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Research Article

ANALYSIS OF SOIL ENZYMES DURING THE CYCLIC PROCESS OF VINEYARD MANAGEMENT

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Abstract

Keeping soil quality at par is one of the needed things for sustainable development and the existence of millions of living things in biosphere. Soil enzymes are used as soil quality indicators for quick response of changes for environmental stress, pollution and agricultural practices much more sooner (1–2 year) than other soil properties (organic matter); easy to measure (relatively simple procedure), having relations with plant productivity, soil quality parameters (organic matter, soil physical properties, microbial activity, and microbial biomass), and biogeochemical cycle; and being integrative. To assess the detrimental effect of the soil in grape cultivating field we selected four farming sites and various soil enzymes like protease. Urease, cellulose, chitinase, beta glocosidase, phosphatase, amylase, aryl sulphatase and dehydrogenase. As an additional support, we estimated the microbial population in all these fields and in all the stages of the cyclic process of the vine cultivation. Our study showed that the extensive use of the chemical pesticide badly affect the soil microorganism and which in turn badly affect the quality and quantity of the soil enzymes and subsequently the quality of the soil.

Keywords: Soil enzymes; Soil microorganism; Fungicides; insecticides and herbicides

Introduction

Soil is a rich source of many metabolic activities mediated by enzymes. Soil enzymes become a good indicator for monitoring various impacts on soil because of their central role in the soil environment. Soil enzymes acts as an important catalyst of metabolic process including decomposition of organic inputs and detoxification of xenobiotics (Schinner *et al.*., 1996; Dick, 1997). It is also used as an indicator for many soil pollutions like heavy metals, pesticides and hydrocarbons (Schinner *et al.*., 19993; Sparling, 1997; van Beelen and Doelman, 1997; Margesin *et al.*., 2000a, 2000b). Microorganisms are the main source of enzymes in soil (Tabatabai, 1994). It has been proposed that dynamics in the enzyme activities may provide useful hints of changing the quality of soil (Dick, 1992; Visser and Parkinson, 1992). Studies showed that soil enzyme hold potential for assessing the impact of hydrocarbons and of fertilization on soil microorganisms and are useful tool to monitor the early remediation of contaminated soil (Margesin *et al.*., 200a and 200b). The cyclic process in the vineyard management may cause to pollute the soil differentially in different stages. This cyclic variation in the soil contamination might cause variation in the enzymatic activities in the soil. These changes in the enzymatic activities can be assessed by different methods and can correlate to the population of soil microbes and fauna. The information regarding soil enzymes activities can provide guidance of the soil degradation potential (Trasar-Cepeda *et al.*, 2000). The reason to choose the soil enzyme as the monitoring tool for soil contamination of both intracellular and extra cellular is due to the fact that soil enzyme activities is simple, requires low labour costs compared to other biochemical analysis (Ndiaye *et al.*, 2000) and the results are correlated to other soil properties (Klose *et al.*, 1999; Moor *et al.*. 2000; Ndiaye *et al.*, 2000, Trasar-Cepeda *et al.*, 2000). It is also noted that any change in soil management and land use reflected immediately in soil enzyme activity and that leads to change in soil quality and can be detected easily than any other method of soil analysis (Ndiaye *et al.*, 2000). Soil tillage leads to profound changes in the soil enzyme activity also reported many (Kandels *et al.*, 1999, Acosta-Martinez and Tabatabai, 2001) and land use (Staben *et al.*, 1997; Gewin *et al.*, 1999;

