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EVALUATION OF RICE MILL EFFLUENT BIOREMEDIATED BY POLYHYDROXYALKANOATE PRODUCTION ON THE GROWTH OF BROAD BEAN

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ABSTRACT

Treated parboiled rice mill effluent (TRME) was investigated for its utilization in agriculture for the purpose of irrigation. The effluent was treated by the fermentation process of the bacterium *Acinetobacter junii* BP25 (Genebank Accession no – KY072961) for the production of the biodegradable polymer polyhydroxyalkanoates (PHA). Pot culture experiments were conducted to study the effect of TRME on seed germination, chlorophyll content, total carbohydrate, protein and amino acids of broad bean (*Vicia faba,* Variety COGB 14) at different time intervals with varying effluent concentrations. TRME was acidic in nature (pH 5 ± 0.5), with comparatively lesser COD (650 ± 1.2 mg/L) and BOD (312 ± 1.6 mg/L) when compared with the effluent before the treatment process (COD- 6250 ± 2.2 mg/L; BOD- 620 ± 2.5 mg/L). The germination percentage of seed and the biochemical parameters showed a gradual decline with increase in effluent concentration. It has been observed that 10% (pH 7 ± 0.5) effluent concentration had positive impact ($P < 0.05$) on all the parameters of the plant up to 20 days. However, at higher concentrations of TRME, toxic effects were observed after 20th day. This suggests that the neutralized effluent can be used safely for broad bean cultivation, after proper dilution and treatment.

KEYWORDS: **Biodegradable polymer, treated parboiled rice mill effluent, germination percentage, broad bean, effluent concentration.**

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INTRODUCTION

Water is one of the vital life sustaining bio resource of our planet, which all living organisms rely on. World water resources are passing through a stage of transition. This transition is, perhaps, an aftermath of rapid economic development process, coupled with other human activities in various parts of the world.[1] Out of all the water on Earth, saline water in oceans, seas and saline groundwater make up about 97% of it. Only 2.5–2.75% is fresh water, including 1.75–2% frozen in glaciers, ice and snow, 0.5–0.75% as fresh groundwater and soil moisture, and less than 0.01% of it as surface water in lakes, swamps and rivers.[2] But the consumption rate in which we are utilizing this resource would soon lead to a critical condition leading to serious detoriation of nature. Population explosion and pollution have caused relentless damage to all the natural resources. As the process of agriculture requires a massive amount of water for the purpose of irrigation, the usage of effluents from different sectors shall be used as an alternative so as to overcome pollution problems and conserve the water for the upcoming generations. Irrigation with effluent can increase water supply for alternative uses. The utilization of the effluent can effectively prevent the water bodies and agricultural lands from getting polluted, as these are the places where the effluent is being discharged usually.. In addition to these direct economic benefits that conserve natural resources, water contains a lot of nutrients that can serve as an alternative source to chemical fertilizers which are expensive.[3] Direct usage of effluent for the growth of plants shall cause toxicity problems in the cultivated crop and a jeopardic change in the soil parameters and so on. So a careful evaluation on a long time scale is needed to implement an effluent for irrigational purpose. Primarily treatment of an effluent is needed to reduce the organic and inorganic load so that there would be no harm to the physiological or biochemical characters of the plant and the soil. Several studies were carried out with effluents from various industries like sugar mill effluent for the growth of green gram,[4] Nagajyothi *et al*.[5] have studied the effect of biomass power plant effluent on seed germination, seedling growth and chlorophyll content of green gram. Paper mill effluent[6], textile effluent[7] were studied for the growth of different plant varieties. Rice is one among the staple food widely used throughout the world and India ranks top in both the production and consumption of rice.[8] About 50% of the paddy processing is done by an age old technique called parboiling. This method basically involves the use of large quantity of water for soaking and steaming of the paddy, this process ensures an increased nutritive property to the rice.[9] The effluent from these processes are left untreated into the nearby water bodies or agricultural lands leading to long-standing environmental issues. Previous work done by Mahananda *et al*.[10] showed the utilization of untreated rice mill effluent for the growth and biochemical parameters of tomato. Hence the present study focuses on the utilization of parboiled rice mill effluent which is treated biologically through the production of Polyhydroxyalkanoates (PHA) by fermentation process for the growth of broad bean (*Vicia faba*) based on the biochemical and morphometric parameters with respect to different pH (5 ± 0.5 and 7 ± 0.5) conditions. PHA are a family of biopolyesters synthesized by many types of bacteria as carbon and energy reserve materials,[29] thus the biopolymer production helps in the treatment of the effluent effectively.

