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EXPLORATION OF SYMBIOTIC RELATIONSHIP: INVESTIGATING THE PHYSIOLOGICAL AND MICROBIOLOGICAL ACTIVITY OF SUGAR-CANE PRESS-MUD AND HARNESSING AS BIOLOGICAL MANURE.

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ABSTRACT

Bio-compost is a material which contains live micro-organisms exhibiting beneficial characteristics towards the growth of plants and also in soil development making the soil more fertile. Bio- composting of sugar-cane press-mud is a composition of Organic fertilizer and Bio- fertilizers where a unprocessed biological nature. i.e., plant material and living micro-organisms which are agriculturally useful in terms of nitrogen fixation, phosphorus solubilisation or nutrient mobilization have been altered together through mobilization have been altered together through microbiological decomposition processes increase the productivity of the soil or crop. Bio-Composting is the degradation of organic matter by various microbes in a moist, favourable environment. Composting takes place aerobically or an-aerobically in the environment. Five different varieties of plants were grown for enumeration of the quality of the bio-compost prepared in the trial windrow. The varieties of plants grown were namely Tomato, Chili, Cabbage, Brinjal. The Bio-compost was prepared in farm by sugarcane wastes such as bagasse and the dried sludge from Sugar Effluent Treatment Plant was mixed with it. Aerobic condition was maintained by regular tumbling of the trial bio-compost windrow and continuous monitoring of Temperature, pH and Electrical conductivity was done. Physico-chemical and microbiological results were performed once a week or as and when required. After proper degradation of the bagasse the bio-compost was used in the cultivation of the plants in selected plot. The essential factors such as N: P: K was maintained by the selected strains of micro-organisms and it was also checked that no pathogenic activity by any microbes occurred while preparation of the bio-compost.

Keywords: Bio-compost, Physico-chemical, Organic fertilizer, Bio-Fertilizers, Microbiological, Pathogens.

1. INTRODUCTION

Nowadays, farmers are growing diverse food crops and applying agro chemicals or fertilizers such as Urea and D.A.P. in imbalanced ratio as with the increasing population & consumption production capacity of the soil have reduced. Production of enough crops and it's by-products for consumption is a huge challenge in the field of agriculture. Hybridization or genetic development in crops has slowed down the ever growing demand of nutritious food. Waste and microbiology studies are becoming increasingly important for understanding the roles of micro-organisms that play in agricultural productivity. The bio-composting of sugar-cane press-mud with addition of effective microbial strains culture in bio-fertilizer enhances the growth factor of crops, makes the crop disease resistance and helps it tolerate the pollutant activity of soil which may have raised due to earlier use of agrochemicals. The application of bio-compost is considered an important component of nutrient management and an eco-friendly alternative to chemical based farming with cost-effective input and a sustainable way of agriculture crop production. Waste and microbiology studies are becoming increasingly important for understanding the roles of micro-organisms that play in agricultural productivity. Microorganisms

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are playing an important role in the bio-composting process through the generation of enzymes, which help while the degradation of plant based organic waste materials like cellulose, lignin hemicelluloses, etc. (Vargas Garcia et al., 2010) The increase of pH in the initial period of composting might be due to the evolution of ammonia from the nitrogenous sources during composting (Rashad et al., 2010). The C: N ratio of different treatment reflects the organic waste mineralization and stabilization. The microbial population of soils is made up of five major groups including bacteria, actinomycetes, fungi, algae and protozoa, and among these groups, bacteria are the most abundant group and the most important microbe for degrading waste.

