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The Capability of Soil Enzymes *(Bio Stimulants)* in Sustainable Crop Production

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ABSTRACT

Soil quality is a key factor for the growth of crop plants and the deciding factor for the availability of plant nutrients. Sustainable crop production is the current focus of agricultural research across the globe. The use of chemical fertilizers since the green revolution has enhanced food grain production to fulfill the requirement of a huge population.
The soil enzymes (*biostimulants*) are the mediators of organic matter decomposition and soil nutrient transformations. The total enzyme activity of the soil has been found to stabilize soil organic matter through humification, enhance decomposition, increase the availability of plant nutrients, and regularize nutrient cycling. Their patterns of activity concerning environmental factors and management practices help to design sustainable management practices. Thus, knowledge of soil enzymes is essential to design and evaluate new sustainable crop management practices.
The objective of this study was to compare the Physico-chemical characteristics and it was found that the soil physicochemical parameters had a greater impact on enzyme activity. It was suggested that the use of biostimulant formulations is a promising technique in crop production.

Key Words: Soil enzyme, Nutrient cycling, Sustainable crop, green revolution.

INTRODUCTION

Soil enzymes increase the reaction rate at which plant residues decompose and release plant-available nutrients. Sources of soil enzymes include living and dead microbes, plant roots and residues, and soil animals. Enzymes stabilized in the soil matrix accumulate or form complexes with organic matter (humus), clay, and humus-clay complexes, but are no longer associated with viable cells. It is thought that 40 to 60% of enzyme activity can come from stabilized enzymes, so activity does not necessarily correlate highly with microbial biomass or respiration (Kompała - Bąba, A., 2021). Therefore, enzyme activity is the cumulative effect of long-term microbial activity and activity of the viable population at sampling (Dick, R. P. 1994).

Soil enzymes have varying optimum pH and temperature values at which they function most effectively. For example, the activity of phosphatase, arylsulfatase, and amidase involved in phosphorus, sulfur, and nitrogen cycling, respectively, are strongly correlated to variations in soil pH (Sherene, T.,2017). Since enzyme structure and substrate binding can be altered by heat and extreme cold temperature, enzyme activity decreases above and below the optimum temperature. The activity of many enzymes often correlates with soil moisture content, as well. Drought may suppress enzyme activity. It can be said that soil enzymes can be a corresponding ecological indicator for the requirements already mentioned. Soil enzymes can reveal ecosystem perturbations, as they are sensitive to management practices, and have been used as indicators of biogeochemical cycles, organic matter (OM) degradation, and soil remediation. Thus, they can represent soil quality, especially in combination with other physical or chemical properties. Many studies have been published on the use of soil enzyme activities as ecological indicators of soils affected by contamination, such as toxic trace elements (TTEs), stress conditions, and management practices (Lee, S. H.2020). Soil enzymes can be used as biological indicators for diagnosing soil quality because of their stability and sensitivity; they can well indicate whether the biochemical reactions in the soil to which soil enzymes are involved are correctly performed.

MATERIAL AND METHODS

Collection soil samples

Samples were collected from the fields of the Neral, Badlapur, and Ambarnath regions. Random soil sampling was done and samples were collected in polythene bags. Soil samples were harvested at low temperatures till further use.

Physicochemical analysis of soil samples:

Physicochemical characterization of soil samples was carried out which includes physicochemical parameters such as pH, organic content, salini4ty, etc. (Walkely and Black, 1934).

Enrichment of soil samples:

Collected soil samples were inoculated in St. Luria Bertani medium and flasks were enriched at RT on a shaker for 48-72 hrs.

Isolation of soil bacteria

Enriched soil samples were streak inoculated on Sterile Luria Bertani agar and incubated at RT for 24-48 hrs. Well, isolated colonies were checked for their colony characteristics. Further isolates were purified and preserved on a slant for future use.

Screening of enzyme producers:

Extracellular enzyme activities were checked by the spot inoculation method on different media.

Catalase activity:

Catalase test was performed by taking 3-4drops of hydrogen peroxide (H2O2) was added to 48 h old bacterial colony which is grown on Luria Bertani medium. The effervescence indicated catalase activity.

Caseinase (protease) activity:

The qualitative assay for protease production was performed on sterile skim milk agar plates. Isolates were spot inoculated and followed by incubation at a t30C and zone of clearance around the colony indicating the enzymatic degradation of protease (Chaiharn 2008).

Amylase (starch hydrolysis) activity:

The Bacterial Isolates Were Spot Inoculated On Starch Agar medium plates and incubated at 30°C for 48h. At the end of the incubation period, the plates were flooded with iodine solution, kept for a minute, and then poured off. Iodine reacts with starch to form a blue color compound. This blue color fades rapidly. Hence the colorless zone surrounding colonies indicates the colorless production of amylase.

Cellulase activity:

Cellulase production was determined by using the method (Miller G.L 1959). M9 agar medium with yeast extract plates was inoculated with individual bacterial isolates and incubated for 3-5 d at 28C. Bacterial growth surrounded by clear halos was considered a positive indication of cellulase production.