MATERIALS AND METHODS

Effluent and seed collection

Parboiled rice mill effluent (RME) from KDP Rice mill, Thiruvanamali district, Tamilnadu, India, having milling capacity 10T day^{-1} was collected in sterile containers and stored at 4°C until future use. Seeds of broad bean (*Vicia faba,* Variety COGB 14, kind LAB LAB) were collected from the Department of vegetable crops, Horticulture College and Research Institute, Tamil Nadu Agricultural University, Coimbatore.

Treatment of the effluent

The filtered, heat treated effluent was used as a cheap substrate for the production of PHA by *Acinetobacter junii* (Genebank Accession no – KY072961). After the recovery of all the bacterial cells for the extraction of PHA, the effluent was autoclaved, which serves as the treated rice mill effluent (TRME) used for further studies carried out for the growth of the plant.

Characterization of effluent

The physicochemical characteristics of the effluent like COD, BOD, TDS, TSS, Phosphate, sulphate etc,were analyzed before and after the treatment process, following the procedures recommended by American Public Health Association (1998).[11] The total carbohydrate and protein content was analyzed by anthrone method[12] and Lowry's method[13] respectively. TRME had a pH of 5 ± 0.5 after the process of fermentation. Hence the effects of acidic pH and neutral pH on the growth of the plant were studied with two different pHs, 5 ± 0.5 and 7 ± 0.5. The pH adjustments were done using 0.1M NaOH and HCl.

Pot culture experiments

Pots of same size were filled with equal amounts of sandy loam soil and 10 seeds of broad bean were sown in each pot. The pots were irrigated with selected concentrations (10%, 15%, 25%, 50%, 75% and 100%) of the TRME. For each treatment, 100ml of TRME was applied to the respective pot at 2 to 3 days interval, throughout the study period. Control sets, irrigated with distilled water with pH 5 and 7 for the corresponding sets of experiments were also maintained for comparison. All the experiments were done in triplicates. The pot culture of broad bean was analyzed for the seedling growth for the experimental period of 30 days.

Analysis and assays

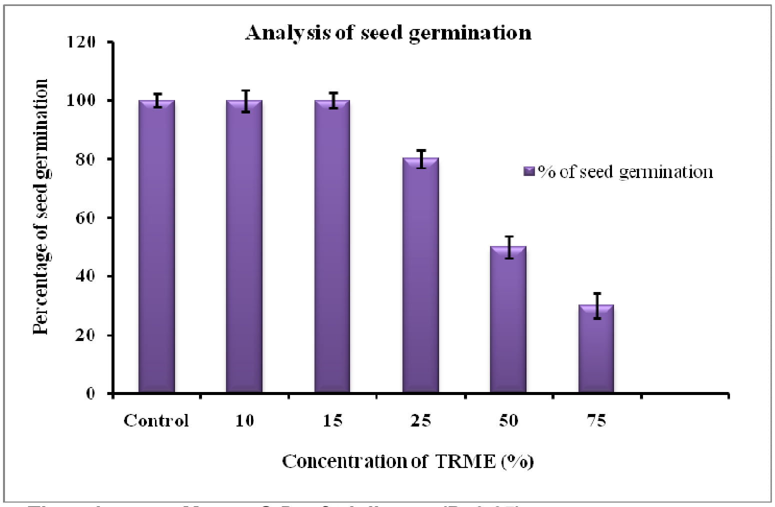
The germination percentage, shoot length, root length, chlorophyll, total carbohydrate, protein content and total free amino acids were all analyzed and estimated by standard methods.[14]

Statistical analysis of data

Analysis of variance (ANOVA) and Duncans multiple range test (DMRT) were carried out to test the significant variation between the treatment pots. All the statistical tests were carried out using the Statgraphics Plus Statistical Package version 6.0.

