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STUDIES ON THE KINETICS OF SOIL PROTEOLYTIC ENZYME ACTIVITY OF VINEYARD SOIL AFTER AND BEFORE HARVESTING

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ABSTRACT

Vineyard cultivation profoundly affects the soil quality. Soil quality is determined by the nutrient content and micro-organisms which produce multitudes of enzymes. Soil enzymes are very sensitive to various environmental conditions and soil chemicals including micro and macro nutrients. The growth of the vine is depending on the nutrient mobility and its absorbance by the root. To avail the nutrient various process like releasing of nutrient from the bounded state is essential and is performed by enzyme. The enzyme took for the present study is protease. The study showed that soil protease work well in the temperature around 40°C and at the dilution of toluene around 0.16 and 0.8 respectively for before and after harvest process of cyclic process in the vineyard cultivation. P^H plays an important role in the activities of protease. The P^H optima showed around 4.6 to 5, so acidic medium is better for vineyard cultivation regarding the enzyme protease but the P^H optima for other enzyme also should be considered to reach the generalization of soil P^H.

Key Words: Vineyard, soil P^H, Protease, Temperature optima, P^H optima, cyclic processing of vineyard cultivation.

Introduction

It is believed that nitrogen in the form of inorganic is the only the source of nitrogen for plants (Paungfoo et al, 2008, Vitousek et al, 1997, Galloway et al, 2008, Grubor and Galloway, 2009). Decomposition plays an important role in the separation of minerals in the organic detritus by the activity of microorganisms and macro-organisms like earthworm (Haider et al, 1975, Bond-Lamberty and Thomson, 2010, Berbeco et al, 2012). Nitrogen is available to plants by the decomposition process. The nitrogen content in the vineyard in India is very high, about 32% (Ahlawat and Sindhu, 1990; Conradie and Saayman, 1989; Motsara, 2002; Negi, 1999). This evaluation suggests that hydrolysis of this nitrogen can made available of nitrogen to the vineyard which normally starving for nitrogen (Hirschfelt,et al, 1992; Pool,, 1990). Soil released proteolytic enzyme, which play important role in process of amino acid production, mineralization and uptake of nitrogen by microbes and plants (Kielland , 1994;Keilland et al, 2007; Chapin et al, 1988). But it is observed that the protein depolymerization is mostly rate limiting due to reduced enzyme activity and unavailability of nitrogen by leaching process (Schimel, 2005; Rilling et al, 2007), sample disturbance and immobilization during incubation is also a challenge for the proteolytic enzyme assay(Schimel, and Bennett, 2004). Ichishima (1972) developed a method for proteolytic enzyme but later modified by Watanabe and Hayano in 1995. Many workers used this method and provide data on proteolytic actual activity at different localities in the world (Kielland et al, 2007; Hofmockel et al, 2007; Weintraub and Schimel, 2005). Potential activities are also made by adding toluene to inhibit the uptake of amino acid by microbes. Weintraub and Schimel (2005) used 1% toluene for the assay but immobilization was not absolute, hence Julia et al (2011) performed the same experiment by increasing the concentration of toluene in order to prevent absolute immobilization of the amino acids. But by increasing the concentration may cause adversely to the microorganisms (Julia et al, 2011). So we decided to perform the experiment by using the concentration preferred by Weintraub and Schimel (2005) and precede the kinetic study also to find out the effect of toluene concentration on the kinetics of proteolytic activities and a few proteolytic inhibitors and activators in vine yard soil located in the Sangli district Maharashtra. Our observation agrees with Julia et all concerned with increased concentration of toluene prevent the mobilization of amino acids to an extent.

