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Screening different microbial flora and their enzymatic activities during tea waste composting

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Abstract— Proper management of solid waste is a major problem in most of the metropolitan areas. Composting is one of the oldest and simplest methods of organic waste stabilization. Composting tea waste is not only an environmental safe method of its disposal but also enhances the growth of the plants as it contains nutrients and tannic acid which create a more fertile environment. In the present study composting of tea waste with variations in type of soil, type of cattle dung and type of tea leaf waste was studied. From this physical, chemical, biological activities and soil enzymes such as cellulase, pectinase, proteases, amylases, xylanase activities were studied in various combinations like black soil, red soil, tea waste, green tea leaves waste along with cow dung, buffalo dung and goat manure in a ratio of 2:2:1 for a time period of 30 days. Analysis of soil with tea waste and cattle dung revealed that compost soil underwent changes in all measured physicochemical, biological and enzymatic parameters like moisture content recorded as 1.6%, highest temperature as 46^0 C and percentage of microbial population like bacteria as 60% and fungi as 40% were observed in the compost soil. Higher enzyme activities such as protease, pectinase, xylanase were observed with bacterial isolates comparatively with fungal isolates. PGPR activities such as IAA, Siderophore production, phosphate solubilization and organic acid production were also observed. Improved soil microbial and enzyme activities in cattle dung soil is an indication of improvement in soil fertility. Therefore the produced compost can be used to increase the crop yield and simultaneously decreasing the environmental pollution.

Keywords— *Tea waste, cattle dung, physico-chemical-biological activities, PGPR activities.*

I. INTRODUCTION

Environmental degradation is the major problem of the world due to use of chemical fertilizers which leads to loss of soil fertility due to imbalanced use of fertilizers that has adverse effect on agricultural soil. Solid waste is useless, unwanted and discarded material resulting from day to day activities in the community, due to which solid waste management has become second major issue in the world. These wastes are generally considered to be non-toxic and non hazardous. In Indian cities ~960 million tones of the wastes are produced annually which consist of industrial, mining, agriculture, household waste and municipal waste. Of this about 360 million tonnes are the organic waste from the agricultural sources and ~290 tonnes of the industrial wastes are produced which are hazardous in nature and ~480 million tonnes of the wastes are produced from the urban cities in the country [1]. In India alone, 100 billion cups of tea is consumed per year and producing around 190,400 tonnes of used tea waste [2]. India is the second largest producer of tea; subsequently, the utilization rate is also high. Tea powder is a great source of biodegradable garbage but it can also make a good source of compost as well. The present research is about preparing compost using waste tea powder that is generally thrown away.

Compost is organic matter that has been decomposed by an process called composting, It is nature's way of recycling solid waste which minimizes environmental pollution as compost is the rich source of nutrients with high organic matter content such as nitrogen, phosphorous, potassium, carbon that can be beneficial to improve organic matter status thereby reduces the need for manufacturers fertilizers [3]. As composting is the one of the low cost biological decomposition processes circuited by microbial activity called as microbial farming as this does not require any energy and manpower. In this process various microbes including bacteria and fungi break down organic matter into simpler substances. Generally, composting starts at mesophilic temperature range and progresses into the thermophilic range [4]. The necessary elements required by the composting microorganisms are oxygen, carbon, nitrogen and moisture content. If any of the above elements are in short supply or if they are not available in the standard proportions, the microbes will not grow and will not provide

adequate heat. Therefore by maintaining standard conditions during composting the organic matter converts to fixed compost that is odorless and pathogen free and a poor breeding substrate for flies and other insects.

Soil enzymes are highly involved in the degradation of soil organic matter and nutrient cycling as they catalyze several important reactions which are necessary for the life processes of microorganisms in soils and the stabilization of soil structure, the decomposition of organic wastes, organic matter formation and nutrient cycling [5]. The activities of these enzymes in soils undergo complex biochemical processes and play an important role in agriculture and particularly in nutrient cycling [6]. The important applications of composting is it can improve soil properties that are badly in need of renewal as it can increase the organic carbon contents in the soil compost also acts as a soil intervention in improving soil structure, water filtration rate, water holding capacity and cultivating land. During which no environmental pollution occurs and at the same time the compost improves soil fertility.