RESULTS

The physico-chemical characteristics of effluent before and after the treatment were given in Table1. The fermentative utilization of the effluent by the production of PHA has positively influenced the reduction of COD and BOD which helps in the effective ecofriendly utilization of the effluent. The effect of the TRME on the initial growth and germination of the broad bean seeds were studied on the 10^{th} day after the sowing of the seeds (Figure 1). In 10, 15 and 25% the percentage of seed germination was comparable with the control as all of them showed 100% germination. With increase in effluent concentration, the germination rate decreased and at 100% effluent concentration, the seeds did not even showed signs of germination even after 10 days. The influence of TRME on the shoot and root length of the plant was elucidated in Table 2. Compared to control, the seeds have exhibited significant and appreciable length at 10% TRME (P < 0.05), at 15 and 25% it was considerable, but with 75% the length of the plant was only negligible. The data about the chlorophyll content with varied concentration of TRME at different time intervals were represented in Tables 3. At 10% of TRME, chlorophyll a and b contents were higher than the control at both the pH up to 20th day and decreased there onwards in the broad bean (P < 0.05). At 75% effluent concentration, overall decrease in chlorophyll content was recorded at all days as compared to control. The total carbohydrate present in the control and test plants was given in Table 4. Up to 25 days there was a constant increase of carbohydrate and at 30^{th} day it exhibited a sharp decrease. 10% of TRME supported the high and significant level of carbohydrate, protein and amino acids (Table 5 and 6) in both root and shoot than that of the control (P < 0.05). 75% of TRME could not aid much to the growth and other biochemical parameters of the plant showing only limited results.



The values are Mean ± S.D. of triplicates (P<0.05)

Figure 1
Effect of TRME on the germination of broad bean seeds

Table 1
Physico-chemical characteristics of effluent before and after treatment

S.No	Parameter	Effluent before treatment	Effluent after treatment
1	pH	6.5±8.0	5.0 ± 0.5
2	Colour	Phenolic yellow	Pale yellow
3	COD	6250 ± 2.2 mg/L	650 ± 1.2 mg/L
4	BOD	620 ± 2.5 mg/L	312 ± 1.6 mg/L
5	TDS	970 ± 1.6 mg/L	95 ± 1.9 mg/L
6	TSS	790 ± 2.1 mg/L	110 ± 1.5 mg/L
7	Sulphate	40 ± 1.2 mg/L	15 ± 1.09 mg/L
8	Phosphate	21 ± 1.5 mg/L	5 ± 0.27 mg/L
9	Nitrate	0.5 ± 0.09 mg/L	0.08 ± 0.15 mg/L
10	Chloride	140 ± 0.15 mg/L	80 ± 0.23 mg/L
11	Total phenols	35 ± 0.11 mg/L	12 ± 0.12 mg/L
12	DO	0.9 ± 0.10 mg/L	0.04 ± 0.02 mg/L
13	Turbidity	457 ± 2.3 NTU	255 ± 3.2 NTU
14	Carbon	0.53 ± 0.10%	0.02 ± 0.01%
15	Nitrogen	0.31 ± 0.02%	0.04 ± 0.03%
16	Hydrogen	7.89 ± 0.01%	1.2 ± 0.05%

The values are Mean ± S.D. of triplicates (P<0.05)

COD- Chemical oxygen demand; BOD- Biological oxygen demand; TDS- Total dissolved solids; TSS- Total suspended solids; DO- Dissolved oxygen; mg/L- Milligram per litre; NTU- Nephelometric Turbidity Units

Table 2
Morphometric parameters of broad bean at different time intervals exposed to TRME (pH- 5 ± 0.5 and 7 ± 0.5)