Materials and Methods

Soil samples

Five organic soil samples were collected from the vineyard located (68380N, 149_430W) in Kavathe Mahankal Tehesil, Sangli District. The soil samples were collected randomly from five acre vineyard where vine plants were uniformly grown. The soil samples were preserved at -20°C and sub samples were picked randomly from the thawed soil and also kept at low temperature. These samples were used for determination of C and N content, pH, organic (nitrate) and inorganic (Ammonia) N, total soluble protein (TSP), total free amino acids (TFAA) and proteolytic enzyme activity. Bulk density was also determined (Hobbie and Gough, 2000)

Soil Physico-chemical properties

Soil moisture content, soil pH, total carbon (TC), total nitrogen (TN) total phosphorous (TP, total sulphur (TS) and available nitrogen (INORGANIC) (AN) was analyzed. Soil moisture content was determined by gravimetric method after drying at 105°C. Soil pH was determined in soil: water suspension (1:2 ratio) with a glass electrode. TC, TS, ANS was analyzed as the method described in manual of soil analysis (Magesin and Schinner, 2011).

Determination of Proteolytic activity

Soil protease (E.C.3.4.21-24, PRase) activity was assayed by the method of Ladd and Butler (1972). The fresh soil sample (1.0g) was reacted with 2% Na-casein was precipitated and Tris buffer for 2h(50°C) at optimal pH and the residual casein was precipitated with 10% trichloroacetic acid and filtrate was reacted with $Na2CO_3$ and folin-Ciocalteau reagent. The tyrosine concentration was measured calorimetrically at 700nm after 1 h incubation at room temperature. All the assays were performed at optimal pH. Control was included in each assay by the same protocol for the enzyme assay. Protease enzyme activity is measured in $\mu g\ g^{-1}\ h^{-1}$.

Kinetic study on Soil protease

Effect of varying concentration of toluene on Kinetics of Soil Protease activity

Six concentration of casein (0.1, 0.4.0.6, 0.8, 1.0, 1.5 mol.L-1) and six concentration of toluene (0.1, 0.4.0.6, 0.8, 1.0, 1.5 mol.L-1) was used. The kinetic parameters *Vmax and Km* were calculated by nonlinear regression of the statistical software SPSS version 15.0.

Three toluene concentration were tested: 1) 2g wet soil, 0.16 ml toluene, brought to final volume of 16 ml toluene, brought to final volume of 16 ml with incubation buffer (1.0% V:V). 2) 1g wet soil, 0.4 ml toluene, brought to final volume of 12 ml with incubation buffer

(3.33% V:V). 3) 2g wet soil, 0.8 ml toluene, brought to final volume of 16 ml with incubation buffer (5.0% V:V). Samples without toluene were 1 g wet soil and 12 ml incubation buffer. A formula {(0 or 3.33% activity [µg amino acid-N/12 ml incubation buffer) x (16 ml incubation buffer/2g]/12 ml incubation buffer) x (16 ml incubation buffer/2 g])}was used to correct the variation in mass/volume vs toluene concentration between the two different sample sets with assumption that the effect is linear.

Result and discussions

The soil constituents of carbon, available nitrogen, total nitrogen, available phosphorous and total phosphorous are showed varying response. Carbon content, total phosphorus and total sulphur was decreased slightly after harvesting, while available nitrogen and total nitrogen was observed slight increase. This increasing and decreasing the soil content of nutrient may related with variation in the soil enzyme during the cyclic process of grape cultivation with change in p^H of the soil, because soil p^H is decreased after harvesting. This indicates that lower p^H may favor the lipophilic or hydrophilic adsorption of soil nitrogen to organic and inorganic particles in soil. So to increase the absorption of soil nitrogen by grape vine we recommend increase the p^H of the soil around six, but care should be taken to concomitant factors that affect the absorption of phosphorous and suulphur, because both nutrients are decreased after harvesting process (Table1).

Table1: Soil chemical properties in vineyard soil (AH-after and BH-before harvesting)

	pH	**Total carbon (g/kg^{-1})**	**Available nitrogen (mg/kg^{-1})**	**Total nitrogen (g/kg^{-1})**	**Total Phosphorus (g/kg^{-1})**	**Total Sulphur (g/kg^{-1})**
AH	4.9 ± 0.03	11.23±0.12	39.2±0.32	9.5±0.24	5.6±0.41	4.12±0.21
BH	5.6 ± 0.01	11.93±0.02	37.4±0.37	8.7±0.34	6.2±0.21	5.02±0.41

To find out the soil enzyme activity, we analyzed the effect various toluene on *V*max/*K*m of the enzyme, because toluene is a better solvent commonly used as diluents in the vineyard