Acosta-Martinez *et al.,* 2003b). The literature survey showed extensive works on soil enzymes, but no work was observed till today regarding changes in the soil quality during the cyclic process in vineyard management. Thus we selected this aspect to find out the variation in soil quality during the cyclic process of vineyard management in Sangli District, in Maharashtra state in India. In this we investigate enzyme activities known to play critical roles in organic matter decomposition and mineralization of C, N, P and S nutrients in soil of vineyard at different stages in the cyclic process of vineyard management. Glycosidase are a group of C cycling enzymes which helps in the breakdown of carbohydrates to sugars. Sugars are the main source of energy for soil microorganisms. β-glycosidase activity was studied due to its involvement in cellulose degradation. ά-glycosidase was studied because it involves in hydrolysis of disaccharides, ά-galactopyranosides in soil. The ß-glucosaminidase activity also studied because its involvement in chitin degradation in vineyard soil. β-glucosaminidase is the enzyme involved in the hydrolysis of N—acetyl –β-D- glucoseamine residue from the terminal non-reducing ands of chitoologisaccharides (Parham and Deng, 20000).This type of hydrolysis enables smooth cycling of C and N in soil which is humid as normally observed in vineyard soil (Steveson, 1994, Ekenler and Tabatabai, 2002), microbial biomass C and N, and with fungi populations. We also studied acid phosphatase activity because it catalyzes the hydrolysis of many organic and inorganic phosphomonoesters and hence important in soil P mineralization and plant nutrition. The study was also done on arysulfatase activity to study organic S mineralization in vineyard soil.

So far the studies on soil enzymes activities were concentrated on water logged and temperate areas. Vineyard wherever practiced is in region which is semiarid and water scarcity places like western part of Maharashtra such as tehils like Jath, Atpadi, Kavathe Mahankal, Miraj, Tasgaon and Walva. Maharashtra is the leading grape producer in India (82 hectors and total production is 440M) (Source: http://www.mapsof india.com). The study area for the present work is located in the western part of Maharashtra where rain fall is very rare (Average rain fall is 400-450mm).

Materials and Methods

Experimental site and design

One year long term experiment was initiated in 2011 December at six tehslis of Sangli district- Jath, Kavathe Mahankal, Atpadi, Wlava, Miraj and Tasgaon. Sangli district is located in the western part of Maharashtra- north latitude 16.4 to 17.7º and east longitude 73.43 to 75.00°. Minimum average temperature is about 14.0ºC and maximum was 43°C. The average rainfall was 400-450mm. It belongs to semi-arid region with hot climate and poor rainfall, this make the district better suited for grape cultivation (Fig. 1).

Fig 1: Location map of study

The commonly used verities of grapes in Sangli district are Thompson Seedless and its mutants like Tas – A – Ganesh, Sonaka and Manik Chaman and A 17/3 found promising, however, yet to be released; colored seedless varieties like Fantasy Seedless, Sharad Seedless and Crimson Seedless; seeded varieties like Red Globe (found promising but yet to be recommended).

Cyclic process in grape cultivation

***Bud break*:** This is the first stage in the cyclic process of grape cultivation. Depending on the weather condition new leaves will come out within three to four weeks after bud break. This is the period by which the vines maximize the food production by photosynthesis. In this stage the vine is prone to powdery mildew. Farmers apply antifungal spray during this period. The extra leaves are removed during this period (thinning) to divert maximum energy for flowering.

Flowering: After the bud break the vine begins to flowering within 10 weeks. This is usually in May or early June. This is the period of pollination, pollination will complete within one or two weeks.

Fruit stage: The pollinated flowers begins to produce fruits, non-pollinated flowers will drop off. Heavy watering to the vine plant is needed at this stage. Direct and bright sunlight must be avoided at this stage. Veraison is usually doing at this stage. It is stage of development of color to the seed. It is mainly depending on the variety of the vine plant. It continues until late July.

Harvest: It generally takes place 100 days after the flower formation. After the harvest the plant goes to dormant stage, the leaves are fall off. Pruning is critical in this stage as this protect the plants from extreme frost in this stage.

The various pesticides used by grape growers in Sangli district are shown in Table 1.

Table 1: The various pesticides used by grape growers in Sangli district

Fungicides

1. Aureofungin
2. Azoxystrobin
3. Benomyl
4. Captan
5. Carbendazim
6. Cymbopogan
7. Cymoxanil
8. Copper Oxychloride
9. Copper Sulphate
10. Chlorothalonil
11. Dinocap
12. Fosetyl-al
13. Iprodione
14. Kitazin
15. Lime Sulphur
16. Mancozeb
17. Myclobutanil
18. Penconazole
19. Sulphur
20. Triademefon
21. Zineb
22. Ziram Cymoxanil + Mancozeb
23. Metalaxyl+ Mancozeb
24. Dimethomorph
25. Propineb
26. Flusilazole
27. Hexaconazole
28. Fenamidone +Mancozeb