Treatment %	10days				15days				20days				25days				30days			
	Root		Shoot		Root		Shoot		Root		Shoot		Root		Shoot		Root		Shoot	
	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7
Control	5.1 ± 0.06^{c}	5.5 ± 0.03^{c}	24.1 ± 0.11^{d}	26.7 ± 0.12^{d}	6.2 ± 0.05^{d}	6.3 ± 0.56^{c}	28.7 ± 0.21^{c}	38.2 ± 0.25^{c}	6.9 ± 0.05^{c}	6.8 ± 0.04^{b}	35.2 ± 0.26^{c}	40.9 ± 0.14^{c}	8.2 ± 0.02^{c}	8.9 ± 0.26^{c}	42.9 ± 0.39^{bc}	50.3 ± 0.22^{c}	10.4 ± 0.05^{bc}	10.9 ± 0.41^{c}	60.5 ± 0.46^{c}	62.3 ± 0.32^{c}
10	5.5 ± 0.02^{d}	5.9 ± 0.05^{d}	26.9 ± 0.21^{e}	28.7 ± 0.11^{e}	5.9 ± 0.02^{c}	6.4 ± 0.02^{c}	31.4 ± 0.12^{e}	39.6 ± 0.14^{d}	7.4 ± 0.06^{e}	7.6 ± 0.05^{d}	38.5 ± 0.21^{d}	42.5 ± 0.10^{d}	8.8 ± 0.05^{e}	9.2 ± 0.05^{d}	44.6 ± 0.21^{d}	56.7 ± 0.42^{d}	10.9 ± 0.05^{c}	11.2 ± 0.06^{d}	62.5 ± 0.61^{d}	65.6 ± 0.22^{d}
15	5.4 ± 0.01^{d}	5.5 ± 0.02^{c}	24.4 ± 0.32^{d}	26.4 ± 0.31^{d}	5.8 ± 0.06^{c}	6.4 ± 0.05^{c}	30.2 ± 0.19^{cd}	29.2 ± 0.16^{bc}	7.1 ± 0.05^{d}	7.1 ± 0.08^{c}	36.4 ± 0.49^{c}	34.6 ± 0.11^{b}	8.4 ± 0.12^{cd}	8.4 ± 0.12^{bc}	43.2 ± 0.26^{c}	51.4 ± 0.32^{c}	10.7 ± 0.11^{c}	10.8 ± 0.11^{c}	60.9 ± 0.72^{c}	62.7 ± 0.41^{c}
25	4.2 ± 0.06^{b}	4.9 ± 0.06^{bc}	20.1 ± 0.46^{c}	24.6 ± 0.19^{c}	4.8 ± 0.02^{b}	5.3 ± 0.06^{b}	22.1 ± 0.31^{b}	25.7 ± 0.12^{b}	5.4 ± 0.07^{b}	6.5 ± 0.05^{ab}	30.1 ± 0.56^{b}	33 ± 0.36^{ab}	5.9 ± 0.11^{b}	7.2 ± 0.41^{b}	36.4 ± 0.31^{b}	36.1 ± 0.21^{b}	6.6 ± 0.05^{b}	7.6 ± 0.10^{b}	44.7 ± 0.16^{b}	47.6 ± 0.35^{b}
50	3.8 ± 0.09^{b}	4.1 ± 0.04^{b}	16.2 ± 0.51^{b}	18.7 ± 0.11^{b}	4.1 ± 0.05^{a}	4.9 ± 0.07^{a}	17.4 ± 0.26^{a}	20.4 ± 0.17^{a}	4.9 ± 0.06^{a}	5.3 ± 0.09^{a}	20.7 ± 0.27^{a}	24 ± 0.19^{a}	5.1 ± 0.10^{a}	6.4 ± 0.13^{a}	26.9 ± 0.41^{a}	30.2 ± 0.26^{a}	5.7 ± 0.02^{a}	7.1 ± 0.12^{a}	29.9 ± 0.21^{a}	37.6 ± 0.26^{a}
75	1.2 ± 0.08^{a}	1.8 ± 0.06^{a}	5.4± 0.10^{a}	11.2 ± 0.22^{a}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

a,b,c,d,e denote the significant differences within the values provided in the table, which were studied through Duncan's Multiple Range test. Each value is Mean ± SD of three replicates, means with different superscript letters within each column are
statistically significant (P < 0.05). '-' represents the non availability of plant specimen for analysis.

