IMPACT OF GREEN MANURE AND CONSORTIUM BIOFERTILIZER ON AMYLOLYTIC BACTERIAL POPULATION AND THEIR ACTIVITIES IN MAIZE RHIZOSPHERIC SOIL

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ABSTRACT

Microbial population and their activities in soil are important measure of soil biological activities as well as its health. The present study was conducted to access the impact of application of green manure and cellulose degrading bacterial consortium on the soil amylolytic bacterial population and amylase activity in rhizosphere of maize crop in field conditions. Soil amylolytic bacteria and amylase activity exhibited significant changes in response to application of consortium biofertilizer. The highest population of amylolytic bacteria was recorded during vegetative stage of maize crop in treatment T8 with 100%N + green manure+ consortium biofertilizer. Amylolytic population was found to be significantly higher in treatments having inorganic N + consortium biofertilizers as compared to control treatment. Soil amylase activity was significantly influenced by organic manure and vegetative growth stage. Highest amylase activity was recorded in treatment T8, whereas minimum activity was recorded in control (inorganic) treatment. Application of consortium biofertilizers significantly increased the amylase activity over treatments having solitary application of inorganic fertilizers. The results suggested that application of consortium biofertilizers on green manure boosted the colonisation and activities of amylolytic bacteria which directly influenced the available carbon pool as well as soil health.

Keywords- Amylolytic Bacteria, Amylase Enzyme, Consortium, Green Manure, Maize

1. INTRODUCTION

Soil microorganisms play important role in the nutrient matter cycling through enzymatic decomposition and transformation of organic matter. Soils possessing large amounts of microbial biomass usually offer more nutrients owing to the degradation potential of its microbiota [1]. Soil biological activities vary with time and are limited by substrate availability thus may provide useful linkage between microbial community composition as well as carbon processing. The soil organic matter consists of various polysaccharides such cellulose, hemicellulose, starch, xylan, lignins, proteins, fatty acids etc [2]. Starch is a major carbon compound within most plant tissues, its synthesis increases during active plant photosynthesis. It serves as reserve food material in plants during respiration in dark periods. It is a polymer of glucose linked to one another through the C1 oxygen via a glycosidic bond [3].

Starch-hydrolyzing microbes and the associated extracellular enzymes (amylases) in soil are usually inducible as their activity depends on the availability as well as type of substrate. Amylolytic bacteria and amylase enzyme are responsible for the major breakdown of complex polysaccharides (starch) to a readily available form of glucose [4]. Production of these extracellular enzymes from microbes during litter degradation may be influenced by temperature, moisture, and substrate involvement [5]. Substrate formed by incorporation on fresh/dry plant material in form of green manure, have impact on the amylolytic microbial population and amylase activity of soil. Decomposition of green manure is a biological breakdown and transformation of complex organic compounds into simpler organic and inorganic molecules [6].

This can lead to changes in soil amylolytic microbial populations that may ultimately alter the amylase activity during litter decomposition. Therefore, a field experiment was conducted to examine the effects of applications of green manure and consortium biofertilizers on amylolytic bacterial population and their associated amylase activity in maize rhizospheric soil.

2. MATERIAL AND METHODS

2.1 Experimental design

A field experiment was laid out in random block design in triplicate at the experimental area of School of Organic Farming, Punjab Agricultural University, Ludhiana in *kharif* season of 2016. Experiment was conducted to study the effect of different combination of fertilization, in situ green manuring and bacterial consortium on the amylolytic bacterial population and amylase activity of soil. In situ incorporation of *Crotolaria juncea* (Sunn hemp) was done eight days before the sowing of maize crop (in green manured plots). Two levels of the nitrogen fertilization were used 75% and 100%. The bacterial consortium used in the experiment was a dual purpose microbial consortium which has ability to degrade cellulose as well as plant growth promoting activities. This bacterial consortium was sprayed over green manure just before the ploughing; application of this consortium reduced the fallow period between maize sowing to 8days which was usually 14 days. The maize crop (variety – PMH1 and PMH4) were raised by following the crop management practices recommended in Package of Practices, PAU, Ludhiana [7]. A total of 8 different combinations of nitrogen fertilization (75% and 100%), organic amendment (with and without green manure) and bacterial consortium (with and without bacterial consortium) were made which are listed in Table 1.

Table 1: Different combinations of treatments used in the experiment.