An attempt was made in this study to observe the influence of cattle dung and tea waste compost on soil physico-chemical, biological and enzyme properties and assesses its effects on crop yield.

II. RELATED WORK

Tea powder can be a great source of biodegradable garbage but it can make good compost which usually discarded as wet garbage. According to *Diver,2002:Ingham,2005: Kannangara et al, 2006* compost tea has been cited as an option for organic growers which enhance crop fertility by introducing microorganisms that might help in soil nutrient retention etc. similar reports were confirmed by the *Pradeep* and *Narsimha et al 2011,Radha et al,2012.* Dr. Elaine Ingham, a professor at Oregon State University reported the use of compost tea to suppress plant disease and to stimulate plant growth. Therefore in the present study we made an attempt to prepare compost tea along with addition of cattle dung which can improve the quality of compost by long term deposition of organic manure which stimulates healthy plant growth.

III. MATERIALS AND METHODS

Collection of samples
Compost was prepared in used urea bags using tea powder waste. It was collected from houses, tea stalls and hotels.

Construction of compost piles
The piles are prepared using different combination of red soil, black soil, different tea waste, green tea waste with cattle dung i.e. cow dung, buffalo dung, goat manure in a ratio of 2:2:1(Table 1).

The material was allowed to decompose for 30 days. The temperature was monitored regularly to check the process of completion of the decomposition. The temperature rise initially and when the compost was ready, it remained constant. The compost thus made was analyzed for its physico-chemical properties.

Analytical methods for characterization of soil
Measurement of temperature
The temperature is measured periodically using mercury thermometer by placing 10cm deep in compost piles.

Measurement of moisture content
5gms measured soil sample is taken from composting piles and kept in hot air oven for 24hrs. After 24hrs the weight of the soil is measured and noted. The difference between the initial volume taken and final volume gives the moisture content of soil.

Initial volume – Final volume = moisture content

Enumeration of bacteria and fungi
Bacterial and fungal population in soil was enumerated using nutrient agar and potato dextrose agar respectively. After preparation of medium, 20 ml of sterile medium was aseptically transferred to sterile Petri plates and allowed for solidification. After solidification of the medium different aliquots of soil suspension was spread uniformly with the help of sterile glass spreader. The bacterial plates were incubated at 37^0 C for 24hrs while fungal at room temperature for 4-5 days. After incubation, colonies were counted and sub cultured on agar slants for further studies.

Enzyme assays
Soil samples were withdrawn after 10 and 20 days of incubation to determine the soil enzyme activities.

Cellulase/ xylanase /pectinase assay
To estimate the amount of enzymes in the bacterial culture, nutrient broth incorporated with 1% of appropriate substrate for different enzymes is taken whereas for fungal cultures czapeck's broth incorporated with 1% of substrate (starch, xylan, pectin) solution is taken of which 3ml broth is taken into vial and inoculated with bacterial cultures in sterile conditions and incubated for 24 to 48hrs (bacterial cultures) and 48 to 72hrs (fungal cultures).

After incubation the samples are centrifuged for 10mins at 8000rpm, and supernatant is collected in two sets each containing 0.5ml and the volume is made up to 1ml with addition of distilled water. To this solution 1ml of dinitro salicylic acid (DNS) is added to one of the set immediately

and to other set the DNS is added after 10mins of incubation at room temperature (delayed).

Now the solutions are incubated in boiling water bath for 10mins and after cooling add 6ml of distilled water and absorbance is taken colorimetrically at 540nm by using blank. The difference between the immediate and delayed is taken and then plotted on standard glucose graph.

Protease assay

To estimate the amount of enzymes in the bacterial culture, nutrient broth incorporated with 1% of appropriate substrate for different enzymes is taken whereas for fungal cultures czapeck's broth incorporated with 1% of starch solution is taken of which 3ml broth is taken into vial and inoculated with bacterial cultures in sterile conditions and incubated for 24 to 48hrs (bacterial cultures) and 48 to 72hrs (fungal cultures).