Composting can be aerobic and anaerobic. Different species of bacteria are responsible in each case, different chemical changes takes place and different temperatures are reached (Hussain and Naser, 2013). Microorganisms are increasingly becoming an important source in the search for industrially important molecules. In soil 80-90% of microorganisms remain unidentified whereas these biological communities are known to play a dominant role in maintaining a sustainable biosphere. Microorganisms are highly considered valuable as they are used in composting, fermentation processes, in brewing, baking and in production of various antibiotics and vaccines. The composting process always occurs in nature, however many artificial measures have been developed to improve composting efficiency. Over the past years, effective inoculation has been reported by several researchers. Various specialized inoculums have been applied in practice. Several authors have been reported composting using different bulking agents (Gautam et al., 2007; Rosazlin et al., 2011; Tripetchkul et al., 2012; Harrison et al., 2014). Recently addition of microorganisms to speed up composting and increase the nitrogen content in the waste to improve the microbiological degradation is actively investigated (Vargas-Garcia et al., 2007; He et al., 2008; Li et al., 2011).

2. MATERIALS AND METHODS

2.1.1 Sample Collection:

In the present study, isolation of microorganisms were done from soil/root sample, pond water and ETP sludge namely, Cyanobacteria (blue-green Algae), Rhizobium sp. and Aspergillus sp. The leguminous roots were dipped in Ethanol for few minutes and then wiped with tissue paper to remove any foreign particles. Microbes were isolated in Buffered Peptone Water and BG11 broth. Serial dilution technique was performed from 10^{-1} – 10^{-7} dilution to isolate the micro-organisms. Pour plate Method was done in Differential Media namely Nutrient Agar for Cyanobacteria sp. and Aspergillus sp. Sprinkle method and pour plate method was performed to isolate the Rhizobium sp. from roots and soil sample respectively. Pure culture was isolated in Soya bean Casein Digest Agar/ Nutrient Agar for Rhizobium sp., Cyano-Agar for isolation of Cyanobacteria and Sabouraud Dextrose Agar for Aspergillus sp. Further microscopically observation was done for enumeration of morphology of the colonies. Isolated pure cultures were preserved on freshly prepared slant. Shredded sugarcane bagasse was collected from sugar manufacturing industry for the preparation of Bio-compost. Plant seeds were collected from vendor.

2.2.2 Sample Preparation:

The isolated microorganisms preserved in slant were further processed for Microbial Consortium in double stranded Soya bean Casein Digest Medium. The bagasse was laid on the plot scale trial in Windrows. The Microbial Consortium was diluted with sugarcane treated effluent water to check controlled growth of Microbes and to prevent the growth of any pathogenic micro-organisms such as Escherichia coli sp., Salmonella sp., Pseudomonas sp., and Staphylococcus sp. Windrow was sprayed by the diluted Microbial Consortium and tumbling was done by Aero tiller. The windrow was turned on a

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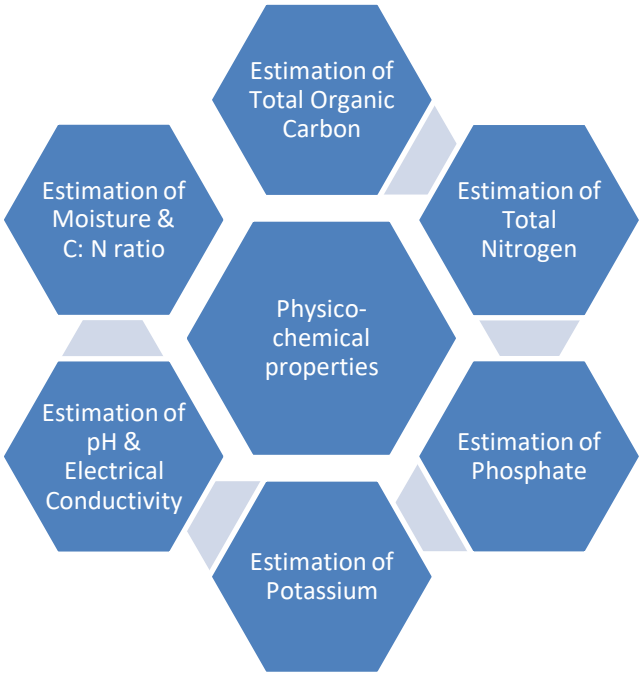
regular basis to improve the oxygen content, distribute heat to regulate temperature and to distribute moisture equally in the windrow.

