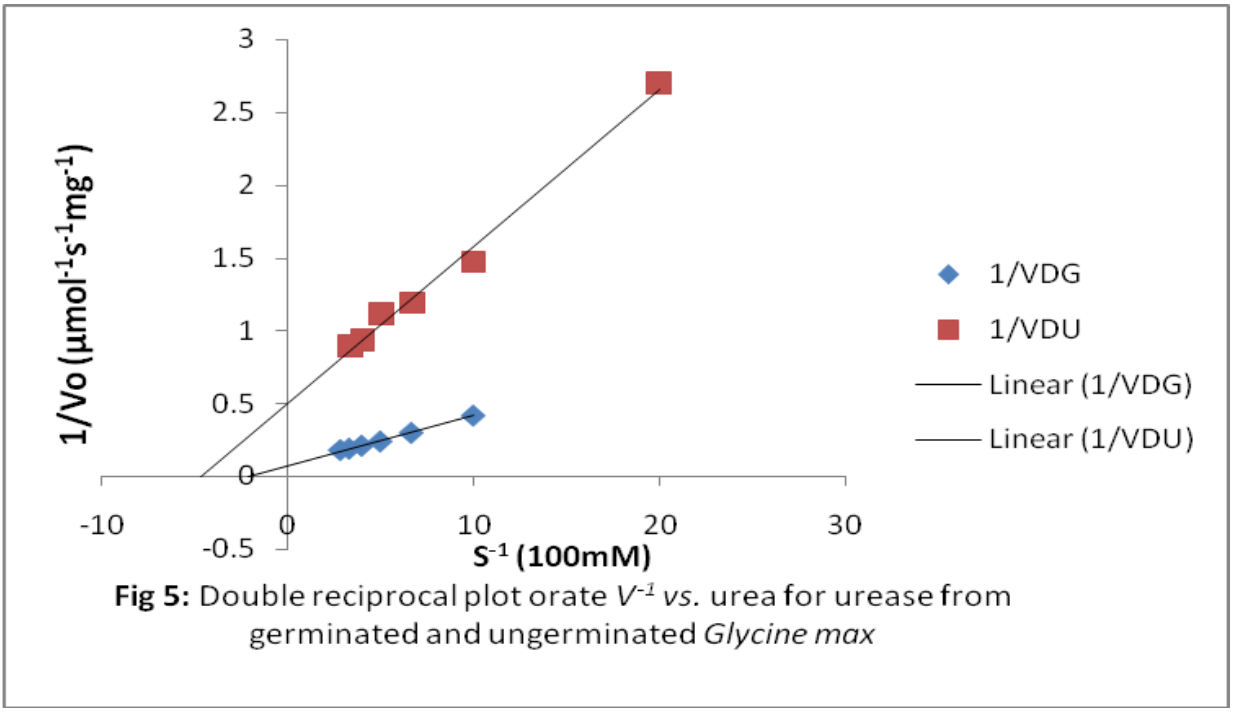

Fig 5: Double reciprocal plot orate V^{-1} *vs.* urea for urease from germinated and ungerminated *Glycine max*