Urease activity:

Urea degrading enzymes were screened and were expressed as µg NH4 + released g-1 dry soil h-1 at 37 ºC, were assayed spectrophotometrically by the indophenol blue method described by Guan (1986).

Determination of plant growth-promoting activity of selected isolates

PGP traits characterization will include the study of phosphate solubilization test described by Gaur (1990), Production of ammonia by Nessler reagent (Cappuccino and Sherman, 1992), Production of HCN by Lorck method(1948), IAA

production Salkowsky's method (Brick et al.1991) and Siderophore production ability by CAS method (Schwyn and Neilands, 1987).

Sustainability of enzyme producers at various physical conditions

The sustainability of enzyme producers was examined at different environmental conditions like temperature, pH, and different salinity.

Application of biostimulant producers as a plant growth promoter

Plant growth promotion activity was observed by pot assay. In the pot, an assay-prepared bioinoculant was added to the soil. Application of inoculants will be done along with the control set. Plant characterization will be done after the specified growth of the plant.

RESULT AND DISCUSSION

Sampling and characterization of soil

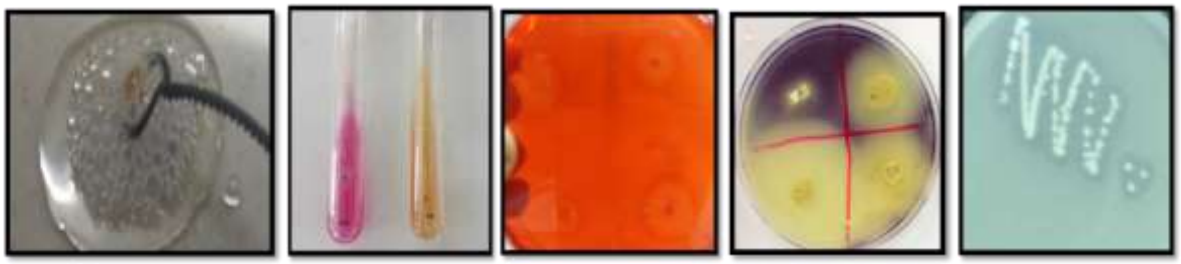
Six Soil samples were collected and immediately checked for their physicochemical characterization. Results of physicochemical characterization are mentioned in table no.1. All samples show pH range from slightly acidic to slightly alkaline, Organic matter ranging from 10-31% and salinity is ranging from 0.2 to 5%.sample no. 5 shows higher salinity than others.

Table 1: Physiochemical characterization of collected soil samples

Soil Samples	pH	Organic matter (%)	salinity
S-1	6.1	23	3
S-2	5.3	31	4.1
S-3	5.3	19	2.5
S-4	6.4	20	2.2
S-5	7.2	28	3.4
S-6	7.4	10	4.1

Screening of enzyme producers

Six soil samples were collected and 56 soil isolates were screened out on their colony and morphological characteristics. All 56 isolates were screened for soil enzyme production ability. Results of screening are shown in Figure no.1.



(a) (b) (c) (d) (e)

Figure 1: Enzyme activity (a) Catalase activity, (b) Urease activity, (c) Cellulase activity (d) Amylase activity (e) Casease activity

Out of 56 isolates 12 isolates shows good enzyme activity which was further screened for quantitative assay. Quantitative enzyme assay of all the isolates shown in Figure No. 2. Isolate No. 3,4,5,6,8,10,11 and 12 shows potential enzyme activity.

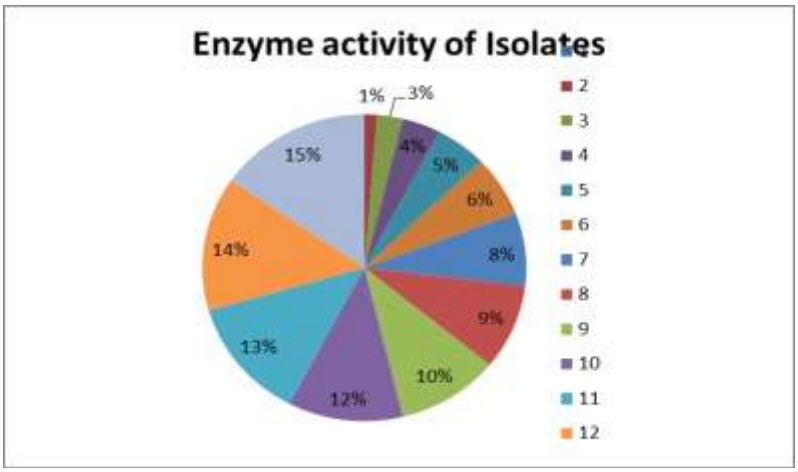


Figure 2: Enzyme activity of selected isolates

8 isolates showed maximum enzyme activity, further screened for plant growth-promoting ability.

