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ZERO WASTE MANAGEMENT FOR INDUSTRY BY PRODUCTION OF LIGNIN BIOFERTILIZERS

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ABSTRACT Lignin is a by product from Kraft and sulphite pulping industry. It is a complex biopolymer obtained after cellulose fibre extraction from plants. Effective treatment of this lignin is essential for reducing the pollution of water bodies. In the present study, the alkalophilic NaOH effluent from an industry was nullified using acid, HCL, treatment. The effluent was treated with fresh strains of, Bacillus subtilis and Trichoderma spp. The efficiency and quality of strains confirmed using plate count. The fertilizer incubated for 14 days and applied to Vigna unguiculata seeds. Biometric evaluations were performed and the yield has found to be increased compared to control and absolute control. This potential treatment of chemical slurry can be compensated for lignite and peat base of phosphate solubilizers, potassium and nitrogen fixers. The GC-MS analysis of the formulated fertilizer detected the presence of components like dineopentylesters which stimulate its biocontrol activity. Plant growth promoting factors like macro nutrients and micro nutrients confirmed by total Kjeldahl digests, UV-Visible Spectrophotometry and Flame Photometer.

KEYWORDS : Industry effluent, Bacillus-Trichoderma, HCl-NaOH, Vigna unguiculata.

Introduction

Lignin spent discharged from pulp and paper industry is a severe turmoil in aquatic systems. The colourful solution when released to fresh water bodies ceases the photolysis in water bodies creating foul smell and toxicity to different levels of food chain [1]. According to current estimate, by 2025 an average of 1.8 billion populations will adversely face scarcity of drinking water [2]. The significance of this alarming situation can be resolved only through proper recycling of recalcitrant lignin lodged to water. Incineration of dry leaves and other plant waste do create long term respiratory disorders. The tropical compound from leaf smoke called benzo(a)pyrene is accountable for this toxicity [3].

Lignin bio fertilizers developed from cosmetic products having antibacterial property is currently available [4]. The formula for utilizing sludge from paper factories to agriculture bio fertilizer is a novel approach in horticulture development. Kraft paper production from dry leaves is an historical approach of waste management during the time war due to shortage of paper pulp [5]. These papers obtained are converted to artefacts for ornamental purposes. The application of 1ml liquid bio fertilizer is equal to 1 kg of 5 month old carrier based bio fertilizer [6]. When the powder formulations were used for the management of plant diseases in horticultural and plantations crops through micro-irrigation techniques, the concerns are raised on blockage of nozzles and distribution of bio-inoculants. In addition, the process of bio-hardening in horticultural plantlets and tissue culture plants, necessitates the development of liquid-based bio-formulations. In addition, it has been demonstrated that the development of liquid formulation has several advantages including high cell count, zero contamination, longer shelf life, greater protection against environmental stresses and increased field efficacy [7][8].

Utilization of plant extract and biomass is another environment friendly way of managing the disease as a source of natural pesticides. Plants are store house of biochemicals that contribute in suppressing phytopathogens [9]. These biochemicals (nitrogen-containing compounds and phenolics) function as a defense and chemical signal molecule against pathogens[10].The present study overlays on the effluent so obtained which is formulated to liquid bio fertilizer on treatment with exponential phase strains of Bacillus subtilis and *Trichoderma* spp which enhances yield of the crop variety. The GC-MS analysis of bio fertilizer developed contain different phases of minerals, compounds and photochemicals that promote growth , insect resistance to plant *Vigna unguiculata.*

Materials and Methods

2.1 Collection of Spent

The paper waste effluent was collected from Dhanalakshmi Paper Mills, Dindigul. The spent was filtered and sterilized at 121◦C for 15 minutes. The alkaline nature of spent nullified by 0.1 N Hcl.

2.2 Preparation of inoculums

Bacillus and *Trichoderma* was revived from *Vigna unguiculata* cultivating fields by spread plate technique. Viability of strains checked every 7 Days. The isolated colony of *Bacillus* transferred to 250 ml Nutrient Broth (Peptic digest of animal tissue 5.0 g/l Sodium chloride 5.0 g/l Beef extract 1.5 g/l) and incubated at (27-30) ◦C for 24 hours. *Trichoderma* is sub cultured in Sabouraud Dextrose Broth (Dextrose: 40 g/l and Mycological Peptone: 10 g/l) and incubated at 25◦C for 15 days. The biomass of bacterial inoculums obtained by fermentation for 14 days at 35◦C. The fungal culture fermented every month.