Treatments	
T1	**75% of recommended N**
T2	**100% of recommended N**
T3	**Bacterial consortium + 75% N recommended**
T4	**Bacterial consortium + 100% N recommended**
T5	**Green manure + 75% N recommended**
T6	**Green manure + 100% N recommended**
T7	**Bacterial consortium + Green manure +75% N recommended**
T8	**Bacterial consortium + Green manure +100% N recommended**

2.2 Soil sampling

Soil samples were collected from rhizospheric soil of maize crop at different growth stages of rice crop 0, 30, 60 and at 90DAS (days after sowing). Plants were uprooted from five random locations from each treatment. Loose soil was shaken off the roots and the soil that adhered strongly, was carefully brushed from the roots and kept as rhizospheric soil. The five rhizospheric samples from each treatment were combined to form one representative samples, that were analysed [8]. At the time of crop sowing, the initial amylolytic population was recorded to be 25, 35, 75 and 92 cfu×10^5/g of dry soil, whereas amylase activity was recorded to be 0.007, 0.212, 2.788, 3.884 µg glucose/hour/g soil in bare field, field with bacterial consortium application, green manured field and field having green manure + bacterial consortium, respectively.

2.3 Enumeration of starch solubilizing bacterial population and assay of soil amylase activity

Enumeration of starch solubilizing bacterial population was done on starch agar medium containing 10% starch using serial dilution spread plate technique. The bacterial colonies appeared on medium were counted and expressed as cfu/g of soil. Amylase activity was accessed using the method developed by Cole [9] and followed by Tu [10] with modifications. Five grams of soil samples were placed in the test tubes; to this 1 ml of toluene was added. All the contents in the tubes were mixed thoroughly; after 15 min, 20 ml of 2% starch in 0.2 M acetate buffer (pH 5.5) was added. Another set of soil samples was treated in the same manner by replacing starch with acetate buffer without substrate. Tubes were incubated for 24h. The suspension was filtered by whatman no. 1 filter paper, and the amount of reducing sugar content in the filtrate was determined by the Nelson–Samogyi method [11] using digital spectrophotometer.

2.4 Statistical analysis

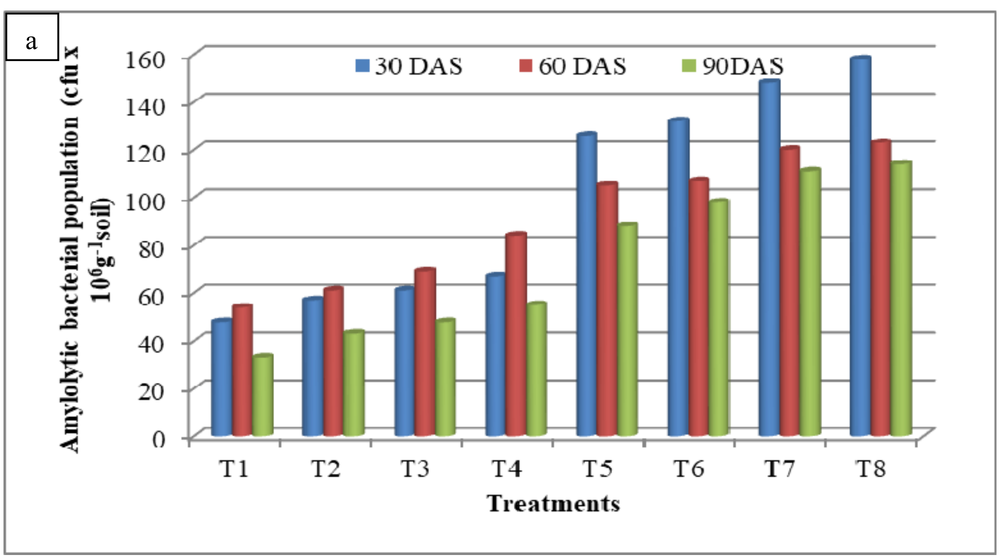
To determine the effect of different levels of nitrogen fertilizer, stages of plant growth and their interaction on soil amylolytic population and amylase activity, two way analysis of variance (ANOVA) was used at P=0.05 level of significance using CPCS1 software [12].

3. RESULT AND DISCUSSION

The relative population of microorganisms and their metabolic activity are assumed to be an important indicator of soil biological activity [13]. Amylolysis is considered as essential microbiological processes in soil. Amylolysis consists of starch hydrolysis through enzymes (amylases) excreted by amylolytic bacterial population. Amylolysis is a very common process among bacteria and fungi [2].

