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Transforming Agricultural waste into Organic liquid NPK Fertilizer using Probiotics: A Fermentation Approach and Its Effect on Brinjal Growth

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Abstract

Brinjal farming appears to be benefiting from Integrated Nutrient Management, that utilizes integrated application of chemical fertilizers and organic fertilizers produced from agricultural waste. In the present study, probiotic microorganisms *Lactobacillus* and *Saccharomyces* were used to ferment nitrogen, phosphorous, and potassium-rich agricultural residue into organic liquid NPK fertilizer. The effectiveness of this process was tested on brinjal under field conditions. Nine distinct agricultural wastes were combined to create 27 factorial formulations that had one N, P, and K-rich substrate and subjected to microbial fermentation in 1 L flasks. Thirty days after fermentation, the fertilizer formulation containing groundnut cake, rock phosphate, and potassium humate recorded the highest total NPK of 3.9%. 29 treatments were made including T1 - control, T2 - 100% inorganic fertilizer treatment (RDF for brinjal), T3 to T29 - organic fermentation liquid in 27 flasks + 50% RDF. The results showed that the integrated treatment T21 (Groundnut Cake + Rock Phosphate + Potassium Humate + 50% RDF) on brinjal recorded significant Plant height of 84.3 cm, 9.33 branches/plant, individual fruit weight 54.2 g and 177.9 kg yield/plot at 75 DAT.

Key words : Agro wastes, Fermentation, Integrated Nutrient Management, Organic Liquid NPK fertilizer.

Chemical fertilizers and pesticides have been widely used to enhance crop growth and yield to provide the required food supplies over the increased demand for food in recent years. Fertilization is essential for enhancing plant attributes and nutrient uptake. It is also essential for supporting plants photosynthetic systems. However, the widespread use of chemical fertilizers has led to a decrease in the dynamic equilibrium of soil, flora, and fauna

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ecosystems as well as contamination of water streams. The need for sustainable and environmentally friendly fertilizers has led to the search for alternative fertilizer sources for use in agriculture[4].

The agricultural sector is a primary source of substantial agricultural solid waste, which can pile indiscriminately, posing a nuisance to world health and a threat to food security, or be utilized as raw materials for the bioeconomy[1]. These agricultural wastes can be effectively transformed into organic fertilizers that are rich in Nitrogen (N), Phosphorous (P) and Potassium (K). These organic fertilizers obtained from agricultural waste can be used in integrated nutrient management with inorganic fertilizers. Thus, they reduce the use of chemical fertilizers ultimately reduce the high cost of chemical fertilizers purchase. Furthermore, the organic compounds lower soil pH and enhance soil structure, aeration, and moisture retention[8]. Also, a fermentation process using decomposing microorganisms like *Lactobacillus* sp., *Rhodopseudomonas*, *Actinomycetes* sp., and *Streptomyces* sp., can enhance soil nutrients without affecting the environment[2].

The warm-weather crop referred to as brinjal eggplant (*Solanum melongena* L.) is mostly grown in tropical and subtropical climates worldwide. Brinjal farming may benefit greatly from integrated nutrient management, which involves using organic manures in addition to inorganic fertilizers. In the production of vegetables, integrated nutrient application has become extremely important.

Thus, the main objectives of the study were to assess the effectiveness of the organic liquid NPK on brinjal. The probiotic microbial consortium also helps in deriving nutrients from the organic sources. This study not only emphasizes the effective utilization of agricultural waste but also in the bioconversion of those waste into eco-friendly organic fertilizer rich in NPK.

Materials :

The experiment was conducted in the Laboratory of Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University, Chidambaram. Nine different agricultural wastes or residues rich in Nitrogen, Phosphorous and Potassium such as Poultry litter, Groundnut cake, Green gram flour, Phosphate rich organic manure (PROM), Rock Phosphate, Bone meal, Potassium humate, Woodash and Pressmud were collected. Microbial strains such as *Azospirillum*, *phosphobacteria* and potash mobilizer were obtained from Tamil Nadu Agricultural University (TNAU).

