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STUDIES ON EFFECT OF ACTINOMYCETES ON DECOMPOSITION OF PLANT RESIDUES

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ABSTRACT

In this study cellulolytic bacterium Actinomycetes were used to decompose plant residue (sugarcane). India produces larger amount of plant residue each year. Nearly half of the residues burned every year . Open burning of sugarcane residue has large impact in regional atmospheric pollution and global climatic change. Actinomycetes culture isolated from different sources : post harvest sugarcane residue , soil collected from village Walunj, Ashti. The results showed that an inoculation of Actinomycetes bacteria provided better decomposition of sugarcane crop residue and enhanced physicochemical properties of soil Actinomycetes could be used sugarcane leaf decomposers resulted in a biofertilizer after the degradation .

Keywords :- Actinomycetes , Decomposition , Isolation , Plant residue

1. INTRODUCTION

India, the second largest agro-based economy with year-round crop cultivation, generates a large amount of agricultural waste, including crop residues (13). In these last years, sugarcane straw (Saccharum officinarum L.) has been viewed as great residues of wealth that is burned(4). practices, approximately 92 seems a very small number of metric tons of crop waste is burned every year in India, causing excessive particulate matter emissions and air pollution. Crop residue burning has become a major environmental problem causing health issues as well as contributing to global warming**(**13).

Agriculture fire is a common practice used by farmers, to control crop diseases and plagues, as well as to prepare the land for the next crop (7). Sugarcane is the only crop which fire is used pre-harvesting in both, developed and developing countries, to expose the cane stalks removing the outer leaves, driving away insects, snakes and other wild animals making easy not only manual, but also mechanical harvesting (14); additionally, after harvesting, sugarcane residues are burned releasing to the atmosphere more harmful pollutants (10).Crop residue burning significantly increases the quantity of air pollutants such as CO2, CO, NH3, Non-methane hydrocarbon volatile organic compounds .This basically accounts for the loss of organic carbon, nitrogen, and other nutrients**(**13). Residue burning may adversely affect soil fertility due to the fact that it causes losses of some nutrients and organic matter over time. Recently, sugarcane producers have been required to adopt alternative sugarcane residue management practices(e.g. cane residue retention), since burning has raised air pollution concerns (3).Burning of sugarcane residues either before or after sugarcane harvest is widely practiced in many tropical countries. Farmers burn sugarcane to reduce the amount of leafy extraneous material, including stalk tops and dead leaves delivered with the cane to the factories for processing and to control pests. Additionally, sugarcane burning facilitates manual harvesting thus reducing labor and production costs. This practice significantly reduces the amount of trash that needs to be dealt with, but pollutes the surrounding neighborhood with smoke and ash. In addition, residue combustion is a source of particulate and gaseous (CO2, NO, NO2 and N2O) emissions to the atmosphere that may contribute to the "greenhouse effect", and the associated global warming. Some studies have reported beneficial effects of crop residue burning(12) . However, residue burning may adversely affect soil fertility due to the fact that it causes losses of some nutrients and organic matter over time. Recently, sugarcane producers have been required to adopt alternative sugarcane residue management practices(e.g. cane residue retention), since burning has raised air pollution concerns(3). Post-harvest sugarcane residue is a lignocellulosic material removed from the cane stalks and deposited on field surface. Average structural polysaccharide percentages on dried sugarcane residue are cellulose (38.1%), hemicellulose (29.2%), lignin (24.7%), ashes (3.4%) and extractives (4.7%) (9). Cellulose is a basic component of all plant materials and its production exceeds that of all other natural substances. Plant residues in soil consist of 40-70 per cent cellulose. Cellulose is made up of chains of β-D-Glucose consisting of about 1900 glucose units (monomers). Cellulose is degraded and utilized well in aerated soils by aerobic microorganisms. Cellulolytic microorganisms are commonly found in the field and forest soil in manure and on decaying plant tissue. They include both aerobic and anaerobic fungi and bacteria, many of which grow under extreme conditions of temperature and ph. Soil microorganisms can decompose residues and offer numerous ecological and economical benefits to growers; however, the process is dependent on the biotic density, diversity and activity in the soil. Further, the decomposition process is unfavourably slow such that sugar yields are adversely affected in emerging ratoons. These laboratory findings suggest that sugarcane residues may decompose at accelerated rates when treated with microbes co-applied with specific nutritive formulations(8). Actinobacteria, one of the most widely distributed phyla among soil bacteria .are well known for their ability to degrade plant residues. However, the extant knowledge regarding the propensity of Actinobacteria to degrade plant residues is mainly based on studies with pure cultures. Plant residues mainly consist of polymers, such as cellulose, hemicelluloses, polysaccharides, and lignin As soil-dwelling microorganisms are the main driving force for their decomposition their fate is largely determined by both the ecological (i.e., community composition and interspecies interactions (15). Actinomycetes are the masters of enzymatic machinery that engineer soil productivity through nutrient cycling, complex polysaccharide decomposition, soil reclamation, and the first line of defense against soil pathogen India, the second largest agro-based economy with year-round crop cultivation, generates a large amount of agricultural waste, including crop residues. In the absence of adequate sustainable management practices, approximately 92 seems a very small number of metric tons of crop waste is burned every year in

India, causing excessive particulate matter emissions and air pollution. Crop residue burning has become a major environmental problem causing health issues as well as contributing to global warming.(Kennedy et al.,1999) The present study were therefore taken under investigation of the soil sample collected from field from Ashti with following aims and objectives. 1.To isolate Actinomycetes with maximum productivity. 2. To determine the role of Actinomycetes in decomposition of plant residues.