2.3 Development of Bio fertilizer

Around 250 ml of spent mixed with 50 ml of *Trichoderma* and 100 ml *Bacillus culture.* Add 50 ml plain nutrient broth to the mixture with 50 ml distilled water. The bio fertilizer so formed is fermented for 45 days with viability check at every 7^{th} day.

2.4 GCMS analysis of Bio fertilizer

The fertilizer and the paper waste effluent were extracted using dichloromethane and hexane in ratio 2:1 in soxhlet apparatus. GC-MS instrument specifications include Shimadzu GC-MS, Model Number: QP2010S. Column: Rxi-5Sil MS, 30 meter length, 0.25 mm ID, 0.25 µm thickness. GCMS Software: GCMS Solutions and libraries used are NIST 11 & WILEY 8. The instrument was designed with following specifications, Column Oven Temp. : 60.0 °C Injection Temp. :260.00 °C Injection Mode :Splitless Sampling Time :2.00 min Flow Control Mode :Linear Velocity Pressure :57.4 kPa Total Flow :54.0 mL/min Column Flow :1.00 mL/min Linear Velocity :36.5 cm/sec Purge Flow :3.0 mL/min Split Ratio :50.0 Oven Temp. Program Rate Temperature (°C) Hold Time (min) - 60.0 0.00 5.00 280.0 5.00[GCMS-QP2010] IonSourceTemp : 200.00 °C Interface Temp. :280.00 °C Solvent Cut Time :5.00 min Detector Gain Mode :Relative Detector Gain :0.70 kV

+0.00 kV Threshold :1000 [MS Table].

2.5 Estimation of Macronutrients in Fertilizer

The macronutrients in the fertilizer such as nitrogen, potassium and phosphorous were identified and reported using Microkjeldhal distillation [11][12], Flame photometry[14] and UV Visible Spectrophotometer[15].

2.6 Statistical Analysis

The biofertilizer was applied as stages to *Vigna unguiculata* seeds in form of seed treatment, followed by seedling dip, and ultimately soil and foliar application. The germination index of Vigna unguiculata seeds calculated immediately after addition of fertilizer to soils before ploughing in the ratio of 400 ml / acre. A comparative study is done with market available organic fertilizer. Treatment I contain *Vigna unguiculata* plants treated with developed fertilizer. Treatment II contains organic fertilizer *(Rhizobium) treated Vigna unguiculata* plants. The efficiency is tested depending on biometric parameters like plant height, number of leaves, number of fruits and yield per plant. The absolute control plants were maintained without any fertilizer application. Analysis of varience was done on the data collected using the statistical package MSTAT [17]. Multiple comparisons among the treatment means were done using DMRT.

Result and Discussion

In case of liquid formulation with *Bacillus subtilis* and *Tricoderma* spp., there is an increase in crop yield of treatment plants compared to absolute control without any bio inoculant, chemical nitrogen or farm yard manure. The observations were taken during harvesting stage and during 60 to 90 days after sowing. The Gas Chromatography-Mass Spectrophotometry analysis of paper effluent indicated components shown in Figure 1 such as 3-Methyl-but-2-enoic acid-26.98%, 1,7-octadien -3-ol-1.65%, oxirane-0.62% ,(1S,3R,4R)-3-(hydroxymethyl)-2-methyl- 2- azabicyclo[2.2.1] heptanes- 1.97% and 0.68% 1H-Imidazole-2,4,5-D3 which act as potential bio control against plant pathogens like *Fusarium*. The other main components