3.1 Amylolytic bacterial population

Statistical analysis revealed the critical difference @ 5% in treatment, environment and interaction as 0.30; 0.62, 0.49; 1.02, 0.85; 1.77 respectively in var. PMH1 and PMH4. Amylolytic bacterial population was significantly affected by organic inputs such as green manure and application of live bacterial culture (cellulose degrading consortium). Significantly higher amylolytic population was observed in treatments having green manure and consortium than the population in treatments with solitary application of inorganic nitrogen or bacterial consortium. Maximum amylolytic population 158× 10^6; 143× 10^6 cfu/g of soil were observed in treatment T8 having Bacterial consortium + Green manure +100% N (Figure 1) in rhizospheric soil of PMH1 and PMH4, respectively. This reflects the positive impact of green manure application on these bacteria. The amylolytic population might be increased due to availability of plant matter which served as substrate for these bacteria. Hence, these microorganisms belong to the zymogenous group as they respond primarily to the addition of fresh carbon substrates thus rely on organic matter for their energy and nutrients requirements.



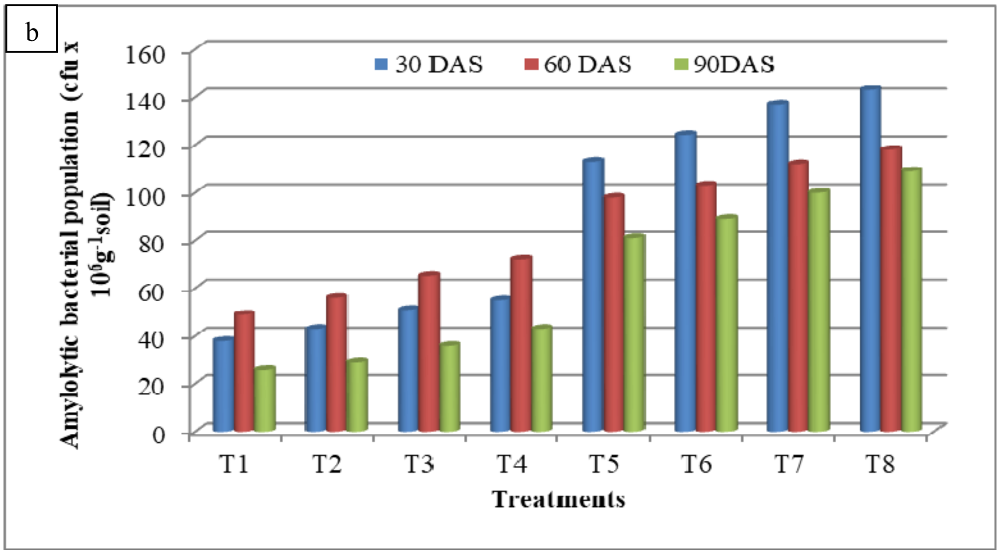


Figure1: Effect of green manure, consortium biofertilizers and inorganic nitrogen on population of soil amylolytic bacteria at different growth stages of maize a) var. PMH1 b) var. PMH4.

The application of bacterial consortium with inorganic fertilizer (T3 and T4) showed significantly higher amylolytic bacterial population than treatment having inorganic fertilizers (T1 and T2). This might be happened due to starch solubilizing character of bacterial consortium. The results were in accordance to Boruta and Paluszat [13], observed that higher count of amylolytic microorganisms was present in the soil cultivated in the organic farming system than the conventional farming system. Myoekow et al. [14] and Perucci et al. [15] had also reported that use of organic fertilizers resulted in the increase of organic C content in soils and introduced enormous amount of organic substance into soil that enriches the microflora of a given soil in terms of quantity and quality compared to conventional farming.

Amylolytic bacterial population was significantly affected by the vegetation stage of crop. Incorporation of plant material directly in soil significantly increased the amylolytic bacterial population at 0 and 30 DAS. Significantly minimum amylolytic bacterial count was observed at 90 DAS in treatment T1, in rhizospheric soil of both maize cultivars. However, treatment devoid of green manure showed higher amylolytic population at 60 DAS that start decreasing as the crop proceeds towards maturity. This might be due to increased availability of root exudates at flowering stage. The root exudates were rich source of available sugars, proteins, macro and micro nutrients at this particular growth stage. Study was supported by Boruta and Paluszat [13] that plant roots stimulate the growth of bacteria showing amylolytic activity. The intensive bacterial growth might have caused by the composition and amount of root excretions released by the plants, which were changing continuously during plant growth.