2. MATERIALS AND METHODS

2.1 Microorganisms Cellulolytic actinomycetes were selected to be used in this research due to their efficiency to breakdown the plant residue.

2.2 B.O.D INCUBATOR provides the required temperature for the growth of microorganisms. And allows to perform the BOD testing.

2.3 Isolation of an Actinomycetes cultures were isolated from samples of soil samples from different localities from decaying wood soil , soil from village of Walunj , Ashti. The strains were cultivated and maintained on selective media. Actinomycetes isolation were done by serial dilution and streak plate technique (Wlliah et al 2004) 1gm of soil was suspended in 9ml of water and then sample were streak on the Actinomycetes Isolation Agar Media . To minimize the other bacterial and fungal growth , Fluconazole (q.s) were added. Then plates were incubated at 30^0C for 4-5 days . The plates were observed intermittently for the actinomycetes growth during incubation . After incubation , actinomycetes colonies which are morphologically distinct were picked from the actinomycetes isolation agar plates and further purified by repeated streak plate method (11).

Once the pure colonies were obtained each colony was further identified based on its characteristics such as earthy smell , colony morphology, the color of hyphae and presence or absence of aerial mycelium and gram staining method. Identification of an actinomycetes was done on the basis of macroscopic and microscopic examination suggested by Bergey's Manual of Systematic Bacteriology, 2nd Edition, Vol5, The Actinobacteria, Part A.

2.4 Gram Staining of Actinomycetes.

Many actinomycetes organisms can be observed via the Gram stain technique owing to their Gram-positive nature. The procedure is as follows:-

1.Transfer drop of obtained culture onto a microscopic slide using inoculation loop. 2. Slide air dried with the help of heat over a gentle flame. The slide should be moved circularly over the flame to prevent overheating or forming of ring patterns in the slide. The heat helps the cell adhesion to the glass slide and prevents the significant loss of culture during rinsing. 3.Crystal violet stain were added over the fixed culture. 4. Washed out the slide with water and administer iodine, which is used as a fixative. 5.Wash the slide and use alcohol as a decolorizer to remove the crystal violet stain from Gram-negative species without affecting the staining of those that are Gram positive. 6. Washed out the slide again and added safranin (the counterstain). 7. Wash for the last time and observe results.

Then selected and identified colonies of actinomycetes were transferred to actinomycetes isolation agar slant and incubated 30^0C.for their growth. After incubation , the slants containing pure actinomycetes isolates were stored at 4^0C for further studies

2.5 Sugarcane crop residue decomposition

Sugarcane residue samples were collected from fields cropped residues utilized in this study was fragmented into small pieces (10-15cm in length). Then devided into two treatments.

1.The Experiment was conducted in Laboratory by using two large size containers .

2.Container one were control sample and one were for further treatments .
3. One container was filled with Agricultural soil collected from the farm and Sugarcane crop residue collected from the same site.

4. Another container was also filled with sufficient amount of soil and sugarcane crop residues 3.For further treatments isolated colonies were picked into flask and homogenized with 100ml water under a aseptic condition very carefully by using a flame.

4. This mixture was sprayed on the residue per day with 100ml and repeatedly mix once to provide aeration. 5. For better result moisture on residue was maintained every day. 6. Residue breakdown was monitored by visual observation.

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of Actinomycetes Actinomycetes bacteria were isolated from soil samples of Walunj, Ashti. The isolated culture was identified as Actinomycetes species. Slants were prepared and preserved in Freeze. These are used for further studies. The isolated microorganisms are identified as actinomycetes by Macroscopic , Macroscopic Characterization. Identification of actinomycetes was done on the basis of macroscopic and microscopic examination The isolated actinomycetes were observed for their aerial mycelium , colony morphology on media ,White colour and white colour amorphous powdery texture. Microscopic examination was performed by cover slip and Gram –staining method.

Fig 1. Culture . Typical colonies of Actinomycetes on Actinomycetes Agar plates Shows white wrinkled colonies .

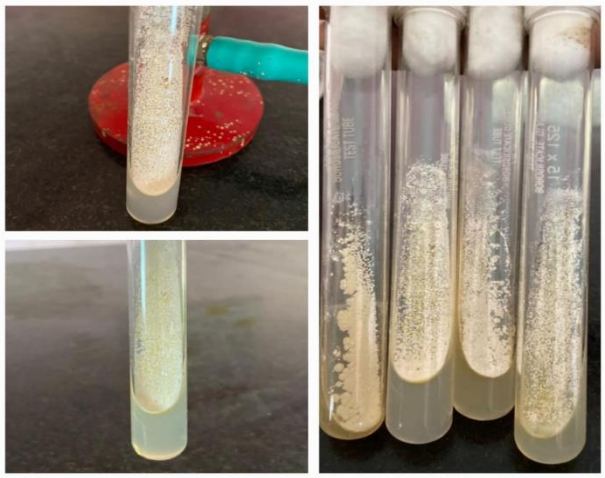

Fig 2. Typical growth of Actinomycetes on Agar slants .