After incubation the samples are centrifuged for 10mins at 8000rpm, and supernatant is collected in two sets each containing 0.5ml and the volume is made up to 1ml with addition of distilled water. To this solution 1ml of casein solution substrate is added and 1ml of follin's reagent is added. The test tube are mixed and kept in room temperature for 30mins and absorbance is taken colorimetrically at 600nm by using blank. The difference between the immediate and delayed is taken and then plotted on standard tyrosine graph.

PGPR activities of isolates

Qualitative estimation of Indole Acetic Acid (IAA) production

The bacterial and fungal cultures which are isolated are inoculated onto the Luria broth in sterile conditions and incubated for 24 to 48hrs. After incubation Salkowasky's reagent is added and observed for color change.

Qualitative estimation of siderophore production

The bacterial and fungal cultures which are isolated are inoculated into the nutrient broth in sterile conditions and incubated for 5 to 10mins. To this add 0.5M ferric chloride solution and observed for color change.

Quantitative estimation of Phosphate Solubilization

The bacterial and fungal cultures are aseptically transferred onto the plates containing picovasky's medium and incubated for 24 to 48hrs at 37°c and observed for clear zone around the colonies.

IV. RESULTS AND DISCUSSION

Microbial properties of compost

Composting is the process of degradation of complex and heterogeneous organic material by a mixture of microorganisms (Figure 1).Among the microorganisms, bacteria play an important role in organic matter decomposition. Various hydrolytic enzymes are believed to control the rate at which various substrates are degraded.So, the population of cellulolytic, pectinolytic, proteolytic, xylanolytic and amylolytic bacteria and fungi which are responsible for hydrolysis of cellulose, pectin, proteins, xylan and starch, respectively were studied in this experiment to understand the degradation of various organic wastes.

The total number of organic matter decomposing bacteria (24 isolates) and fungi (12 isolates) were represented in the form of pie diagram (Figure 2). The higher bacterial and fungal population may be due to alkaline pH and deposition of organic wastes in the soil. The isolated organisms are sub cultured and stored for further use.

Physico-chemical properties of soil

Soil fertility mediated by microorganism is dependent on maintenance of physico-chemical characteristics in soil. The compost soil from complex of cattle dung and tea waste which are decomposed by several microorganisms present in the soil. This compost made the soil pleasant and imparts black colour to soil within 20-25 days. Analysis of soil samples revealed that compost soil underwent changes in all measured parameters.

Effect of temperature on compost soil

The temperature gradually increased at early period of composting as this enters the thermophilic stage and after 20days the temperature enters into the mesophilic stage where the temperature becomes stable and later it enters into the cooling and maturation phase where temperature decreases (Figure 3). The highest temperature noted is 46^0 C on the 15th day of composting and the lowest temperature noted is 26^o C on 25th day of composting.

Effect of moisture content on compost soil

Compost proceeds best at a moisture content of 40% to 60% by weight. At lower moisture content microbial activity is limited, so in order to overcome this problem appropriate moisture content is maintained. The highest moisture content is seen in RGC (red soil, green tea waste, cow dung) and the lowest is seen in RGG (red soil, green tea waste, goat manure).

Effect of enzyme activities on compost soil

There is a considerable interest in the study of enzyme activities of soils; such activities may reflect the potential capacity of a soil to form certain biological transformations of importance to soil fertility. Soil enzymes are highly involved in the degradation of soil organic matter and nutrient cycling (Figure 5,6,7,8,9,10). With increase in soil incubation period xylanase (1198.98 UM/L/MIN),

proteolytic (827.86UM/L/MIN) and pectinolytic (833.22UM/L/MIN**)** activities were improved in compost soil up to 20 days and declined further this is may be due to high organic matter content in cattle dung and tea waste. From the present study the enzymes like xylanase, protease and pectinase played a crucial role in catalyzing and solubilizing the substrates. At the end of the composting (when temperature goes down), complex carbohydrate turns to comparatively simple form of carbohydrates. Thus, the population of xylanolytic isolates is higher than proteolytic and pectinolytic isolates in mature compost.