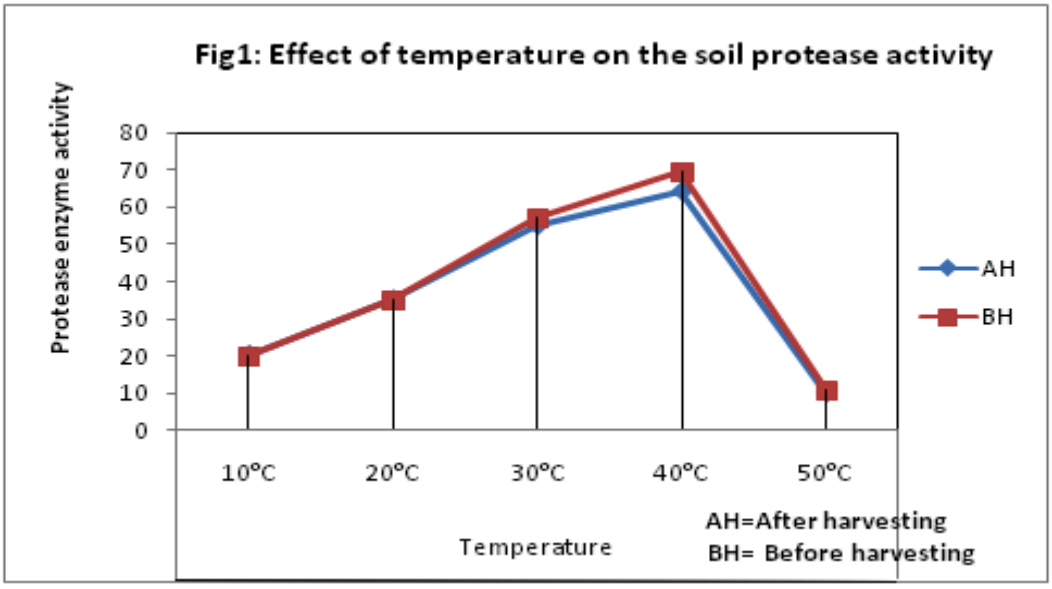
management process. The study showed that toluene concentration 0.8 is better as diluents after harvesting processes and 1.00 process before harvesting (Table.2) The significance of the value is determined by t-test with significance level $\leq$ 0.05.

Table 2. *V*max/*K*m value of soil enzyme, protease, before harvesting (BH) and after harvesting (AH) *K*m: mM; *V* max: μg g -1 h $^{-1}$)

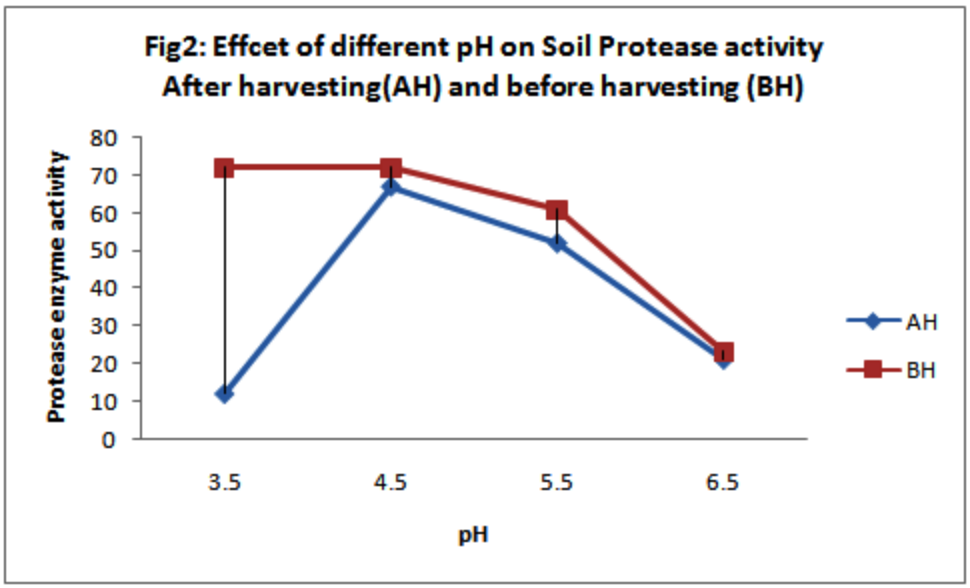
	Toluene concentrations (in ml)				
	0.16	0.4	0.8	1.00	1.2
AH	64.20	67.10	64.1	64.3	64.21
BH	70.12	72.12	70.20	69.32	70.4