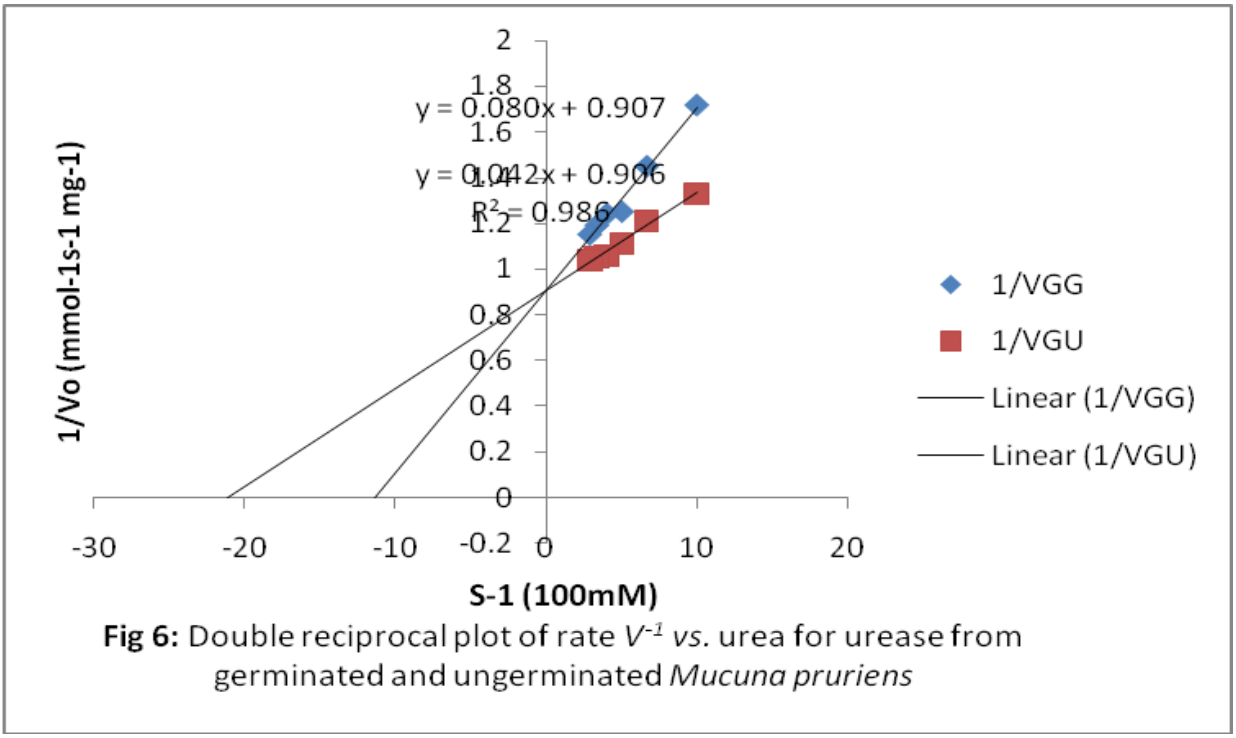

Fig 6: Double reciprocal plot of rate V^{-1} *vs.* urea for urease from germinated and ungerminated *Mucuna pruriens*

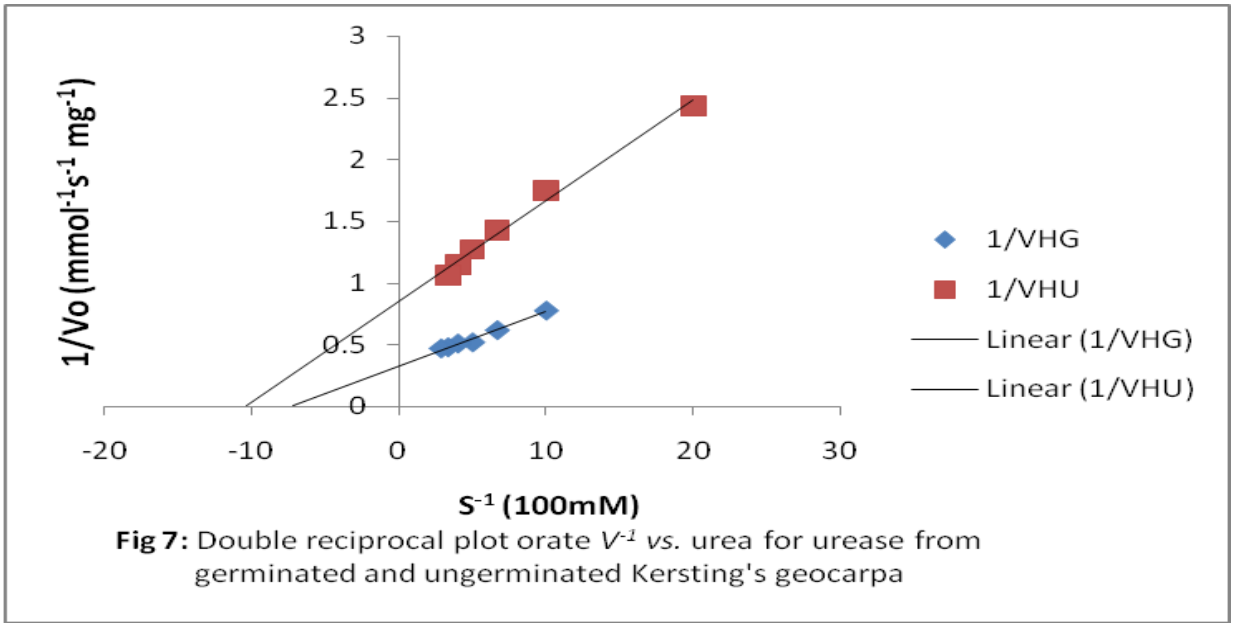

Fig 7: Double reciprocal plot orate V^{-1} *vs.* urea for urease from germinated and ungerminated Kersting's geocarpa