Effect of PGPR activities on compost soil

The most active compound called auxin in plants is Indole acetic acid. IAA is known to stimulate both cell elongation and cell division in plants production. In the present study it was found to be bacterial isolate 6 with highest IAA production (Figure 11) where as siderophore production was found to be highest by fungal isolate 3, 7 & 11(Figure 12) as compared to other isolates. The highest phosphate solubilization i.e, clear zone of 5mm in diameter on Pikovaskya medium is with bacterial Isolate 20 (Figure 13).The phosphate- solubilizing activity characterizes the microorganisms with ability to produce and release metabolites such as organic acids that chelate the cations bound to phosphate, converting them into soluble forms. Among 36 isolates almost all have showed IAA production, siderophore production, Phosphate solubilizing activity (Table 2&3).

Effect of plant growth on compost soil

Plant studies were performed in order to check the impact of compost on soil. when compared with control the *fenu greek* plant growth is faster and taller in soil mixed with compost(Figure 15).Future studies are required to prove the nature of these isolates and to harness their potential as bio-inoculants in agriculture.

V. CONCLUSION

Analysis of soil with cattle dung and tea waste altered the physico -chemical, biological and enzymatic parameters like moisture content, high temperature, organic contents and microbial populations including bacteria and fungi were observed in compost. Higher enzyme activities such as xylanase, protease and pectinase also measured in compost. Improved soil microbial and enzyme activities in cattle dung and tea waste compost is an indication of improvement in soil fertility. Therefore compost made from tea waste and cattle dung had an ability to serve as an alternative of chemical fertilizers for *fenu greek* plant growth. Further studies should be undertaken to generalize the effect of compost for the other popular vegetables.

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Table 1: Different combination of red soil, black soil, different tea waste, green tea waste with cattle dung(2:2:1)

Black soil	Tea waste	Cow dung (BTC)
		Buffalo dung (BTB)
		Goat manure (BTG)
	Green tea waste	Cow dung (BGC)
		Buffalo dung (BGB)
		Goat manure (BGG)
Red soil	Tea waste	Cow dung (RTC)
		Buffalo dung (RTB)
		Goat manure (RTG)
	Green tea waste	Cow dung (RGC)
		Buffalo dung (RGB)
		Goat manure (RGG