P$\leq$ 0.05 determined by t-test

The effect of temperature on protease activity was studied. The kinetic study showed that the soil enzyme protease work well with temperature optima at 40°C. This finding is correlated with the fact that grape cultivation is suited for tropical climate and dried condition. The difference in the response of protease after and before showed no considerable change, may be temperature is the limiting factor (Fif.1)



The effect of p^{H} on the protease activity showed the soil enzyme is suitable at around p^{H} 4.5-5 (Fig.2)



References

Paungfoo-Lonhienne C, Lonhienne TGA, Rentsch D, Robinson N, Christie M, Webb RI, Gamage HK, Carroll BJ, Schenk PM, Schmidt S. 2008. Plants can use protein as a nitrogen source without assistance from other organisms. *Proceedings of the National Academy of Sciences, USA* **105**: 4524–4529.

Vitousek PM, Aber JD, Howarth RW, Linkens GE, Matson P, Schindler DW, Schlesinger WH, Tilman DG. 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications* **7**: 737–750.

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA. 2008. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science* **320**: 889–892.

Gruber N, Galloway JN. 2008. An earth-system perspective of the global nitrogen cycle. *Nature* **451**: 293–296.

Haider, K., Martin, J. P. and Filip, Z. 1975 Humus biochemistry.*In* Soil Biochemistry**4**. Eds. E. A. Paul and A. D. McLaren. Marcel Dekker Inc. New York, pp 195–244.

Berbeco MR, Melillo JM, Orians CM (2012) Soil warming accelerates decomposition of fine woody debris. Plant and soil 344: 10–1007

Bond-Lamberty B, Thomson A (2010) Temperature-associated increased in the global soil respiration record. Nature 464: 579–583.

Ahlawat VP, Sindhu SS (1990), Nutritional survey of grape orchards of Haryana. Haryana J. Hort. Sci. 19(1-2):96-100.

Conradie WJ, Saayman D (1989), Effect of long-term nitrogen, phosphorus and potassium fertilization on Chenin Blanc vines. Am.J. Enol. Vitic. 40(2):85-90.

Motsara MR (2002), Available nitrogen, phosphorus and potassium status of Indian soils as depicted by soil fertility maps. Fert. News. 47:15-21.

Negi JP (1999). Indian Horticulture Database. National Horticulture Board, Gurgaum, 37

Hirschfelt, D. J., L. P. Christensen, W. L. Peacock, and M. L. Bianchi. 1992. The effects of vineyard floor management on vine growth, production and quality. Vol 19 in Report of research for fresh table grapes. Fresno: California Table Grape Commission.

Pool, R. M., R. M. Dunst, and A. N. Lasko. 1990. Comparison of sod, mulch, cultivation, and herbicide floor management practices for grape production in nonirrigated vineyards. Journal of the American Society for Horticultural Science 115(6):872–877

Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85, 591-602..

Kielland, K., McFarland, J.W., Ruess, R.W., Olson, K., 2007. Rapid cycling of organic nitrogen in taiga forest ecosystems. Ecology 10, 360e368.

Hofmockel, K.S., Schlesinger, W.H., Jackson, R.B., 2007. Effects of elevated atmospheric carbon dioxide on amino acid and NH4þ-N cycling in a temperate pine ecosystem. Global Change Biology 13, 1950e1959.

Weintraub, M.N., Schimel, J.P., 2005a. Seasonal protein dynamics in Alaskan arctic tundra soils. Soil Biology & Biochemistry 37, 1469e1475.

Kinetic studies of proteolytic enzyme activity of arctic soils under varying toluene concentrations Julia B. Reiskind*, Martin Lavoie, Michelle C. Mack, Soil Biology & Biochemistry 43 (2011) 70e77