Insecticides

1. Carbaryl
2. Chlorpyrifos
3. Dicofol
4. Malathion
5. Phosalone
6. Methomyl
7. Buprofezin

Plant growth regulators

1. Gibberllic Acid
2. Hydrogen cyanamide
3. Forchlorfenuron
4. Alpha-napthyl acetic acid
5. Chlormequat chloride

Herbicides

1. Diuron
2. 2,4-D Sodium Salt
3. Paraquat dichloride

These chemicals after its spray will reside as its final abode in the soil of vineyard. These chemicals hence will alter the quality of the soil. Since the enzymes are highly sensitive to the organic or inorganic residues from the various pesticides the enzyme activities will indirectly or directly will give warning signal about the quality of the oil. The various chemicals used by the farmers are different in different stages of vine cultivation. Hence in this we took different enzymes to assess the changes in the soil quality in different stages of the vine cultivation.

Assessment of enzyme activities

Protease enzyme activity was performed by the method described by Rosen (Rosen, 1957) with modification by Ladd and Butler (1972). Total bacterial count was pore plate dilution method (Cappuccino and Sherman, 2006). Total bacterial count was pore plate dilution method (Cappuccino and Sherman, 2006). Chitinase activity was measured by determining the release of p-nitrophenol from p-nitrophenyl-D-N acetylglucosaminide (PNG) on the basis of the method of Roberts and Selitrennikoff (1998) with modification. Dehydrogenase activity was measured by Klein *et al.* (1971) rapid evaluation method. Cellulase activity is determined by its effect on microcrystalline cellulose with respect to glucose formation as described in Worthington Enzyme Manual (1993). Amylase enzyme activity was determined by DNS method described by Mandels *et al.* (1976) using starch as the substrate. The analysis of soil arylsulphatase activity was based on the colorimetric determination at 400 nm of p-nitrophenol (PN) released when 1 g air-dried soil was incubated with 4 mL of 0.5 M acetate buffer, 0.25 mL toluene and1mLof50mM p-nitrophenylsulfate solutionat 37ºC for 1 h (Tabatabai and Bremner, 1970). β-glucosidase activity was determined according to EIVAZIand TABATABAI(1988).

Results

Twenty bacterial species were isolated (Table 2). The mean total bacterial count is 14.22^5 cfu/g of soil. The highest TBC was for *E. color* and the lowest was for *Staphylococcus aureus.*

The urease enzyme activity was analyzed, the activity was found more in the bud stage of the cyclic process of grape cultivation and least found in the harvestation stage (Fig. 1). Flowering stage and fruiting stage showed intermediary between other two extremes. The trend for phosphatase (Fig. 2) showed gradual increase in activity from budding stage to harvesting stage in all the farm land studied. Amylase activity (Fig. 3) observed in the form fluctuating pattern from budding to harvesting period, but the overall trend is decreasing with maximum activity in the budding stage and least in the harvesting period. With respect to aryl sulphatase (Fig. 4) the activity showed consistent pattern in all the stages of the grape cultivation. Beta glucosidase activity (Table 3)) observed maximum in the budding and harvesting stages as compared to other stages, but maximum activity is found in farm.No.2.

Table 2: Bacterial species were isolated during study

Bacteria species	Mean total count x 10^5			
	Framland-1	Framland-2	Framland-3	Framland-4
Escherichia coli	33	30	29	26
Pseudomonas flavescens	22	19	16	18
Pseudomonas pseudoalcaligenes	21	20	19	17
Neisseria elongat glycolytica	19	20	21	18
Neisseria lactamica	17	20	18	16
Neisseria polysaccharea	14	18	16	15
Neisseria canis	11	10	9	7
Yersinia mollareti	9	8	9	7
Staphylococcus aureus	7	8	7	6
Citrobacter rodentium	8	9	7	8
Aeromonas salmonicida	8	7	9	7
Moraxella lacunata	6	5	7	6
Moraxella boevrei	6	4	6	7
Moraxella catarrhalis	5	3	6	5
Providencia stutzer	5	4	5	6
Azotobacter	4	3	4	6
Azospirillum	4	4	5	3
Agrobacterium	4	3	5	4
Bacillus subtilis	4	2	3	5
Flavobacterium	3	3	4	3
Herbaspirillum	3	3	2	2
Thiobacillus	3	2	3	3