Figure 1: Composting piles prepared in bags

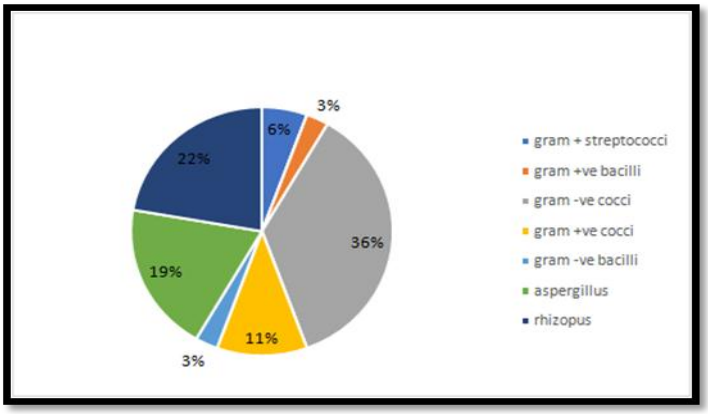

Figure 2: Total number of bacteria and fungi isolated from compost soil

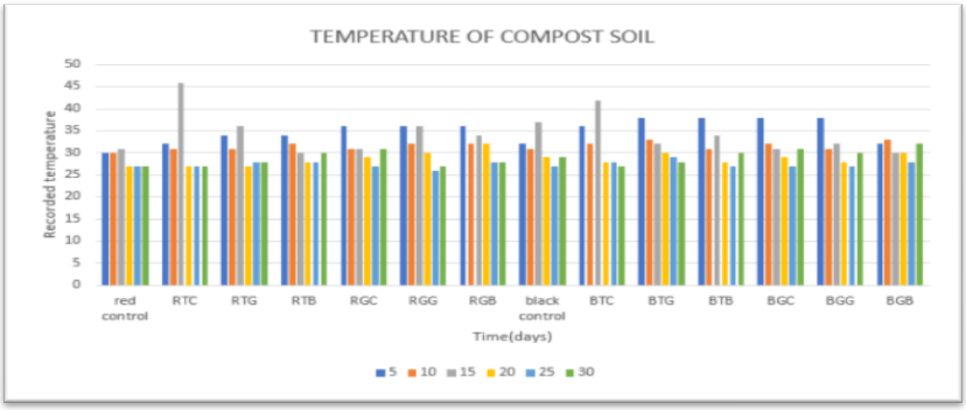

Figure 3: Temperature of different compost soil samples

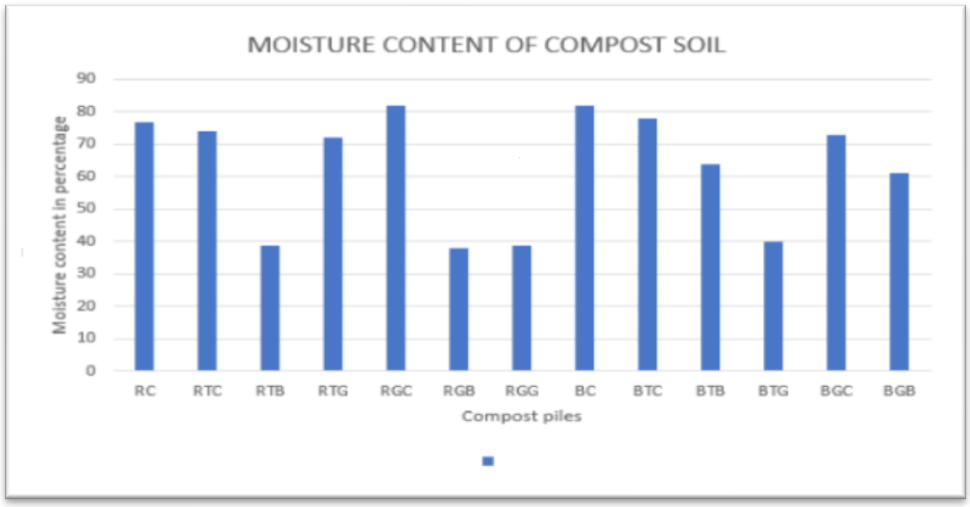

Figure 4: Moisture content of the different compost soils

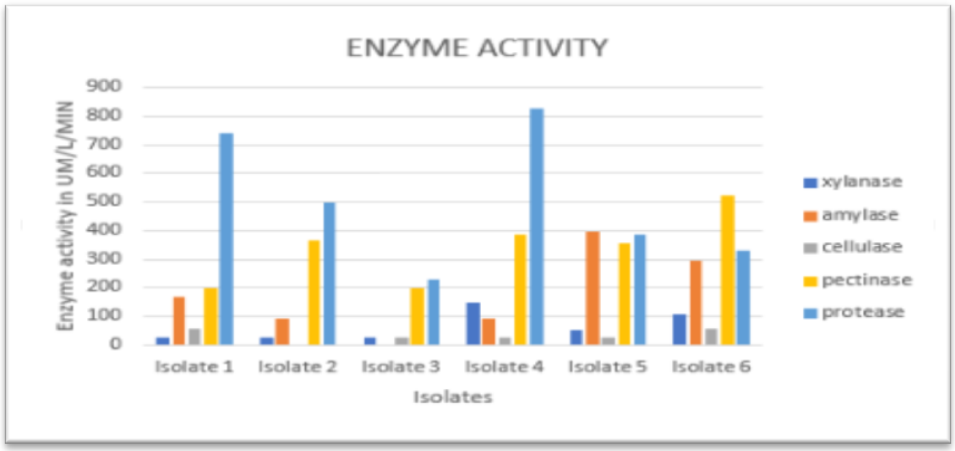

Figure 5: Comparative studies of different enzyme activities where bacterial Isolate 4 has shown the highest protease activity 827.86UM/L/MIN when compared with all other enzymes

