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Upgrading Manure Value of Fermentation Effluent in Combination with Tendu Waste

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Abstract: Organic matter is a valuable resource on which the sustainability and productivity of soils relies heavily. Soil amendment with nutrient rich fermentation effluents after suitable dilution is currently being practiced. However this requires large amount of water for dilution and bulk volume transportation. Nutrient stripping using a solid biodegradable waste was attempted in this study. Organic matter rich effluent from a fermentation based drug industry was subjected to adsorption by tendu (*Diospyros melanoxylon*) leaves waste from local cigarette industry. The nutrient enriched tendu waste was evaluated for phytotoxic effect in pot culture experiments as a soil amendment (0 to 200 t ha^{-1}) on the growth of mungbean. Total plant dry matter, chlorophyll, soluble proteins, peroxidase and superoxide dismutase were measured as indicators of phytotoxicity. Total dry matter, chlorophyll and soluble protein levels did not show significant changes whereas a significant decrease in peroxidase activity ($P<0.001$) and increase in superoxide dismutase ($P<0.001$) at 200 t ha^{-1} application level was observed. This suggests that the plant's defense system against reactive oxygen species is active in unstressed condition as well. The enriched tendu biomass is well tolerated under experimental conditions by mungbean and can be used as bio-manure up to 100 t ha^{-1} application level.

Key words: Organic nutrient stripping % Fermentation effluent % Tendu waste

INTRODUCTION

Organic wastes when applied correctly to soil have beneficial fertilizing effect, resulting in cost savings and reduced inorganic fertilizer requirement. It also includes environmental benefits like soil conditioning and indirectly resource conservation. Nitrogen and other nutrients are mostly bound to organic matter and are not immediately available, thus in effect it acts as a slow-release fertilizer. Alternatives for disposing of effluents rich in organic matter are currently being sought as a valuable resource for soil amendments [1, 2]. Molasses fermentation effluents and post biomethanation effluents rich source of potassium besides high concentration of organic pollutants and cannot be discharged directly. They were shown to be safe for fert-irrigation using 10 to 50% dilutions with irrigation water [3]. However practical difficulties in handling bulk volume in liquid form and monitoring of proper dilution rendered such organic rich effluent unacceptable to the farmers, besides its obnoxious odor [4].

A synergetic waste disposal solution was sought using a biodegradable solid waste as a biosorbent to strip nutrients from fermentation effluent. Tendu (*Diospyros melanoxylon*) leaves refuse; a solid waste from local crude cigarette (called *Bidi*) industry has no direct application except landfill. It is available in large quantities and was shown to remove COD from a molasses fermentation effluent of a bulk drug industry [5]. In present study, tendu leaves refuse, after exhaustive biosorption of nutrients from a molasses fermentation effluent was dried and characterized for its fertilizer value. The use of higher plants in environmental risk assessment has recently gained importance [6]. *Phaseolus aureus* (mungbean), widely cultivated bean in temperate region was used for phytotoxicological assessment of soil amended with spent tendu leaves refuse biomass as a bio-manure. Recycling of nutrients from waste are generally in line with sustainable development as it helps to increase crop production, maintain the organic pool in soils and reduces use of mineral fertilizers besides offering eco-friendly waste disposal technologies.

MATERIALS AND METHODS

Effluent and Tendu Waste: Experiments were conducted on untreated effluent obtained from a drug unit manufacturing ephedrine hydrochloride by biotransformation of benzaldehyde using molasses based fermentation method. The tendu leaves refuse (TLR) were

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obtained from the dumping sites near *bidi* industries in the town of Solapur, India. They were cut in to small pieces and were thoroughly washed with distilled water to remove dirt and were dried at 80°C till constant weight.

Nutrient Stripping on Tendu Waste: The raw effluent was treated with TLR, by adding 2 kg of TLR to 10 L of effluent, wherein TLR swelled and filled entire volume. It was kept overnight at room temperature with intermittent stirring to complete adsorption. The TLR enriched with organic matter from effluent was separated by filtration through cheese cloth. The spent TLR after above biosorption process was air dried, powdered, passed through a 2 mm sieve and used in further experiments as spent tendu leaves refuse (STLR). The fertilizer nutrient contents of STLR were analyzed using standard methods [7]. The soil was passed through a 2-mm sieve to discard non-soil particles. Various properties of the soil used in the greenhouse study were measured using standard methods. Three STLR application rates were used in the greenhouse study. Each was applied on a dry weight basis to achieve mixtures with soil in a proportion of 0, 1, 5 and 10% (w/w) of STLR simulating field applications of biomass equivalent to 0, 20, 100 and 200 t /ha. The mixed soil samples were left for 4 weeks to allow for mineralization before sowing the seeds.

Pot Culture Experiments: Eight seeds of mungbean, (*Phaseolus aureus* Roxb.) were sown in plastic pots of 10 cm diameter containing 400 g soil with and without STLR amendments. Treatments were replicated four times and arranged in randomized complete block design in a greenhouse and watered every third day with deionized water to avoid input of any additional nutrients and minimize any chance of leaching. The plants were harvested 45 days after emergence and were dried at 55EC for 3 days and weighed to determine dry matter yield. Concentrations of chlorophyll were determined in 80% acetone extract of leaf using the method described by Lichtenthaler and Wellburn [8].