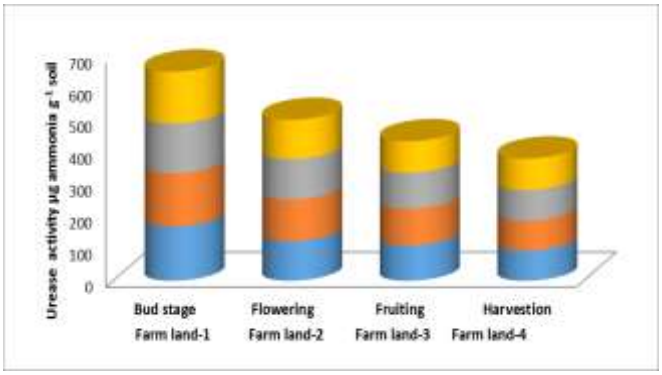

Fig. 1: Activity of urease soil at different stages in the cyclic process of grape cultivation (µg Ammonia g^{-1} soil)

Phosphatase

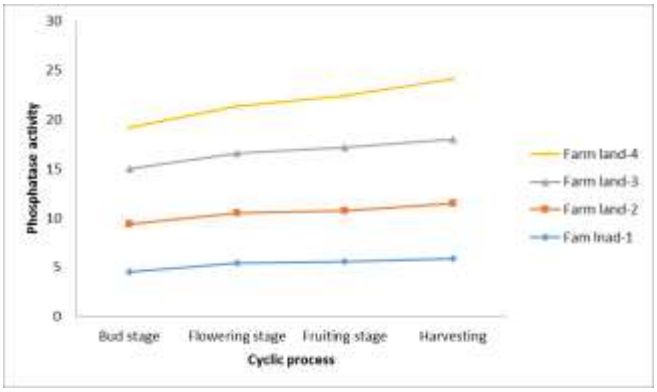

Fig. 2: Activity of phosphatase (µg ammonia g^{-1} soil) at different farmland under cyclic process of vineyard management (One unit of enzyme activity was described as the degradation of 1mM substrate in the standard assay conditions)

Amylase

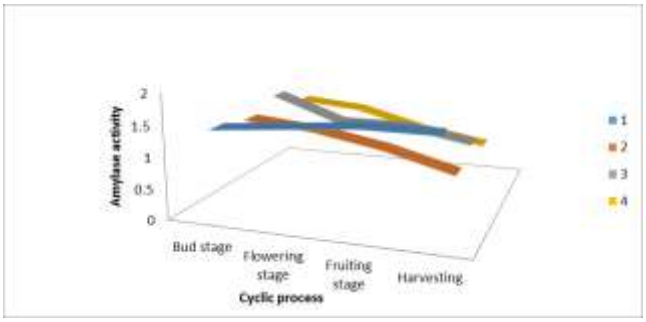

Fig. 3: Activity of amylase U/ml at diferent farmland under cyclic process of vineyard management (One unit of enzyme activity was described as the degradation of 1mM substrate in the standard assay conditions)

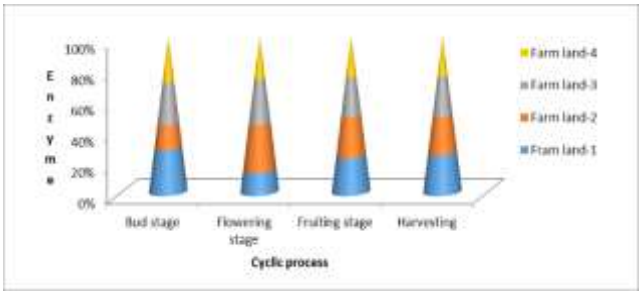

Fig. 4: Aryl sulphatase activity (µM pNP g^{-1}, h^{-1})

Chitinase activity (Table 4) also showed maximum in the budding stages but maximum observed in farm N.2 at flowering stage. Protease activity (Table 5) is found uniformly in all the farmland and in all the stages of the grape cultivation. Cellulase activity (Table 6) is found maximum in budding stage then decreased in the following two stages and increased in the final harvesting stage. Dehydrogenase activity (Table 7) also found maximum in the budding stage and slight increase in harvesting stage after a slight decrease in flowering and fruiting stage.