Methodology for Microbiological Analysis:-

- Enumeration of Total Bacterial Count and Total Fungal Count as per IS 5402:2012, RA 2018
  - Homogenized the sample manually then took 10g of the sample in 90 ml diluents (0.1 % Buffered Peptone Water) to make initial dilution (1: 10).
  - Transferred 1 ml of the above stock to 9 ml of the diluents making it to 10^{-2} dilution repeated same procedure up-to 10^{-7} dilution.
  - Transferred 1 ml from each dilution to sterile Petri plate.
  - Poured about 15-20 ml of melted Nutrient Agar media, Cyano-Agar & Sabouraud Dextrose Agar into the Petri plates.
  - Mixed the inoculums with media by gentle rotation and allowed to solidify.
  - Incubated the plates in inverted position at 37º C for 24 – 48 hours for bacterial isolation & 25˚C for fungus.
  - Recorded the observation of the appeared colonies in the petri plates.
  - Counted the number of colonies in the range of 10-100 colony forming units by using Quebec Colony Counter and report in Cfu/g.

- Methodology for Physico-chemical analysis:
  - Choice of Solvents: Water & Ethanol were used as Solvents for determination of the Physico chemical properties of the sugar-cane press-mud.
  - Different tests for Physico-chemical properties:



3. Results and Discussions:

The prepared bio-compost was analysed microbiologically to determine Total Viable Count, Cfu/g including TBC (Total Bacterial Count) & TYMC (Total Yeast and Mould Count) and Pathogens were also tested to

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determine the biological properties of the sugar-cane press-mud. The plants were observed to grow well and yield in the plants were also observed to be quiet productive.

A. Microbiological analysis results of Sugarcane press-mud:-

S. No.	Microbiological parameters	Obtained Values "0" day	"15" day	"30" day	"45" day	Specified/ Desired Limits (As per FCO)	Comments
01	Total Bacterial Count, cfu/g	25 x10^7	45 x 10^7	46 x 10^7	80 x 10^7	NLT 50 X 10^7	Complies with the limits mentioned in FCO
02	Yeast & Mould Count, cfu/g	10 x 10^7	70 x 10^7	55 x 10^7	60x 10^7		
03	Escherichia coli/25g	Absent	Absent	Absent	Absent	Absent	Complies
04	Pseudomonas aeruginosa/25g	Absent	Absent	Absent	Absent	Absent	Complies
05	Staphylococcus aureus/25g	Absent	Absent	Absent	Absent	Absent	Complies
06	Salmonella/25g	Absent	Absent	Absent	Absent	Absent	Complies
07	Isolation and Identification of Bacteria	Cyanobacteria sp. and Rhizobacterium sp. Isolated & Identified	-	-	-	-	Isolated from the soil and pond water sample
08	Isolation and Identification of Fungus	Aspergillus sp. Isolated & Identified	-	-	-	-	Isolated from waste sample

The prepared bio-compost was analysed to determine the physico-chemical properties were experimented to determine the chemical properties of the manure up-to 45 days at different time intervals i.e. 0, 15, 30 & 45 days finally. Temperature was monitored in regular intervals to understand the metabolic activity in the press-mud.

B. Physico-chemical analysis results of Sugarcane press-mud:-

S. No.	Physicochemical parameters	Obtained Values				Specified/ Desired Limits (As per FCO)	Comments
A	Physicochemical Analysis	"0" day	"15"day	"30" day	"45" day		
01	pH	7.0	8.0	7.6	7.3	6.5-7.5	Complies with specified values
02	Moisture, % by mass	65.3	48.7	14.2	8.7	25% Max	Complies with specified values
03	Total Organic Carbon, % by mass	39.8	39.5	39.2	38.0	14 % Min.	Complies with specified values