3.2 Amylase activity

Enzymatic activity is likely to be the potential index that can fully reflect the changes of the soil biology, fertility and quality. Soil microorganisms together with soil enzymes promote the cycling and transformation of various organic matters that maintains normal soil metabolic functions. Amylase is an enzyme of great value to soil health because it hydrolyses starch and transforms them into available sugar which enhances beneficial rhizospheric microbes. Statistical analysis revealed the critical difference @ 5% in treatment, environment and interaction as 0.23; 0.96, 0.37; 0.76, 0.67; 0.13 respectively in var. PMH1 and PMH4. Amylase activity recorded to be lowest in the control treatment T1 (75% N) at all the time interval of maize crop. The application of bacterial consortium with inorganic fertilizer (T3 and T4) showed significantly higher amylase activity than treatment having inorganic fertilizers (T1 and T2). This might be due to starch solubilizing character of bacterial consortium. The amylase activity was found to be significantly higher in treatments with integrated application of green manure and inorganic fertilization relative to activity of this enzyme in treatments with solitary application inorganic nitrogen (Figure 2). Maximum amylase activity 27.989 µg glucose/hour/g soil and 26.783 µg glucose/hour/g soil were observed in treatment T8 having bacterial consortium + green manure +100% N at 30DAS in rhizospheric soil of PMH1 and PMH4, respectively. The green manure and bacterial culture incorporation stimulated microbial growth that might have elevated the level of amylase enzymes thereby contributing to the available carbon pool of soil. The study was in accordance to Boruta and Paluszat [13] that organic fertilization favoured the development of starch decomposing microorganisms, which testifies an increased soil enzymatic activity with organic farming. The study was also supported Zantua et al. [16] who observed that most of the variation in amylase activity observed in soils was due to organic matter. The soil amylase activity was found to be statistically higher in treatments with integrated application of organic matter and inorganic nitrogen. Therefore, the application of organic fertilizers increased nutrient turnover through both increased microbial biomass and activity.

