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EFFECT OF TEMPERATURE ON SOIL ENZYME UREASE ACTIVITY-PRODUCTIVITY

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ABSTRACT

Soil enzymes play a major role in the mineralization of nitrogen, phosphorus and sulphur. Mineralization is the process of transformation of organically bound elements into mineral from which will readily take up by plants and is crucial to plant nutrition and indirectly plays a role agriculture productivity. The abiontic enzymes present in the soil play an important role in catalyzing several important reactions necessary for the life processes of microorganisms in soils and their by stabilizing soil structure, the decomposition of organic wastes, organic matter formation and nutrient cycling. When the temperatures are increasing due various changes caused by global warming and other aspects they have a profound influence on soil enzymes and indirectly on agricultural productivity. Every enzyme has its own optimum temperature below the optimum temperature the enzyme activity is less due to inactivation and above the optimum temperature the enzyme activity decreases due to denaturation. Due to increase in temperature the enzymes are denatured and nutrient availability is decreasing and indirectly effecting productivity. To study the effect of temperature on soil enzyme activity four different soil samples were collected and incubation studies were carried out at different temperatures ranging from 20 ^{OC} to 70 ^{OC} with two Alfisols and two vertices. Ureas enzymes have shown to posses highest activity at 70°C, which converts Urea present in the soil to ammoniacal nitrogen readily accepted by plants and which indirectly plays a role in productivity is greatly influenced by climate change IE: - temperature. The enzyme activity at different temperatures is as follows where the activity is measured as µg of NH_4^+ released g^{-1} soil h^{-1}, at 20°C 0.9, at 30°C 2.16, at 40 ^{OC} 5.61, at 50°C 14.63, at 60°C 26.32, at70^{o}C 52.67, at 80°C 23.21 and at 90°C 15.45.

KEYWORDS: Alfisol, Vertisols, Urease, Temperature, Climate Change and Productivity

INTRODUCTION

Agriculture is influenced by climate change, temperature being one of the key components. While farmers are often flexible in dealing with weather and by their experience choose highly adaptive varieties to the local climate and in the soils of arid and semi arid tropics, the soil available nitrogen is grossly inadequate for sustainable agriculture unless it is replenished with the mineralization of organic nitrogen. Nitrogen mineralization is one of the most important part of nitrogen cycle that includes mostly a group of hydrolases like L-asparaginase, urease L- glutaminase etc. These enzymes play key roles in overall process of organic matter decomposition and organic nitrogen in soil system which are important reactions necessary for the live processes of microorganisms in soils and stabilization of soil structure decomposition of organic waste, organic matter formation and nutrient cycling (Dick *et al.*, 1994). During the decomposition of organic matter these enzymes are constantly synthesized, accumulated, inactivated and decomposed in soils, hence they play an important role in Agriculture (Tabatabai 1994, Dick, 1997 and Vandana 2012) soil enzymes have potential to provide unique interactive biological assessments of soils because of their relationship to soil biology ease of measurement and

rapid response to change in soil management (Dora *et al.*, 2008).

Among the different facets of soil enzymes the insitu behaviour of soil enzymes in heterogeneous environment of the soil system in respect of their thermal sensitivities, pH effects, kinetics and moisture effects are of prime importance. Hence the present investigation was designed for studying the effect of temperature on soil enzyme urease activity.

Urease (urea amidohydolase, EC 3.5.1.5) is the enzyme those catalyses the hydrolysis of urea to CO_2 and NH_3:

$NH_2CONH_2 + H_2O = CO_2 + 2NH_3$

It acts on C-N bonds other than peptide bonds in linear amides and belongs to a group of enzymes that includes glutaminase and amidase. Since two C-N bonds are broken in hydrolysis of urea by urease; it is evident that the stoichiometric relation in the equation is the result of component reactions. A number of studies have been conducted to determine the mechanism of urease action and the work by Blakeley *et al*. (1969) has provided convincing evidence that carbamate is the intermediate in a two-step reaction. The reaction is summarized by Reithel (1971) as follows:

$$\mathrm{O} = \mathrm{C}_{\mathrm{NH_2}}^{\mathrm{NH_2}} + \mathrm{HOH} \rightarrow \left[\mathrm{O} = \mathrm{C}_{\mathrm{OH}}^{\mathrm{OH}} + \mathrm{NH_3} \leftrightarrow \mathrm{O} = \mathrm{C}_{\mathrm{NH_2}}^{\mathrm{ONH_4^+}}\right]_{12} + \mathrm{HOH} \rightarrow \mathrm{H_2CO_3} + 2\mathrm{NH_3}$$