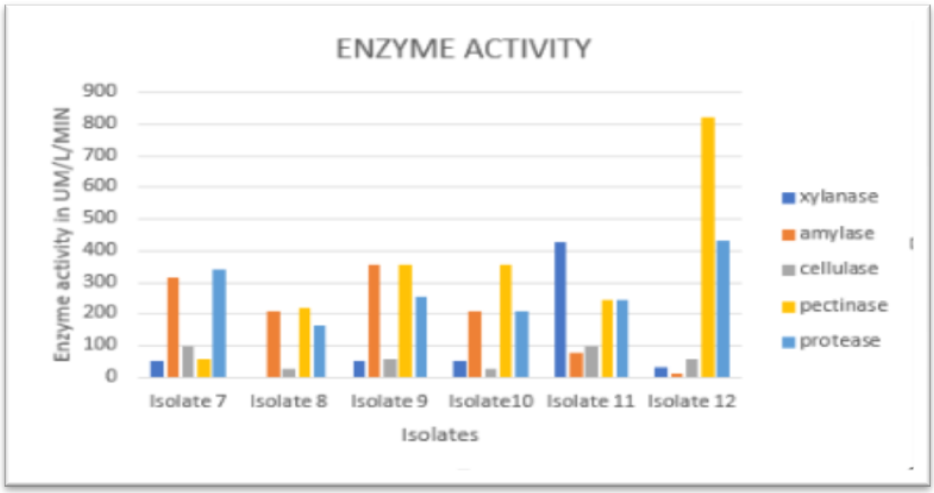

Figure 6: Comparative studies of different enzyme activities where bacterial Isolate 12 has shown the highest pectinase activity 822.22UM/L/MIN when compared to all other isolates.

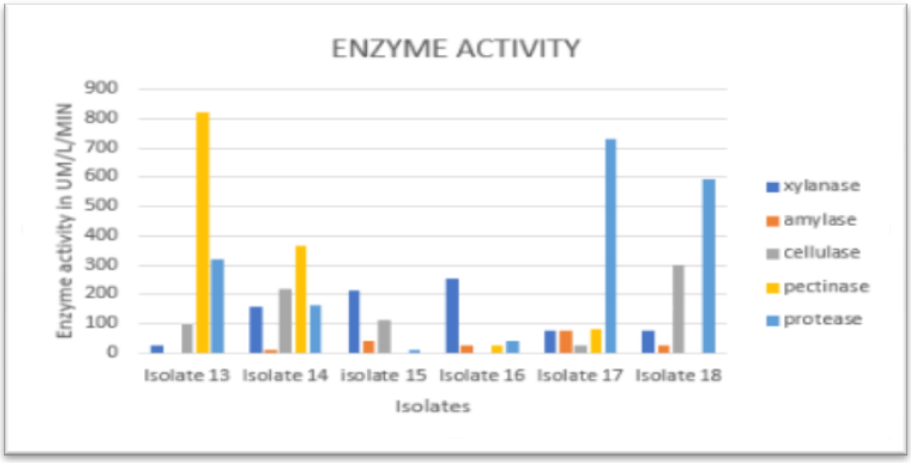

Figure 7:Comparative studies of different enzyme activities where bacterial Isolate 13 has shown the highest pectinase activity 822.22UM/L/MIN when compared with all other enzymes