Isolation of Probiotic strains :

Probiotic strains *Lactobacillus* and *Saccharomyces* used in the study were isolated from milk and bread samples respectively. Serial dilution of 1 mL of milk sample upto 10^{-6} dilutions were made. de Man Rogosa and Sharpe (MRS) agar plates were made and 0.1 mL of milk sample from 10^{-6} dilution was inoculated into the plates and spread using sterile spreader. The plates were incubated at 37°C for 48 hours. *Lactobacillus* colonies developed were transferred to fresh MRS plate for purification[7]. For the isolation of *Saccharomyces,* a small amount of bread

sample was streaked onto Yeast Extract Peptone Dextrose Agar (YPDA) plates and the plates were incubated at 25°C up to 5 days. *Saccharomyces* colonies developed were transferred to fresh YPDA plates[3]. 5 different strains of *Lactobacillus* LF1, LF2, LF, LF4, LF5 and *Saccharomyces* SCB1, SCB2, SCB3, SCB4, SCB5 were isolated. The strains of bacteria and yeast were tested for acid production and CO_2 Production respectively to identify the best strains for utilization in the fermentation Process (Table-1). The best performing strains LF1 and SCB-1 which shows maximum acid production 3.8 pH and CO_2 Production 2.4 mL were selected and sent to Medox Biotech Pvt Ltd., Bangalore for 16s rRNA and ITS sequencing for bacteria and yeast. These two strains were used in further studies.

Fermentation Setup :

The wastes or residues used in the study were sterilized before use. They were subjected to dry heat in oven for 60°C for 2 hrs to reduce the moisture content to 10-12%. Then they were grinded to reduce their particle size to less than 1 mm which reduces their surface area in the Fermentation liquid and enables the microbial strains to break them faster. Finally, the wastes were pasteurized by passing steam and raising the temperature upto 60°C to kill the undesirable microbes such as *Salmonella*, *E. coli* and many fungi which were more likely to present in Poultry litter, Pressmud, Groundnut cake, wood ash, bonemeal, and Rock phosphate. These wastes were made into 27 factorial formulations containing each one of Nitrogen, Phosphorous and Potassium rich substrates. The fermentation set up was made up using 27 one litre flasks. 600 mL of molasses medium containing 18g molasses, 0.6 g yeast extract and 0.6g peptone in each flask were sterilized in autoclave at 121°C for 15 minutes under 15 lbs pressure. To the sterilized molasses medium in 27 flasks 30 g of each substrate of the formulations were weighed and added. The entire fermentation process was maintained at temperature between 30 ± 2°C and around pH 6.5-7.0.

The isolated probiotic strains *Lactobacillus* LF1 and *Saccharomyces* SCB-1 each 1mL were added to the Fermentation medium. Along with that, *Azospirillum*, *Phosphobacteria* and Potash mobilizer maintained in the selective broth such as Nitrogen free bromothymol malate broth (NFB) for *Azospirillum*, Pikovaskya broth for Phosphobacteria and Glucose Yeast Extract Calcium carbonate broth (GYC) for Potash mobilizer were also added each 1mL into the flasks. In addition to the microbial cultures, Growth promoting substances and the sources of natural hormones auxin, cytokinin, such as coconut milk 20 mL, coconut water 10 mL, curd 5 mL, seaweed gel 5 mL and gibberellic acid 0.01% were also added. The flasks were covered with cotton plug to promote aerobic fermentation. All the flasks were shaked twice in a week upto 30 days.

The Organic liquid fertilizer after 30 days of fermentation was filtered and tested for the presence of nitrogen, Phosphorous and potassium. Nitrogen was tested using Kjeldhal method, Phosphorous content was testing using atomic absorption spectrophotometry, meanwhile, potassium was tested using Flame photometry.

Brinjal Field trials :

Field trial to study the efficacy of fermented liquid fertilizer on brinjal PLR (Br.) 2 variety was conducted in **Kottapakathuveli region of Villupuram District** in 30 cents land area. A randomized Block Design with 29 treatments and 3 replications for each treatment were made. 35 days old brinjal seedings PLR (Br.) 2 variety were planted at spacing of 90cm x 60 cm between rows and plants. Organic NPK fertilizer was sprayed at ideal plant growth stages such as planting, vegetative growth, flowering, and fruiting stages. Plant growth parameters such as plant height, Number of branches per plant, individual fruit weight and Total yield per plot were measured at 75-85 days after trans-planting (DAT).

Molecular identification of Probiotic strains:

Comparative 16s rRNA gene sequence analyses revealed that *Lactobacillus* sp. LF1 strain had shown more similarity with ***Limosilactobacillus fermentum*** (PQ222458) and ITS sequencing results revealed *Saccharomyces* SCB-1 strain shows more similarity with ***Saccharomyces boulardii*** (PQ269747).