Evidences from kinetic data suggest that urease forms a carbamoyl complex as one of the ES complexes, and presumably water is the acceptor in the carbamoyl transfer reaction. Therefore, carbamate is the obligatory substrate for the second step in the overall reaction (Reithel, 1971). Since the proposed mechanism is based on kinetic studies, direct evidence for this mechanism is desired. Urease is very widely distributed in nature. It has been detected in microorganisms, plants and animals. Its presence in soil was first reported by Rotini (1935). Studies by Conrad (1940; 1942; 1943) provided the basic information about this enzyme in the soil system. Urease was the first enzyme protein to be crystallized in 1926 by Sumner (1951).

METHODS

Reagents

Urea (0.2M): This was obtained by dissolving 1.2 g of urea in 80ml of distilled water and volume was made up to 100ml.

THAM Buffer (0.1M): 12.28 g of THAM (Tris hydroxyl methyl amino methane) was dissolved in 800 ml of distilled water and the pH was adjusted by the addition of 0.1N HCl and 0.1N NaoH to obtain the desired pH, then the volume was made up to 1litre.

Potassium chloride (2.M) + Silver Sulphate (100ppm) KCl- Ag_2SO_4 solution: 100 mg of Ag_2SO_4 was dissolved in 700 ml distilled water to which 300 ml of water containing 149 g of KCl was added.

MgO: Magnesium oxide was heated in an electrical furnance at 500°C for an hour and the powder was collected in a dessicator and stored in a tightly stoppered bottle.

4% Boric acid: 40gms of Boric acid was dissolved in a beaker containing hot distilled water about 800ml. Then 5ml bromocresol green and 15 ml of methyl red was added and the volume was made up1 littered with hot distilled water.

0.005 N H_2SO_4: This solution was prepared by taking 5 ml of 1N H_2SO_4 is taken in a 1 liter volumetric flask and

make up to the mark by the addition of distilled water.

PROCEDURE

Soil sample (5 g) was taken in a 25 x 150mm capacity screw capped tubes. 9 ml of 0.1M THAM buffer of desired pH or distilled water and 1 ml of 0.2M urea was added, and then the contents were gently shaken for few seconds and covered with polythene paper. The contents were incubated at 37± 0.5^{OC} for 2 hours in BOD incubator. After incubation, the reaction was terminated by the addition of 50 ml of KCl-$AgSO_4$ solution. The contents were agitated on a mechanical shaker for 30 min to release all NH_4^+ formed and the suspension was allowed to settle and filtered. The NH_4^+ released was determined by the steam distillation method. In the controls the same procedure as described above was followed by the addition of urea solution after termination of the reaction with KCl-$AgSO_4$ solution.

The activity of urease was assayed by Steam distillation method. In this method thirty ml of the supernatant with KCl-$AgSO_4$ extract was taken and transferred to Kjeldahl flask. To this, a pinch of MgO was added which was kept at one end of the distillation unit. During steam distillation for 4 min, the solution containing MgO was heated and the ammonia was released into boric acid containing mixed indicator through a tube dipped in the solution. The ammonia released would change the color of the solution from pink to pale green at the end of the distillation. This was titrated against standardized 0.005N H_2SO_4 and the amount released was calculated and expressed as µg of NH_4^+ released g^{-1} soil h^{-1}.

RESULTS

These results also indicated that urease activity increased from 20 to 70°C and then drastically decreased thereafter with further increase in temperature. Though the inactivation of soil urease was detected in 65 – 70°C it was not completely destroyed when the soils are heated up to 105°C (Zantua and Bremner, 1977). Soil enzymes were reported to show higher resistance to thermal denaturation in a heterogeneous soil system as compared to their behavior in purge system (Tarafdar and Chhonkar, 1978, Pal and Chhonkar, 1979 and Vandana, 2012). The greater thermal stability of urease in soils has been attributed to the complexing of urease by organic colloids or adsorption on clay colloids which offer protection against heat denaturation (Burns, 1978). The considerable variation within the soils in the stabilities of urease enzyme has been observed and it suggests that these differences were principally due to soil pH status and adsorptive properties of the soils (O'Toole and Morgan, 1984).