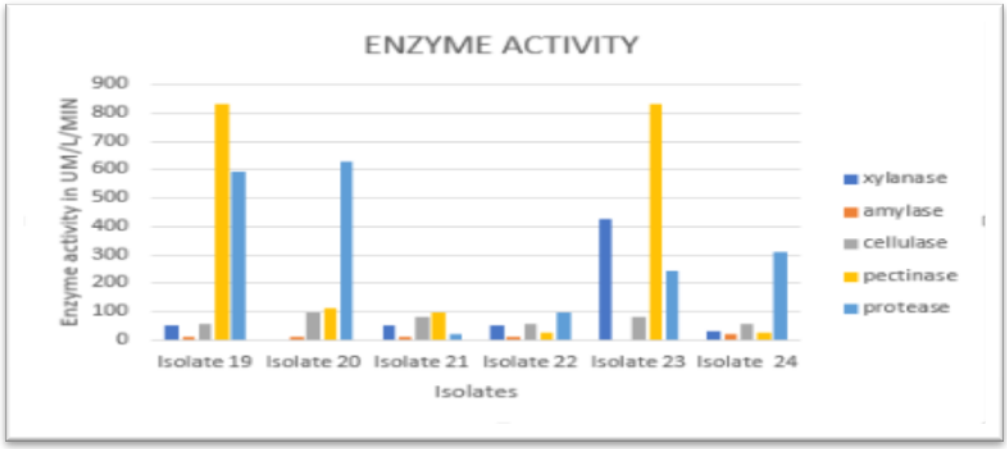

Figure 8: Comparative studies of different enzyme activities where bacterial Isolate 19 has shown the highest pectinase activity 833.22UM/L/MIN when compared with all other enzymes.

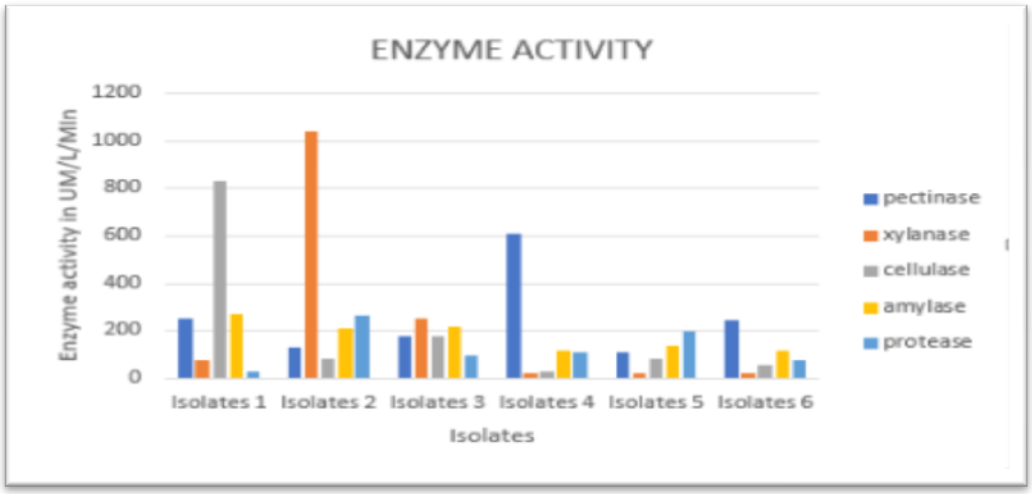

Figure 9:Comparative studies of different enzyme activities where fungal isolate 2 has shown the highest xylanase activity 1039.38UM/L/MIN when compared with all other enzymes.

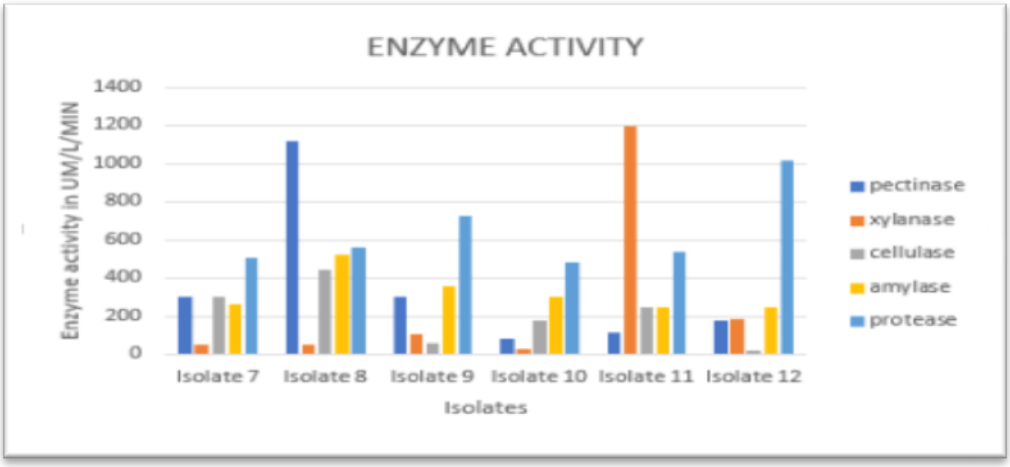

Figure 10: Comparative studies of different enzyme activities where fungal Isolate 11 has shown the highest xylanase activity 1198.98 UM/L/MIN when compared with all other enzymes