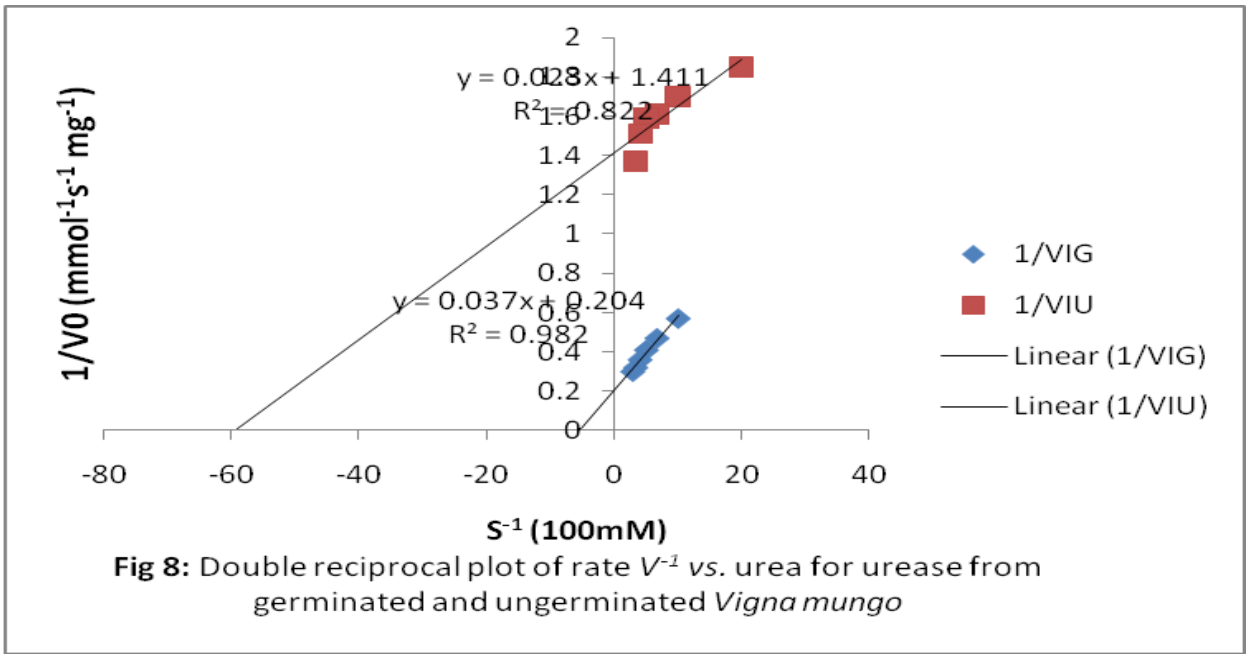

Fig 8: Double reciprocal plot of rate V^{-1} *vs.* urea for urease from germinated and ungerminated *Vigna mungo*

However, urease from control showed lower *Km*, when compared to the germinated. This result showed that the control bean is much more sensitive to substrate than the germinated, but with germinated beans, there is higher urea hydrolysis.

Table 2: Kinetic parameters of urease enzyme from germinated and ungerminated beans

Sample	Km (Germinated) mM	Vmax (Germinated) µmol/smg-1	Km (Control) mM	Vmax (Control) µmol/smg-1
Glycine max	0.45	13.33 x10-4	0.21	2.00 x10-4
Mucuna pruriens	0.09	11.34 x10-4	0.05	11.04 x10-4
Kerstings geocarpa	0.13	3.04 x10-4	0.1	1.18 x10-4
Vigna mungo	0.18	4.90 x10-4	0.02	0.17 x10-4

The behavior of the reaction velocity (Vmax) and the Michelis-Menten constant (Km) from substrate concentration profile ([S]) evaluated. From figures 1-8, it is seen that the reaction velocity was lower in urease from control when compared ($p<0.05$) to germinated beans at the same substrate concentration. With substrate concentration increasing, the reaction velocity increased, and then kept constant with a maximum value when substrate concentration increased to a certain value (figures showing substrate concentration profile), which fulfilled the basic conditions of Michaelis-Menten equation. Therefore the kinetic parameters are in accordance with Michelis-Menten rate reaction. On a general note, kinetic parameter varies with substrate concentration profile and dilution factor of the enzyme. Notwithstanding, Michelis-Menten constants for enzymes usually range between 10-2 and 10-5 mM for most enzymes (Wolfgang, 2007).

Additionally, results (Figures 9 and10) show that, germination increased the Km and Vmax of the enzyme when compared ($p<0.05$) to the control. This feature might be due to the fact that, urease enzyme under germination was metabolically active by making use of seedling's conserved protein to properly fix nickel at the active site of the enzyme to enhance increased hydrolysis of internally generated and external urea (fertilizer) (Polacco & Holland, 1994; Mobley et al., 1995).