It is an empirical practice to estimate Q_{10} values for enzyme reaction over short temperature intervals of 10°C is given in (Table 1). This gives the information regarding the temperature at which the process of denaturation occurs. The range of temperature coefficient in different soils is as follows for soil urease enzyme activity is 0.35 to 2.8.

Denaturation occurred beyond 70 ^{o}C. for the present study both Alfisols and Vertisols were taken higher activity was observed in Alfisols, the range observed in different soils is as follows in Vertisol I was 0.90 µg of NH_4^+ released g^{-1} soil h^{-1} at 20 ^{o}C and increased to 2.16 µg of NH_4^+ released g^{-1} soil h^{-1} at 30 ^{o}C and further increased to 5.61 µg of NH_4^+ released g^{-1} soil h^{-1} at 40 ^{o}C and increased to 14.63 µg of NH_4^+ released g^{-1} soil h^{-1} at 50 ^{o}C and increased to 26.32 µg of NH_4^+ released g^{-1} soil h^{-1} at 60 ^{o}C and increased to 52.67 µg of NH_4^+ released g^{-1} soil h^{-1} at 70 ^{o}C and then when the temperature is increased beyond their optimum temperature its activity decreased to 23.21 µg of NH_4^+ released g^{-1} soil h^{-1} at 80 ^{o}C and further decreased to 15.45 µg of NH_4^+ released g^{-1} soil h^{-1} at 90 ^{o}C and in Vertisol II, the range of enzyme activity was as follows 1.10 µg of NH_4^+ released g^{-1} soil h^{-1} at 20 ^{o}C and increased to 2.40 µg of NH_4^+ released g^{-1} soil h^{-1} at 30 ^{o}C and further increased to 6.73 µg of NH_4^+ released g^{-1} soil h^{-1} at 40 ^{o}C and increased to 16.45 µg of NH_4^+ released

g^{-1} soil h^{-1} at 50 ^oC and increased to 30.73 µg of NH_4^+ released g^{-1} soil h^{-1} at 60 ^oC and increased to 58.43 µg of NH_4^+ released g^{-1} soil h^{-1} at 70 ^oC and then when the temperature is increased beyond their optimum temperature its activity decreased to 27.31 µg of NH_4^+ released g^{-1} soil h^{-1} at 80 ^oC and further decreased to 17.24 µg of NH_4^+ released g^{-1} soil h^{-1} at 90 ^oC. In case of Alfisol I it was observed that the enzyme activity increased as follows 1.60 µg of NH_4^+ released g^{-1} soil h^{-1} at 20 ^oC and increased to 3.80 µg of NH_4^+ released g^{-1} soil h^{-1} at 30 ^oC and further increased to 8.45 µg of NH_4^+ released g^{-1} soil h^{-1} at 40 ^oC and increased to 20.73 µg of NH_4^+ released g^{-1} soil h^{-1} at 50 ^oC and increased to 47.45 µg of NH_4^+ released g^{-1} soil h^{-1} at 60 ^oC and increased to 87.31 µg of NH_4^+ released g^{-1} soil h^{-1} at 70 ^oC and then when the temperature is increased beyond their optimum temperature its activity decreased to 32.73 µg of NH_4^+ released g^{-1} soil h^{-1} at 80 ^oC and further decreased to 19.47 µg of NH_4^+ released g^{-1} soil h^{-1} at 90 ^oC and in Alfisol II, the range of enzyme activity was as follows 1.90 µg of NH_4^+ released g^{-1} soil h^{-1} at 20 ^oC and increased to 4.10 µg of NH_4^+ released g^{-1} soil h^{-1} at 30 ^oC and further increased to 9.27 µg of NH_4^+ released g^{-1} soil h^{-1} at 40 ^oC and increased to 25.35 µg of NH_4^+ released g^{-1} soil h^{-1} at 50 ^oC and increased to 57.31 µg of NH_4^+ released g^{-1} soil h^{-1} at 60 ^oC and increased to 99.67 µg of NH_4^+ released g^{-1} soil h^{-1} at 70 ^oC and then when the temperature is increased beyond their optimum temperature its activity decreased to 35.31 µg of NH_4^+ released g^{-1} soil h^{-1} at 80 ^oC and further decreased to 21.45 µg of NH_4^+ released g^{-1} soil h^{-1} at 90 ^oC. beyond 90 ^oC of temperature, negligible increased was observed in case of activity because the thermal stability of the enzyme was completely lost. the temperature coefficient of the enzyme was calculated. The results pertaining to temperature coefficient were given in the Table (1). Temperature coefficient values (Q_{10}) were calculated in the temperature range of 20 to 90^oC. These values depend on the type of soil which varied from 0.44 to 2.61 in case of Vertisol I and 0.47 to 2.8 in case of Vertisol II in Alfisol I Coefficient was observed to range from 0.37 to 2.45 in Alfisol I and 0.35 to 2.73 in case of Alfisol II.