Plant growth-promoting activity of isolates

It was observed that 5 isolates show potential plant growth-promoting activity which includes IAA production, ammonia production, Siderophore production, Catalase activity, and HCN production ability. Results of plant growth promotion are shown in Figure 3.

(a) (b) (c) (d) (e)

Figure 3: Plant growth promotion activity: (a) IAA production (b) Ammonia production (c) HCN production (d) Siderophore production (e) Phosphate Solubilization

Sustainability of enzyme producers at various physical conditions

Out of 5 isolates two isolates were good enzyme producers at various environmental conditions such as pH, Temperature and at different salt concentrations.

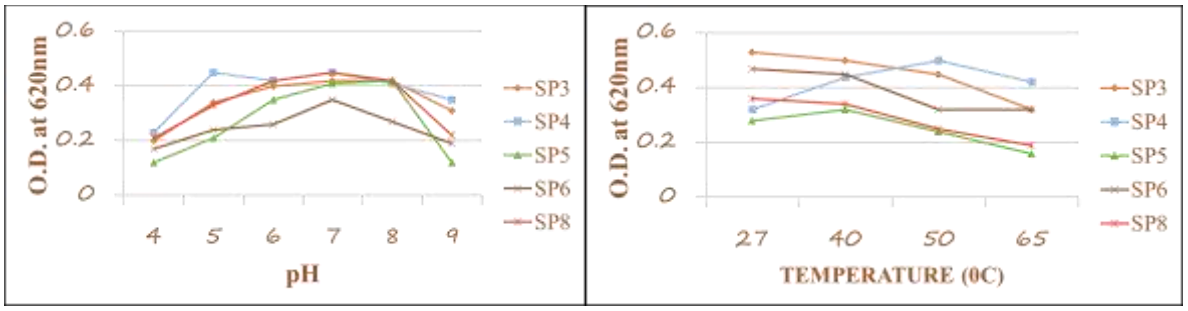


Figure 3: Growth of isolates at different pH **Figure 4: Growth of isolates at different temperature**

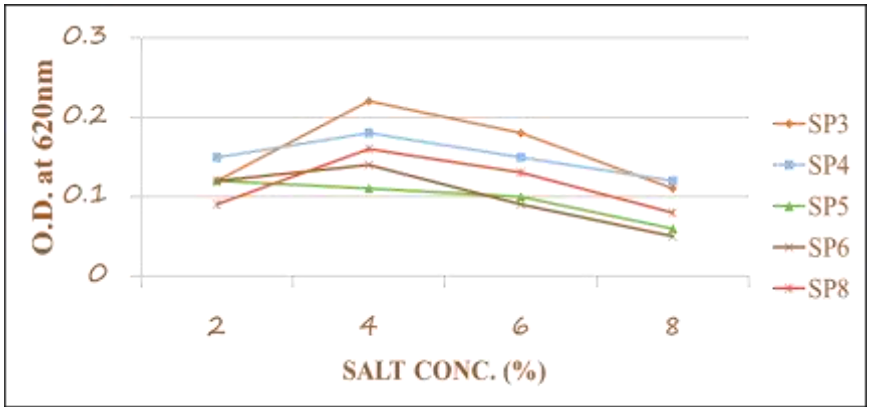


Figure 4: Growth of isolates at a different salt concentration

Application of biostimulant producers as a plant growth promoter

S 3 and S 4 shows potential PGP activity as well as sustainability at a broad range of physical conditions. Hence, both the isolates are characterized morphologically as well as biochemically. It was observed that S - 11 belongs to Bacillus spp. and S - 12 belongs to Pseudomonas spp.

Bacillus sp. and Pseudomonas sp. were used for pot assay to check their influence on plant Vignaradiata. Combination of Bacillus sp. And Pseudomonas sp. Shows better results than individual sp. Results of pot assay are shown in Table no.2.

Table no.2: Effect of biostimulant producers on plant root growth, shoot growth, and biomass by Pot assay

Strain	Root length (cm)	No.of root branches	Shoot length (cm)	No. of shoot branches	Biomass (gm)
Control	5.0	5	12	1	0.158
Bacillus sp	5.3	7	12.5	2	0.345
Pseudomonas sp.	5.2	9	14.7	4	0.564
Bacillus and Pseudomonas	6.1	12	15.4	4	0.840

CONCLUSION

This study showed that the application of the biostimulant improved the plant morphology and growth. Our results also showed differences in the response of the individual and the combined effect of biostimulant producers on plant growth. Biostimulants offer an opportunity to circumvent the excessive use of chemical fertilizers, which lead to a deterioration of

the structure and biological composition of soils and thus contribute to environmental protection. In summary, we conclude that biostimulant treatments can help to preserve soil health and ecosystems, and enhance plant growth, which contributes to improved plant development and productivity. The smart strategies of soil amendments with biostimulants can support sustainable agriculture and contribute to environmental protection. In future work, we would like to study the effect of biostimulants on the crop under stressed conditions.

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