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04	Total Nitrogen, % by mass	1.9	2.1	2.3	2.5	1.2 Min	Complies with specified values
05	Organic C/N ratio	20.9	16.3	16.2	15.2	<20	Complies with specified values
06	Total Phosphates as $P_2 0_5$, % by mass	0.014	0.231	1.01	1.5	1.2 Min	Complies with specified values
07	Total Potash as K_2O, % by mass	0.31	0.32	0.91	1.2	1.2 Min	Complies with specified values

C. Monitoring of Temperature of Sugar-cane press-mud:-

S. No.	Press-mud Windrow	Obtained Values "0" day	"10" day	"15" day	"20" day	"30" day	"35" day	"40" day	"45" day
01	Temperature observed	55ºC	62ºC	68ºC	70ºC	68º C	70º C	72º C	69º C
02	Aero tilling	Performed by Aero tiller	-	Performed by Aero tiller	-	Performed by Aero tiller	-	Performed by Aero tiller	-

Fig. 1: Line Graph representing the Temperature of press-mud:

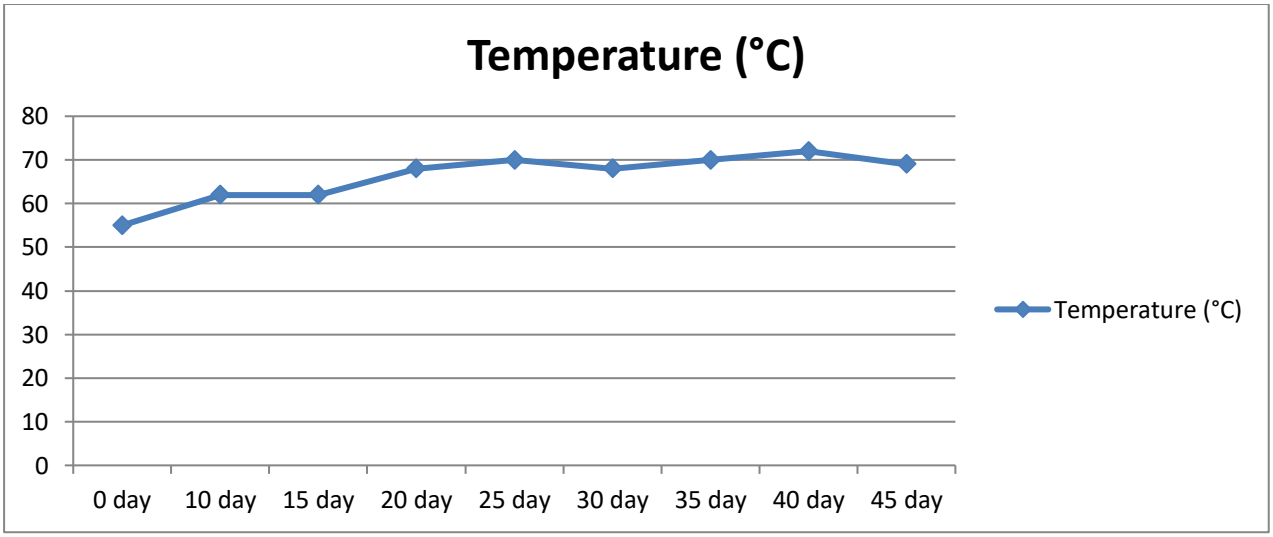

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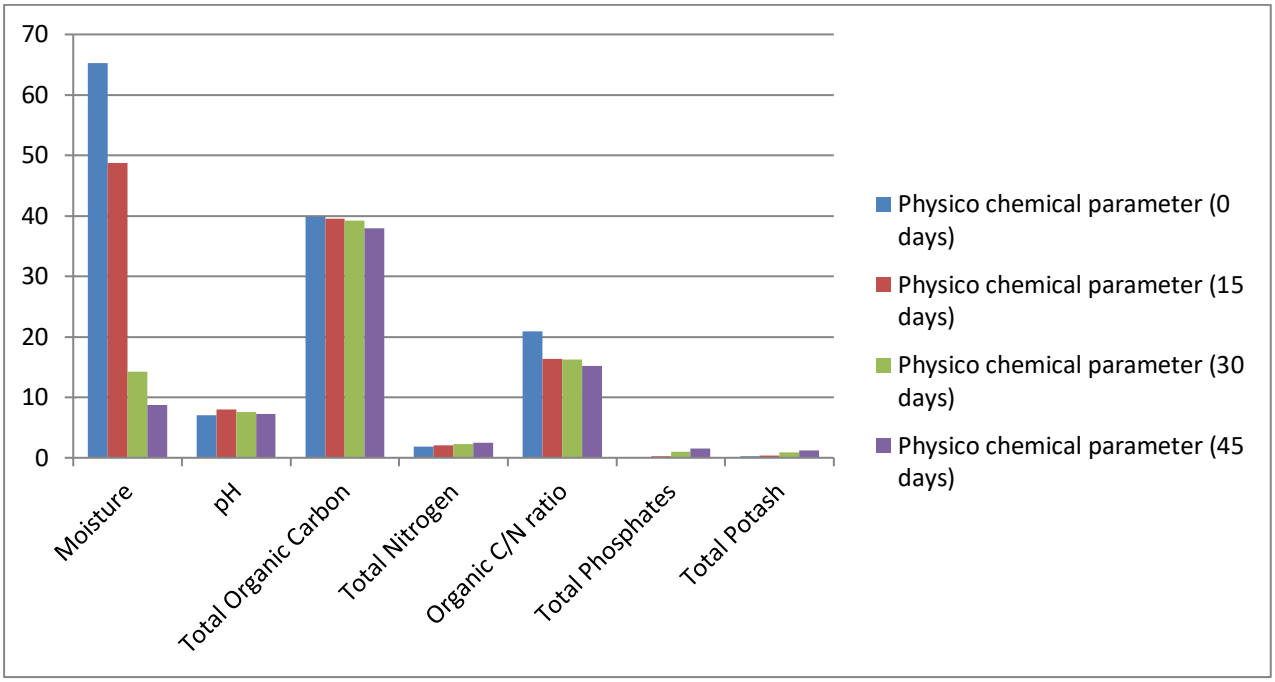
Fig.2: Bar Graph representing the Physico –chemical parameters:



Conclusion:-

After completion of 45 days, Pathogenic parameters were found to be absent in the press-mud and complied within the specified and desired range, as prescribed in Fertilizer Control Order for Organic manure. After completion of the 45 days, it was observed that the Total Bacterial Count increased from $25x10^7$ to 80 $x10^7$ and the Yeast and Mould count also increased from 10 $x10^7$ to 60 x 10^7 CFU/g as given in Table A. which signifies the conversion of press-mud samples into organic matter.