DISCUSSIONS

Temperature has a profound effect and control soil enzyme activities, changing enzyme kinetics and stability, substrate affinity and enzyme production because it can influence the size and activity of microbial biomass. As soil hydrolytic enzymes are the main drivers of soil organic matter (SOM) degradation and litter decomposition, the dependence of these enzymes on global changes, including warming, precipitation, drought and associated soil moisture will assist in understanding the relationships among SOM stock, global carbon cycle and microbial nutrient demand. Moreover, the possible interference of nitrogen demand in soil has also to be considered, being nitrogen a fundamental element not only for several metabolic routes but mainly because involved in protein and therefore enzyme synthesis. Urease activity in all the soils increased from 20 to 70^oC and with further increase in temperature, the activity decreased rapidly. Similar results have been reported by several workers (Rao, 1989, Juan *et al*., 2010, Zantua 1977, Sahrawat, 1984, and Srinivas, 1993). These results also indicated that urease activity increased from 20 to 70^oC and then drastically decreased thereafter with further increase in temperature. Though the inactivation of soil urease was detected in 65 – 70^oC it was not completely destroyed when the soils are heated up to 105^oC (Zantua and Bremner, 1977). Soil enzymes were reported to show higher resistance to thermal denaturation in a heterogeneous soil system as compared to their behavior in purge system (Tarafdar and Chhonkar, 1978, Pal and Chhonkar, 1979 and Vandana, 2012). The greater thermal stability of urease in soils has been attributed to the complexion of urease by organic colloids or adsorption on clay colloids which offer protection against heat denaturation (Burns, 1978). The considerable variation within the soils in the stabilities of urease enzyme has been observed and it suggests that these differences were principally due to soil pH status and

adsorptive properties of the soils (O'Toole and Morgan 1984).

Temperature is an important factor affecting enzyme catalyzed reactions. The rate of enzyme catalyzed reactions, increased with increase in temperature until at some temperature the rate begins to decrease due to denaturation of enzymes. Acid phosphatase and urease activity of soils increased with temperature from 20°C to 70°C and decreased constantly with further increase in temperature to 90°C (Rao, 1989).

The temperature dependence of soil hydrolase activities was described by the Arrhenius equation (Cepeda *et al*., 2007). They measured the Q_{10} of nine different enzymes in three different soils and found that the Q_{10} at 20°C exceeded 2.0 only for B-glucosidase in one of the soils. All other soil enzymes in that study had a Q_{10} closer to 1.5, corresponding to an Ea of 0.3 EV.

A study was carried to evaluate the effect of temperature on soil microbial biomass and enzyme activities (Joa *et al*., 2010). Urease activity decreased gradually with temperature and time. When soil temperature was high, organic matter

Decomposed easily in soil, but some organic matter was resistant against decomposing process because of its constituents. Nutrient release from organic matter and soil microbial activity were affected by soil characteristic, temperature, and organic matter type.