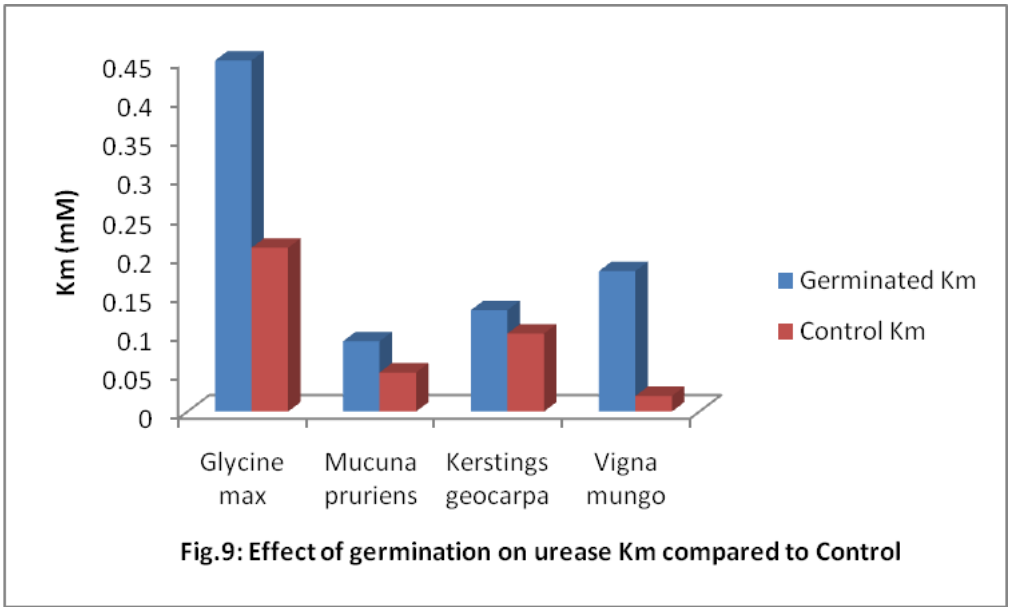

Fig.9: Effect of germination on urease Km compared to Control

Metabolically active urease possessed higher activity (*Vmax*) in order to facilitate hydrolysis of high concentration of urea in plant under germination and also prevent leaf necrosis which usually occurs owing to accumulation of urea in growing plant (Eskew et al., 1983; Krogmeier et al., 1989; Stebbins et al., 1991). This phenomenon reduced the sensitivity (high Km) of the enzyme for urea compared ($p<0.05$) to the control. The urease from control beans (non-germinated) was not performing any metabolic activity, it remain inactive in its natural state before it was isolated. As result it happen to be more sensitive because urea concentration was lower than that of the germinated beans.

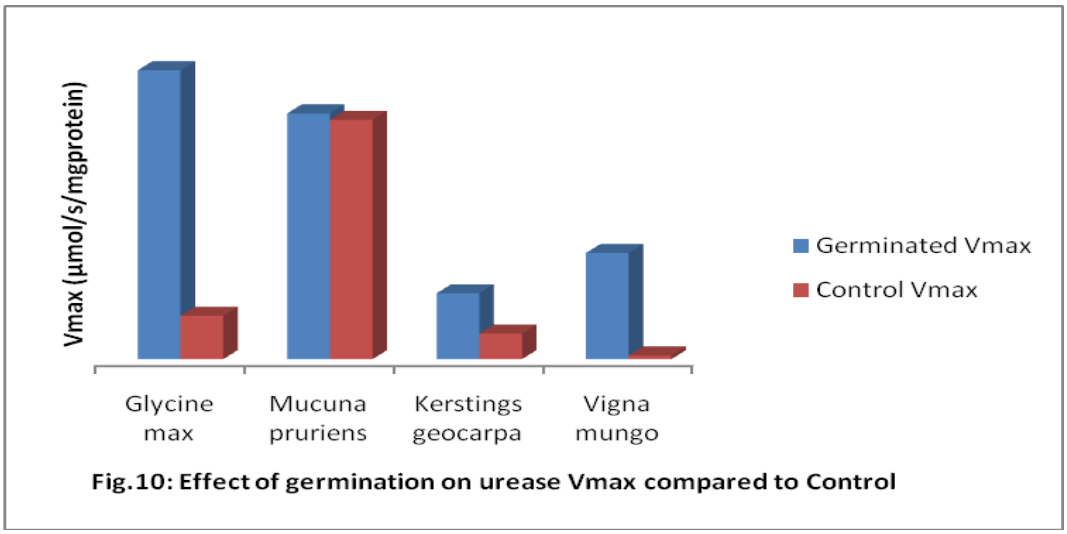

Fig.10: Effect of germination on urease Vmax compared to Control

Conclusion

The result of this work shows that, germination tend to increase the *Vmax* and *Km* of urease, thereby increasing the activity of the enzyme for higher concentration of urea, most especially in growing plant, and also making the enzyme moderately sensitive to substrate.

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