Data Collection: The extracts used in the determination of superoxide dismutase, peroxidase and total soluble protein analyses were prepared by homogenizing 0.5 g of frozen leaf material in 3 ml of cold solution containing 50 x 10^{-3} M phosphate buffer (pH 7.8), 1 x 10^{-3} M EDTA and 2% (w/v) PVPP. The homogenate was centrifuged at 0°C for 30 min at 13,000 rpm. Peroxidases (EC 1.11.1.7) activity (POX) was determined by the method of Herzog and Fahimi [9], using 1 mM 3, 3' diaminobenzidine tetrahydro-chloride as a substrate. The concentration of H_2O_2 in the reaction mixture was 1.3 mM. The kinetic of the reaction was monitored at 465nm. Activity was expressed as changes in optical density at 465nm per 1 min on a protein and fresh weight basis, respectively. Superoxide dismutase (EC 1.15.1.1) activity (SOD) was determined by measuring its ability to inhibit the photo reduction of nitro blue tetrazolium (NBT) according to the methods of Giannopolitis and Ries [10]. The reaction solution (3 ml) contained 50 µmol NBT, 1.3 µmol riboflavin, 13-mmol methionine, 75 mmol EDTA, 50 mmol phosphate buffer (pH 7.8) and 20 to 50 µL enzyme extract. The reaction solution was irradiated under fluorescent lights for 15 min. The absorption by the reaction mixture at 560 nm was measured. One enzyme unit was the volume of extract, which corresponds to 50% inhibition of the reaction carried out without addition of enzyme. Total soluble protein content in the enzyme extracts was determined using bovine serum albumin as standard [11]. One-way ANOVA was applied to each dilution, the significance of the difference between control and three treatments were assessed according to Tukey's multiple comparison test.

RESULTS AND DISCUSSION

Nutrient Stripping as Primary Treatment: Primary treatment of effluent from a molasses fermentation based bulk drug industry with tendu leaves refuse from bidi industry has resulted in 76% reduction in the volume of effluent. Comparison of various fertilizer parameters of TLR and STLR after being saturated contact with the fermentation effluent is shown in Table 1. The results indicate significant nutrient stripping from the effluent and its adsorption onto the solid waste biomass resulting in the significant enhancement of nitrogen, phosphorus, potassium and iron levels. The properties of soil used during pot culture experiment for soil amendment experiment are shown in Table 2. The results of five different growth parameters for mungbean grown in STLR amended soil was compared with reference soil are shown in Table 3.

Dry matter of the mungbean plants grown in control and three treatments did not show any statistically significant changes. There was no statistically significant changes in total chlorophyll content and soluble proteins, indicating unaltered photosynthesis and non-induction of stress-induced proteins. However, very important biochemical mechanism of plant reaction to different harmful changes in the environment is the defense

Table 1: Fertilizer Properties of TLR and STLR

Parameters	TLR	STLR	% Enrichment
PH	6.81	6.25	-
Organic carbon (%)	21.7	28.6	31.8
Total Nitrogen (%)	1.03	1.52	47.57
Phosphorus (%)	0.94	1.29	37.23
Potash (%)	0.52	1.85	255.77
Calcium (%)	1.06	1.45	36.79
Magnesium (%)	0.2	0.24	20
Iron (ppm)	34	101	197.06
Manganese (ppm)	27	35	29.63
Phenols (%)	ND	0.038	-

Values are mean of four replicates

Table 2: Properties of Soil used in the trial

Parameters	Value
pH	7.36
Electrical Conductivity (S/cm)	530
Texture	Clay loam
CaCO3 (%)	3.5
Organic Carbon (%)	1.4
Total Nitrogen (mg/100g)	23
Water Holding Capacity (%)	41.6
Total Salts (mg/100 g)	560

Values are means of four replicates

Table 3; Effect of STLR amendment mungbean growth

Treatment	Dry mass (%)	Total Chlor-ophyllmg g^{-1}	Proteinmg g^{-1}	POX(IU)	SOD(IU)
Control	18.068	4.253	133.78	0.1648	1.823
1% STLR	17.990 ns	4.428 ns	132.60 ns	0.1623 ns	1.968 ns
5% STLR	18.118 ns	4.254 ns	131.33 ns	0.1603 ns	1.983 ns
10% STLR	18.275 ns	4.383 ns	131.98 ns	0.1515 ***	2.667***

Values are means of four replicates, ***P<0.001; ns: non-significant

enzyme complex, including peroxidases and superoxide dismutase [12]. Leaf POX activity was significantly decreased at 10% soil treatment with STLR. Peroxidase is studied as an enzyme, reacting very fast with changes in its functional activity to external signals and extreme environmental situation [13]. SOD activity in leaves was significantly increased above 100 t ha^{-1} application level than control treatment suggesting that the plant's defense system against reactive oxygen species is active in unstressed conditions as well [14]. It is well known that SOD is an essential component of the defense mechanism in the plant cell against the reactive oxygen species produced as a consequence of many stress conditions [15, 16].

CONCLUSION

The study indicates effective stripping of nutrients from organic rich effluent onto a solid waste from bidi industry. The volume of effluent was significantly reduced for further disposal or dilution. The fertilizer value of solid tendu waste was enhanced and nutrients adsorbed from effluent were made available for transporting to distant locations as adsorbed onto the solid tendu waste. During the pot culture studies shows that STLR is well tolerated by mungbean, under present experimental condition, up to the 100 t ha^{-1} level as biomass fertilizer amendment. Applications of STLR based on agronomic principles such as fertility recommendations have the potential to increase yields, resulting in economic and environmental benefits. This study presents an environmentally sound use of a solid waste from one industry for primary treatment of organic rich effluent of another industry, generating a value added biofertilizer and reducing effluent volume and pollution load simultaneously.

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