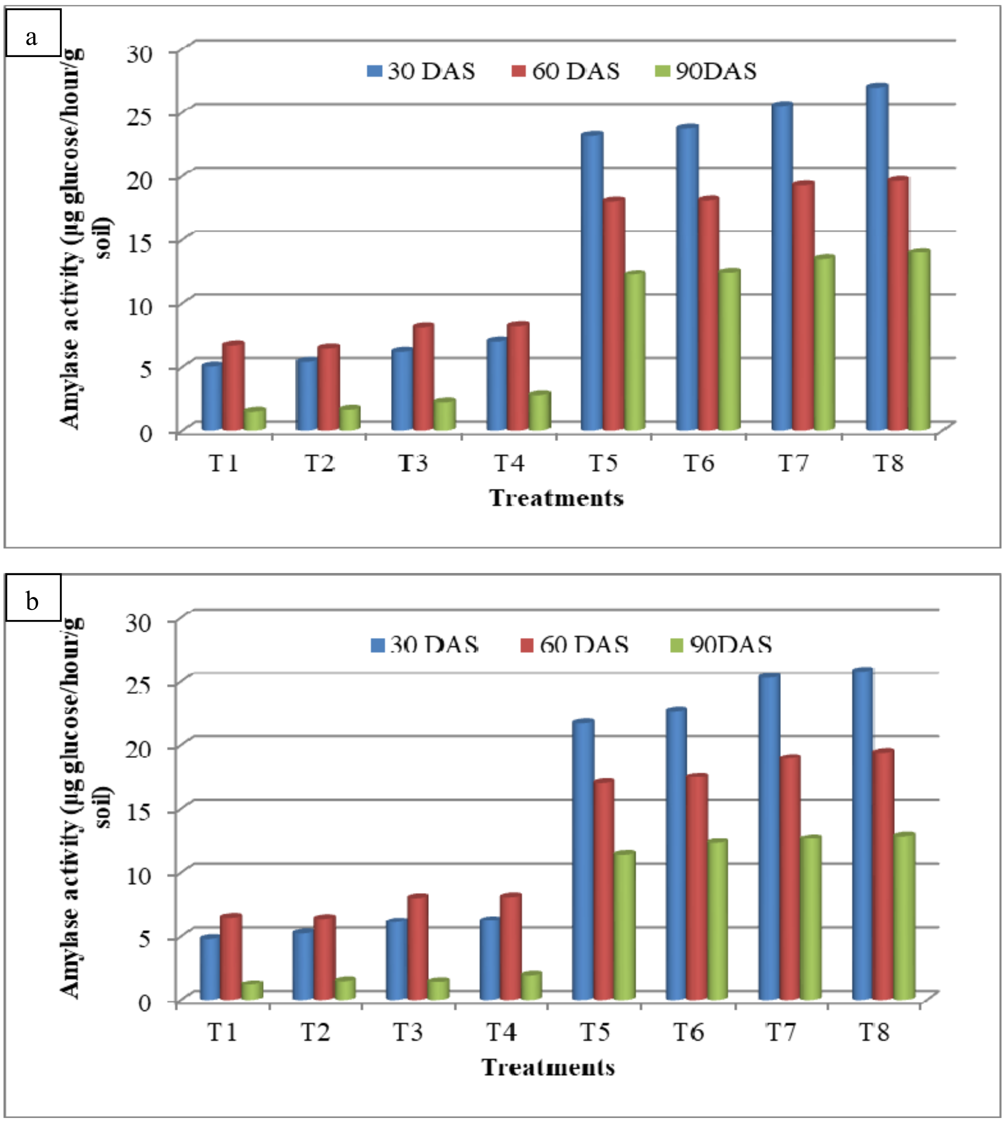


Figure 2: Effect of green manure, consortium biofertilizers and inorganic nitrogen on soil amylase activity at different growth stages of maize a) var. PMH1 b) var. PMH4.

4. CONCLUSION

Application of cellulose degrading bacterial consortium in green manured fields significantly enhanced the amylolytic bacteria and their activities in soil; that benefits the soil health and its properties by stimulating green manure degradation. The population of amylolytic microorganisms and the intensity of processes catalysed by them depend especially on the content of assimilable compounds of carbon and nitrogen thus fertilization has significant impact on soil biological properties.

REFERENCES

1. Akpor OB, Okoh AI, Babalola G O. Culturable microbial population dynamics during decomposition of *Theobroma cacao* leaf litters in a tropical soil setting. J Biol Sci 2006; 6(4):768-774.
2. Khatoon H, Solanki P, Narayan M, Tewari L, Rai JPN. Role of microbes in organic carbon decomposition and maintenance of soil ecosystem. Int J Chem Stud 2017; 5(6): 1648-1656.
3. El-Fallal Amira, Dobara AB, El-Sayed A, Omar N. Chapter 21- Starch and Microbial α Amylases: From Concepts to Biotechnological Applications in Carbohydrates Comprehensive Studies on Glycobiology and Glycotechnology; 2012.
4. Singaram P, Kumari K. Effect of continuous application of different levels of fertilizers with farm yard manure on enzyme dynamics of soil. Mad Agric J 2000; 87 (4-6): 364-65.
5. Maddela NR, Golla N, Vengatampalli R. Chapter 7: Soil amylase in Soil Enzymes: Influence of Sugar Industry Effluents on Soil Enzyme Activities., Pp 31-39 *Springer Briefs in Environ Sci*, Springer International Publishing Switzerland; 2017.
6. Meena BL, Fagodiya RK, Prajapat K, Dotaniya ML, Kaledhonkar MJ, Sharma PC, Meena RS, Mitran T, Kumar S. Legume green manuring: An option for soil sustainability in *Legumes for soil health and sustainable management*, Springer Nature Singapore Pte Ltd; 2018.
7. Package and practices for kharif crops, Punjab Agricultural University, Ludhiana, Punjab, India; 2016.
8. Kaur J, Gosal SK, Walia SS. Correlation of microbial population with enzymatic activities and nutrient levels of soil during paddy growth. Curr J Appl Sci Technol 2018; 29(6): 1-9.
9. Cole MA. Lead inhibition of enzyme synthesis in soil. Appl Environ Microbiol 1977; 33:262-68.
10. Tu CM. Influence of pesticides on activities of invertase, amylase and levels of adenosine triphosphate in organic soil. Chemosphere 1982; 2: 909-14.
11. Nelson N. A photometric adaptation of the somogyi method for the determination of glucose. J Biol Chem 1944; 153:375-80.
12. Cheema HS, Singh B. Software Statistical Package CPCS-1. Developed at Department of Statistics, Punjab Agricultural University, Ludhiana, India;1991.
13. Boruta BB, Paluszak Z. Occurrence of amylolytic microorganisms in soil depending on the type of cultivation. Ecohydro hydrobiol 2006; 6:(1-4): 175-80.
14. Myoeków W, Stachyra A, Ziêba S, Masiak D. Biological activity of soil as an index its fertility. Rocz Glebozn 1996; 47(1/2): 89- 99.
15. Perucci P, Bonciarelli U, Santiloschi R, Bianchi AA. Effect rotation, nitrogen fertilization and management of crop residues on some chemical, microbiological and biochemical properties of soil. Biol Fertil Soils 1997; 24:311-16.
16. Zantua MI, Bremner JM. Stability of Urease in Soils. Soil Biol Biochem 1977; 9:135-40.