Physico- chemical parameters were found within the specified/ desired range within specific limits & optimal temperature given in table's B & C, as prescribed in Fertilizer Control Order for Organic Manure. The organic Carbon Nitrogen C/N ratio also reduced from 20.9 to 15.2 in completion of 45 days due to action of microbes inoculated in the form of Consortia.

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References:-

1. Goyal S.,Dhull, S.K. Kapoor, K.K.,2005. Chemical and biological changes during composting of different organic wastes and assessment of compost maturity. Bioresour. Technol.96(14),184-1591.

2. He, J., T. Jiang, D. Wang, J. Li and B. Lv, 2008. Effect of feeding efficient microbial community on aerobic composting ofmunicipal waste and excrement. Int. J. Biotechnology., 10:93-103.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved,** **UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022**

3. Hussain, I., Naser R., 2013. A Text Book on Environmental Biology. Discovery Publishing House Pvt. Ltd. New Delhi. 276-279

4. Kashif Khan, M. and Khalid, Monowar Alam, 2017. Treatment of Sugar Mill Effluent by Biocontrol Agents (Trichoderma harzianum and Pseudomonas fluorescens) and its effect on the Growth Parameters of ChickPea. Journal of Chemical and Pharmaceutical Research, 2017, 9(10): 117-124.