Fig. 11: IAA production

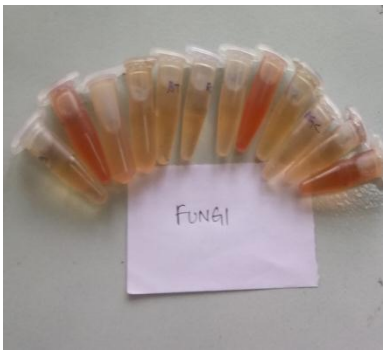

Fig.12: Siderophore production

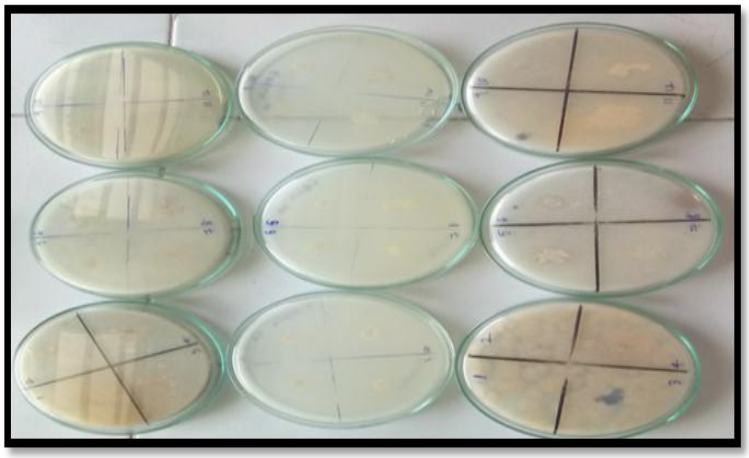

Figure 13: Phosphate solubilization plates showing the highest phosphate solubilization i.e, clear zone of 5mm in diameter is with bacterial Isolate 20

Table 2: PGPR activities of bacterial isolates

BACTERIAL CULTURES	IAA	SIDEROPHORE	PHOSPHATE SOLUBILIZATION
isolate 1	+	+	+
isolate 2	++	+	+
isolate 3	++	+	+
isolate 4	+	+	+
isolate 5	++	+	++(2mm)
isolate 6	++(ppt)	+	++(2mm)
isolate 7	++	+	++(1mm)
isolate 8	++	+	++(2mm)
isolate 9	+	+	+
isolate 10	+	+	+
isolate 11	+(ppt)	+	+
isolate 12	+	+	+
isolate 13	++	+	+
isolate 14	+	+	+
isolate 15	+	+	+
isolate 16	+	+	+
isolate 17	+	+	+
isolate 18	+	+	+
isolate 19	+	+	+
isolate 20	+	+	+++(5mm)
isolate 21	++(ppt)	+	+
isolate 22	+	+	+
isolate 23	+	+	+
isolate 24	+	+	+

Table 3: PGPR activities of fungal isolates

FUNGAL CULTURES	IAA	SIDEROPHORE	PHOSPHATE SOLUBILIZATION
isolate 1	+	+	+
isolate 2	+	++	+
isolate 3	+	+++	+
isolate 4	+	+	+
isolate 5	+	++	+
isolate 6	+	++	+
isolate 7	+	+++	+
isolate 8	+	++	+
isolate 9	+	+	+
isolate 10	+	++	+
isolate 11	+	+++	+
isolate 12	+	+	+

Where,

+++ = very high activity, ++ = high activity, + = low activity, - = no activity

Figure 15: Increased root and shoot length of compost soil plants compared to control