Table 3: β-glocosidase (mM pNP kg^{-1} h^{-1})

Farm land	Bud stage	Flowering stage	Fruiting stage	Harvesting
1	0.022	0.011	0.020	0.022
2	0.012	0.023	0.022	0.021
3	0.021	0.022	0.021	0.022
4	0.022	0.021	0.022	0.022

Table 4: Chitinase activity (mM pNP kg^{-1} h^{-1})

Farm land	Bud stage	Flowering stage	Fruiting stage	Harvesting
1	6.17	5.21	5.11	5.92
2	6.42	5.23	5.12	5.9
3	6.12	4.92	4.75	6.00
4	5.93	4.99	4.34	5.8

Table 5: Protease activity (µg Tyr g^{-1}soil 2 $^{-1}$)

Farm land	Bud stage	Flowering stage	Fruiting stage	Harvesting
1	362.4	360.1	360	355
2	348.5	348	338	333
3	352	342	345	344
4	362	353	350	349

Table 6: Cellulase activity (µg glucose g^{-1} soil 24 h^{-1})

Farm land	Bud stage	Flowering stage	Fruiting stage	Harvesting
1	34.2	25.1	28.4	33.4
2	33.2	24.3	26.3	33.1
3	34.4	26.4	27.5	33.9
4	36.1	22.9	26.8	35.9

Table 7: Dehydrogenase activity (mg/g of oven dried soil)

Farm land	Bud stage	Flowering stage	Fruiting stage	Harvesting
1	0.18	0.14	0.16	0.17
2	0.19	0.15	0.17	0.18
3	0.17	0.13	0.16	0.16
4	0.18	0.16	0.16	0.17

Discussion and Conclusion

Soil quality can be defined as, "the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation"(Karlen *et al.*, 1997). Keeping soil quality at par is one of the needed things for sustainable development and the existence of millions of living things in biosphere. Soil enzymes are used as soil quality indicators for quick response of changes for environmental stress, pollution and agricultural practices much more sooner (1–2 year) than other soil properties (organic matter); easy to measure (relatively simple procedure), having relations with plant productivity, soil quality parameters (organic matter, soil physical properties, microbial activity, and microbial biomass), and biogeochemical cycle; and being integrative. Grape cultivation is one of the leading agricultural practices in the region of western Maharashtra. To increase the productivity farmers use wide array of chemical insecticides, fungicides, and other most harmful chemicals (Table1). Most of these chemicals are remain as residual particles in the field. The budding stage is one of the crucial stage where widespread chemicals are used by farmers to protect the young floral buds. The application of these chemicals followed by water sprinkling enhances the quantity of the pesticides in the soil many times more than other stages of the grape cultivation. Any change in soil chemistry should affect the microbial

population adversely and soil microbial communities, maintaining critical functions may ultimately be more important than maintaining taxonomic diversity. One essential microbial function in soils is the processing and recovery of key nutrients from detrital inputs and accumulated soil organic matter. This often re- quire the activity of extracellular enzymes to process complex organic compounds into assimilble subunits (sugars, amino acids, NH_4^+, PO_4^{-3}). In this study, we observed that the most badly affected season in the cyclic process of vine cultivation is the fruiting stage and harvesting stage. This finding is correlated with the widest application of various chemicals in the field during these seasons. The residual chemical particle inhibits the microorganism that in turn badly affects the enzyme activity and the soil become un-bearable. Soil enzyme activities have been related to soil microbial community structure (Waldrop *et al.*, 2000; Kourtev *et al.*, 2002). The subsequent year's cultivation needed more application chemical fertilizers and the soil became badly affected further. So we strongly recommend that application of chemical pesticide should be minimum and the use of organic farming concept should be encouraged to maintain the soil quality of the soil and future use of the soil for good productivity.

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