Nutrient Composition of Fermented Liquid Formulations :

Out of the 27 liquid formulations tested for NPK, the formulation containing **Groundnut cake+ Rock phosphate+ Potassium humate** recorded the maximum values of 3.9% NPK **(N- 1.1%, P- 2.2%, K- 0.6%).** The formulation which produced maximum NPK % was tested for the presence of micronutrients. 5 mL of the sample was pipetted into Kjeldhal flask. To that 10 mL of conc. HNO_3 was added and the sample was let to stand for 15 mins. After that the entire set up was heated in a hot plate at 120°C for 30 minutes. 2-3 drops of H_2O_2 were added to the solution and continued to be heating until the solution developed light yellow colour. Then the solution was let down to cool and transferred to 50mL volumetric flask where it was diluted to the appropriate volume with deionized water. Appropriate dilutions for different instrument calibration range were prepared. Iron, Manganese, zinc, copper, and cobalt were determined by Atomic Absorption Spectrometry. Boron was tested using Azomethine - H colorimetric method at 420nm. Molybdenum was determined by colorimetric method by measuring absorbance at 820nm. This specific formulation which produces maximum NPK recorded micronu-trients contents (% w/w) of Iron 0.0600, Manganese 0.0250, Zinc 0.0020, Copper 0.0012, Boron 0.0040, Molybdenum 0.00015 and Cobalt 0.00050 (Fig. 1).

Plant growth performance in field trials:

The results of the Field trial conducted to test the efficacy of the fermented organic liquid NPK fertilizer on brinjal PLR (Br. 2) variety shows that T21 produces maximum Plant height of 84.3 cm, 9.33 branches per plant, fruit weight of 54.2 g and 177.9 kg yield/ plot (Fig. 2). Followed by T21, treatment 12 (Greengram flour + Rock phosphate + Potassium humate) recorded second maximum Plant height of 81.9 cm, 8.03 branches per plant, fruit weight of 52.4 g and 170.4 kg yield/ plot. These results were similar to[6] who reported the influence of integrated application of humic acid and inorganic fertilizers on the growth and yield of brinjal var. KKM1.

Table 1: Acid Production and CO_2 Production test for *Lactobacillus and Saccharomyces* Isolates

Lactobacillus Isolates	Acid Production (Final pH after 24 hrs)	Saccharomyces Isolates	CO_2 collected in 24 hrs (mL) in Durham's tube
LF1	3.8	SCB -1	2.4
LF2	4.2	SCB-2	1.9
LF3	4.1	SCB-3	1.1
LF4	4.0	SCB-4	1.5
LF5	4.6	SCB-5	0.8

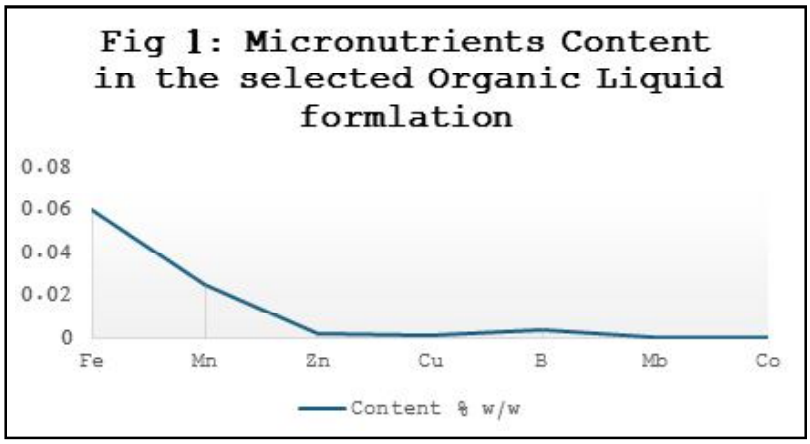

Figure 2: Brinjal Field Studies

This study demonstrated the effective bioconversion of agricultural residues into NPK rich organic liquid fertilizer under controlled fermentation using beneficial microbial consortia. The probiotic organisms used in the study plays an important role in breaking down the organic matter and make the nutrients in them to available form. The utilization of agricultural wastes for fermentation also reduces environmental pollution caused by the improper disposal of agro wastes. The results of this study suggest that the integrated nutrient management on brinjal has a favourable and beneficial impact on increasing growth and yield. Among all the treatments, T21 provided an enhanced crop growth and yield. This integrated nutrient management reduced the use of synthetic fertilizers thus favour eco-friendly and sustainable agriculture.

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