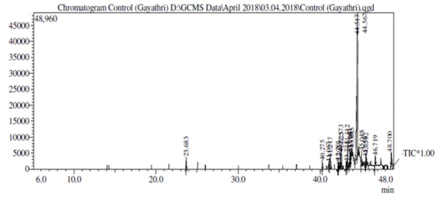

Figure 1: GCMS Chromatogram of Paper Effluent

N– Allyloxymethylacrylamide - 0.65%, 2,4-dimethylvaleronitrile-3.54%, 4-Chloro-3-Hydroxy-1-Butene-1.08%, (S)-4-Iodo-1,2-Epoxybutane-2.12% and 1-(2-hydroxyethoxy) Tridecane-3.73% act as source of nitrogen, carbon and micronutrient such as sulphur in bio fertilizer. Similar data was obtained for bio fertilizers with additional components as shown in figure 2 like Dichlorodioxo-, (T-4)- 3.26%, Docosane-5.63 %, N-(1,1,3,3 Tetramethylbutyl) Bis (Trifluromethyl)Sulfin-imide- 8.54%, 1,3,7,11-Tridecatetrene-1,1-D2, 4,8,12-Trimethyl-, (E,E)- 9.99% and 1,5-Heptadiene-3,4-diol-3.71% which are volatile

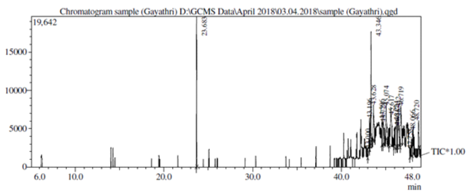

Figure 2: GCMS chromatogram of Biofertilizer

Residues functioning as soil additive, micronutrient source, pH equilibrant and post harvest preservative of fertilizer.

Table 1: Macronutrients Present in Biofertilizers and Paper Effluent

Sample Name	Microkjeldahl (Nitrogen %)	Flame Photometry (Potassium %)	UV-Photometry (Phosphorous %)
Paper Effluent	49	12	37
Bio fertilizer	63	26	46

Table 2: Effect of Fertilizer on Plant height, Number of Leaves and Number of Fruits of *Vigna unguiculata*

Treatment	Plant Height (DAP)			No of leaves (DAP)			No of fruits (DAP)		
	30	60	90	30	60	90	30	60	90
T_1(Biofertilizer)	60.40^{c}	155.4^{d}	201.0^{b}	19.0^{c}	42.6^{b}	74.0^{c}	9.0^{bc}	29.4^{b}	55.6^{b}
T_2(Paper Effluent)	45.40^{de}	109.2^{f}	151.8^{d}	8.2^{f}	29.2^{e}	63.8^{e}	4.40^{c}	20.4^{d}	45.6^{e}
T_3(Rhizobium formulation)	56.20^{c}	124.8^{e}	181.4^{e}	17.4^{d}	35.2^{d}	67.2^{d}	7.60^{bc}	26.4^{c}	49.4^{d}
T_4 (Absolute Control)	41.50^{e}	102.8^{g}	141.8^{e}	12.2^{e}	25.4^{f}	59.2^{f}	5.4^{c}	19.4^{d}	38.0^{f}
T_5(T1+ 75%NPK)	54.20^{cd}	167.0^{c}	202.6^{b}	20.2^{bc}	39.2^{c}	76.6^{b}	11.4^{ab}	29.4^{b}	51.0^{c}
T_6(T1+50%FYM)	74.60^{b}	175.2^{b}	202.4^{b}	21.4^{b}	35.2^{d}	74.2^{c}	11.8^{ab}	29.4^{b}	51.2^{c}
T_7(T1+75%NPK+50%FYM)	85.00^{a}	195.4^{a}	216.8^{a}	26.0^{a}	46.2^{a}	79.2^{a}	14.8^{a}	35.8^{a}	59.2^{a}

Table 3: Effect of fertilizer on yield per treatment in kilogram

Treatment	T1	T2	T3	T4	T5	T6	T7
Yield(kg)	7.83	5.86	7.19	4.77	7.65	8.36	11.23

Biometric characters were observed and recorded 30,60 and 90 Days after Planting(DAP). The data in table 2 prove that microbial inoculants containing paper effluent had significant effect on growth of Vigna unguiculata compared to absolute control. Seeds treated with Rhizobium formulation triggered faster germination compared to all other treatment. The T7 formulation performed better than all treatment combination. In general, from table 3, the application of biofertilizer along with 75% N+ 50 % FYM recorded significantly higher yield per plant then fertilizer alone. Several ash fertilizers are generated from paper and pulp industry waste [18]. Here we have generated maximum yield from the liquid spent of paper and pulp industry waste as reported so far.

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