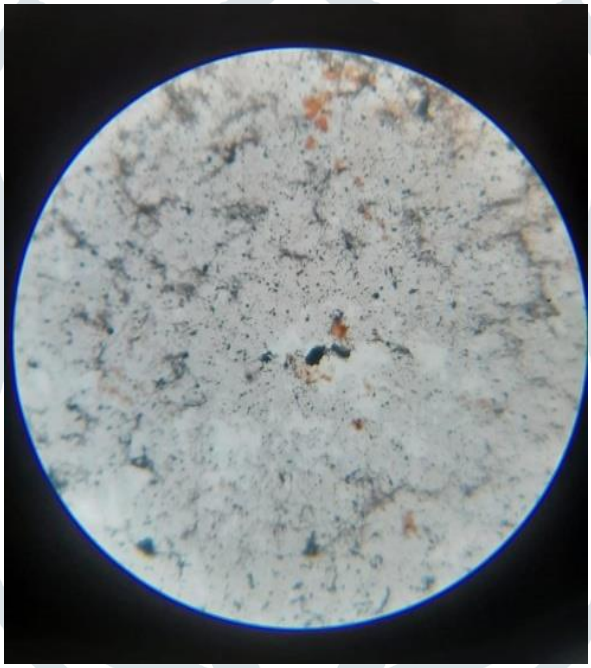

Fig 3. Microscopic observation of isolated actinomycetes. The microscopic observation of actinomycetes using the Gram-staining method. Gram-positive Blue color bacteria were observed .with branched network of hyphae are observed under the microscope.

3.2 Decomposition of sugarcane crop residue by isolated actinomycetes There were two treatments carried for studies. One is for control without bacteria were added in residue and another is used for further studies where the soil were added in plant residues. In the mixed soil and residue samples the mixture of actinomycetes bacteria and water were spread every day continuously through the 8 week time period of the study. Moisture also maintained for getting better degradation results . These samples were kept aerobic condition . microbial growth rates are kept high by allowing the soil microbes maximum access to the cellulose substrate that is kept moist by its contact with soil . microbial contact and substrate moisture are two factors that help facilitate residue microbial consumption . Decomposition of plant residue by the bacteria actinomycetes occurs within 8 week . Actinomycetes bacteria work in decomposition of sugarcane residue , provided the residue mixed with soil. The inoculation of thermophillic actinomycetes significantly increases the degradation of cellulose , hemicelluloses, and lignin.

The treatment where the Actinomycetes colonies mixture over residue were not spread was not significantly effective. In control , no significant degradation of sugarcane crop residue was observed. Hence , we observed that actinomycetes plays an important role in the decomposition of various plant residue in minimum time .In the treatment there was represent the low overall microbial growth that occurred when microbial population not added in the control samples.

The results of sugarcane residue decomposing efficiency of actinomycetes isolates recorded that by inoculation treatment the inoculation of actinomycetes significantly increases the degradation of sugarcane crop residue over uninoculated control.

When soil and microbial population were not mixed various factors affect the microbial decomposition of cellulose. Those are as

1. Minimum contact between bacteria and the cellulose substrate .

2. Crop residue dried rapidly and no water available for microbial metabolism. Gowda (1996) reported that during decomposition of wastes, the microbes consume more O2 to break down the organic compound and release heat energy through respiration process, which caused the temperature to raise in decomposition of organic matter(6).

Fig 4. Control: Treatment in which actinomycetes colonies were not added in plant residue.

Fig 5 . treatment with actinomycetes colonies added in the plant residue and mixed with soil.

4.CONCLUSION

This study showed that the naturally occurring soil bacteria in agricultural soil can induce post-harvest sugarcane waste to decompose extensively. The increase in soil microorganisms, rise in soil cellulase activity, fall in soil cellulose content, and rise in soil organic carbon were all signs of decomposition. This laboratory study provides evidence that the Actinomycetes colonies mixed in soil help the decomposition process of sugarcane residue . The treatment where the Actinomycetes colonies mixture over residue were not spread was not significantly effective. In control , no significant degradation of sugarcane crop residue was observed. The inoculation of thermophillic actinomycetes could significantly increase the degradation of cellulose , hemicelluloses, and lignin.Future field studies should be conducted using these various microbial forms with goal of optimizing shelf life and ease of applicaton for farmers .once the sugarcane stalk is harvested , the next years crop and residue left on ground. It is doubtful that microorganisms that have evolved to be plant residue decomposers would have negative effect on living plants but this should be verified scientifically. This would ensure farmers that application these cellulolytic microbes not cause harm to the crop yield .This would represent an increase in soil fertility and lead to maintained or increased crop yield while lessening the external fertilizer applications.

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