5. Li L.M.Ding, X.L., Qian, K. Ding and Yin, Z.J. 2011. Effect of microbial consortia on the composting of pig manure. J. Animal and Veterinary Advances 10(13): 1738-1742.

6. Manjula G., and T.Meenambal, 2012. Aerobic composting of organic fraction of Municipal solid waste and sugar mill waste. Poll Res. 31 (2): 229-223(2012).

7. Saliha, B.B., Balaji, T., Haroon, A.R.M.., 2011. Recycling composted wastes of sugar industry to enrich soil fertility, Proceedings of the Ninety eight session of the Indian Science Congress, Chennai.

8. Singh, J., Prasad, R., Kalamdhad, A.S., 2013. Effect of natural zeolite on bio-availability and leachability of heavy metals during rotary drum composting of green wastes. Res. J. Chem. Environ. 17.26-34.

9. Tripetchkul, S., Pundee, K., Koonrisik, S., & Akeprathumchai, S.2012. Co-composting of coir pith and cow manure, initial C/N ratio Vs physicochemical changes. International Journal of Recycling of Organic Waste in Agriculture, I (15). http://dx.doi.org/10.1186/2251-7715-1-15.

10. Vargas-Gracia, M.C., Surez-Estrella, F.F., Lopez, N.J., Moreno, J.,2010. Microbial population dynamics and enzyme activities in composting processes with different starting materials. Waste Manage. 30(5), 771-778.

11. Wang, Q., Li, R, Caia, H., Awasthi, M.K., Zhang, Z., Wang, J.J., Ali, A, Amanullah, M., 2016. Improving pig manure composting efficieny employing Ca-Bentonite. Ecol. Eng. 87, 157-161.

12. Wei, L., Shutao. W., Jin. Z., Tong, X., 2014. Biochar influences the microbial community structure during tomato stalk composting with chicken manure. Bioresour. Technol. 154. 148-154.

13. Wong, J.W.C., Fung, S.O., Selvam, A., 2009. Coal fly ash and lime addition enhances the rate and efficiency of decomposition of food waste during composting. Bio-resource. Technol. 100, 3324-331.

14. Awasthi, M.K., Pandey, A.K., Bundela, P.S., Wong, W.C. Li, R., Zhang, Z., 2016. Composting of gelatine industry sludge combined with organic fraction of municipal solid waste and poultry waste emploting zeolite mixed with enriched nitrifying bacterial consortium. Bioresour. Technol. 213, 181-189.

15. Chan, M.T., Selvam A., Wong, J.W.C.,2016. Reducing nitrogen loss and salinity of “struvite” food waste composting by zeolite amendment. Biresour. Technol. 200, 838-844.

16. Gautam S.P., Bundela, P.S., Kapoor. A., Aswathi, M.K., Sarsaiya, S., 2010. Analysis of a gelatine factory farm product (Wheat). Aust.J.Appl.Sci. 4, 4972-4795.

17. Harrison, I. A. 2014. Managing physico chemical parameter in compost systems to enhance degradation of petroleum waste from a sludge dam. African Journal of Biotechnology. Vol. 13(7), pp. 857-865.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved,** **UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022**

18. Huet, J.,Druilhe, C., Tremier, E., Benoist, J.C., Debene st, G., 2012. The impact of compaction, moisture content, particle size and type of bulking agent on initial physical properties of sludge-bulking agent mixtures before composting. Bioresour. Technol. 114, 428-436.

19. Itelima, J.U., Ogbonna, A.I., Onyimba, I.A., Nwadiaro, P.O., Nyam, M.A and Agbala, C.C.2015. Growth and yield response of cowpea (Vignaunguiculata) to Bio-fertilizers produced from Aspergillus niger and Animal Waste materials. > 10, 47-51.

20. Karak T., Sonar, I., Paul, R.K., Das, S., Boruah, R.K., Dutta, A.K., Das, D.K., 2014. Composting of cow dung and crop residues using termite mounds as bulking agent. Bioresour. Technol. 169, 731-741.