In a laboratory simulation test conducted by Zhang *et al.* (2010) to study the kinetic and thermodynamic characteristics of urease and phosphatase, there is an increase in V_{max} with increase in temperature. K_m also increases with increase in temperature. The energy of activation for urease ranged from 30.10 to 173.89 KJ $K^{-1}mol^{-1}$ and enthalpy ranged from 27.58 to 171.48 KJ $K^{-1}mol^{-1}$. Stone *et al.* (2011) found V max and Km is highly temperature sensitive and value increased significantly with temperature. The Q_{10} values found to be ranged from 1.04 -1.93. In a study conducted by Juan *et al.* (2010) for studying the kinetic characteristics of soil urease found that Q_{10} of urease ranged from 1.32 to 1.42. The lower Q_{10} values for soil hydrolysis indicate a small thermodynamic effect on enzyme velocity. The temperature coefficients of enzyme catalyzed reactions were always < 2 suggesting that enzyme catalyzed reactions are less sensitive to temperature changes than uncatalyzed counterparts.

It has been observed by Davidson *et al.* (1977) that L-asparaginase activity from *Pseudomonas ascidovaries* under goes thermal deactivation when exposed to 50°C for 10 minutes. It is know that the temperature needed to deactivate enzymes in soils is about 10 ^{o}C higher than the temperature needed to inactivate the same enzyme in absence of soil. This has been generally attributed to the immobilization of soil enzymes on soil colloids and cell debris (Tabatabai 1982, Srinivas 1993, Raman and Reddy 1998, Srinivas and Raman 2000 and Vandana 2012). Changes in temperature not only effect the enzyme production but also effect enzyme degradation rates in the environments. Biological responses include changes in enzyme production rates with shifts in microbial population and composition. The variation in these values may be due to heterogeneity in composition and the state of enzymes at temperature above 70°C. When the Q10 values were less than 1 which indicates the deactivation of the enzymes set in at that temperature. Recent increases in climate variability may have affected crop yields in countries across Europe since around the mid-1980s (Porter & Semenov 2005) causing higher inter-annual variability in wheat yields. This study suggested that such changes in annual yield variability would make wheat a high-risk crop in Spain. Even mid-latitude crops could suffer at very high temperatures in the absence of adaptation. In 1972, extremely high summer averaged temperature in the former Soviet Union (USSR) contributed to widespread disruptions in world cereal markets and food security (Battisti & Naylor 2009).

Changes in short-term temperature extremes can be critical, especially if they coincide with key stages of development. Only a few days of extreme temperature (greater than 32°C) at the flowering stage of many crops can drastically reduce yield (Wheeler et al. 2000). Crop responses to changes in growing conditions can be nonlinear, exhibit threshold responses and are subject to combinations of stress factors that affect their growth, development and eventual yield. Crop physiological processes related to growth, such as photosynthesis and respiration show continuity and nonlinear responses to temperature, while rates of crop development often show a linear response to temperature to a certain level. Both growth and developmental processes, however, exhibit temperature optima. In the short-term, high temperatures can affect enzyme reactions and gene expression. In the longer term these will impact on carbon assimilation and thus growth rates and eventual yield. The impact of high temperatures on final yield can depend on the stage of crop development. Wollenweber et al. (2003) found that the plants experience warming periods as independent events and that critical temperatures of 35°C for a short-period around an thesis had severe yield reducing effects. However, high temperatures during the vegetative stage a severe yield to have significant effects on growth and development. Reviews of the literature (Porter & Gawith 1999; Wheeler et al. 2000) suggest that temperature thresholds are well defined and highly conserved between species, especially for processes such as anthesis and grain filling. Although groundnut grows in semi-arid regions which regularly experience temperatures of 40°C, if after flowering the plants are exposed to temperatures exceeding 42°C, even for short periods, yield can be drastically reduced (Vara Prasad et al. 2003). Maize exhibits reduced pollen viability for temperatures above 36°C. Rice grain sterility is brought on by temperatures in the mid-30s and similar temperatures can lead to the reverse of the verbalizing effects of cold temperatures in wheat. Increases in temperature above 29°C for corn, 30°C for soya bean and 32°C for cotton negatively impact on yields in the USA. Release of nutrients in soil by means of organic matter degradation

CONCLUSIONS

The impacts on productivity may depend more on the magnitude and timing of extreme temperatures because these affect the release of nutrients in the soil by means of organic matter degradation by soil enzymes. In case of urease the substrate urea becomes volatile when temperature increases even the substrate availability decreases as we increase the temperature which even decreases the productivity due to less availability of nutrients the fertilizer provided is not efficiently utilized as temperature increases.

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