Table 3
Effect of TRME (pH- 5 ± 0.5 and 7 ± 0.5) on the chlorophyll content of broad bean

Treatment %	10days				15days				20days				25days					30days		
	Chlorophyll a		Chlorophyll b		Chlorophyll a		Chlorophyll b		Chlorophyll a		Chlorophyll b		Chlorophyll a		Chlorophyll b		Chlorophyll a		Chlorophyll b	
	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7
Control	1.67 ± 0.05^{d}	1.87 ± 0.14^{d}	0.80 ± 0.05^{d}	0.84 ± 0.12^{d}	2.72 ± 0.07^{bc}	2.82 ± 0.07^{d}	0.96 ± 0.04^{d}	0.97 ± 0.04^{d}	3.17 ± 0.05^{d}	3.37 ± 0.25^{d}	1.26 ± 0.05^{c}	1.36 ± 0.05^{d}	2.61 ± 0.07^{d}	0.86 ± 0.35^{c}	0.79 ± 0.05^{bc}	0.86 ± 0.35^{d}	1.27 ± 0.04^{c}	1.47 ± 0.11^{d}	0.56 ± 0.10^{c}	0.61 ± 0.10^{c}
10	1.78 ± 0.04^{d}	1.97 ± 0.06^{d}	0.79 ± 0.05^{c}	0.86 ± 0.01^{d}	2.81 ± 0.10^{c}	2.92 ± 0.09^{d}	0.99 ± 0.06^{d}	1.02 ± 0.04^{e}	3.26 ± 0.05^{d}	3.67 ± 0.10^{e}	1.41 ± 0.05^{d}	1.45 ± 0.10^{d}	2.81 ± 0.06^{e}	0.87 ± 0.11^{c}	0.82 ± 0.05^{c}	0.87 ± 0.11^{d}	1.40 ± 0.05^{d}	1.56 ± 0.07^{d}	0.58 ± 0.10^{c}	0.66 ± 0.27^{c}
15	1.15 ± 0.01^{c}	1.26 ± 0.06^{c}	0.61 ± 0.12^{c}	0.61 ± 0.11^{c}	2.42 ± 0.04^{b}	2.67 ± 0.08^{c}	0.85 ± 0.07^{c}	0.86 ± 0.07^{c}	2.02 ± 0.06^{c}	2.72 ± 0.05^{c}	1.14 ± 0.14^{c}	1.12 ± 0.16^{c}	2.2 ± 0.16^{c}	0.68 ± 0.10^{b}	0.64 ± 0.15	0.68 ± 0.10^{c}	1.20 ± 0.14^{c}	1.26 ± 0.07^{c}	0.36 ± 0.18^{b}	0.32 ± 0.17^{bc}
25	1.09 ± 0.07^{c}	1.12 ± 0.05^{bc}	0.31 ± 0.08^{b}	0.42 ± 0.06^{bc}	1.37 ± 0.09^{a}	1.8 ± 0.12^{b}	0.46 ± 0.05^{b}	0.65 ± 0.04^{b}	1.96 ± 0.11^{b}	2.2 ± 0.09^{b}	0.86 ± 0.10^{b}	0.91 ± 0.21^{b}	1.12 ± 0.07^{b}	0.59 ± 0.05^{a}	0.47 ± 0.05^{a}	0.59 ± 0.05^{b}	0.87 ± 0.17^{ab}	0.92 ± 0.05^{ab}	0.20 ± 0.17^{ab}	0.24 ± 0.18^{b}
50	0.93 ± 0.05^{b}	1.03 ± 0.01^{b}	0.15 ± 0.03^{b}	0.21 ± 0.01^{b}	1.29 ± 0.12^{a}	1.68 ± 0.22^{a}	0.36 ± 0.16^{a}	0.47 ± 0.27^{a}	1.72 ± 0.16^{a}	1.92 ± 0.16^{a}	0.62 ± 0.17^{a}	0.73 ± 0.27^{a}	0.96 ± 0.04^{a}	0.42 ± 0.06^{a}	0.36 ± 0.02^{a}	0.42 ± 0.06^{a}	0.16 ± 0.18^{a}	0.23 ± 0.16^{a}	0.15 ± 0.05^{a}	0.18 ± 0.06^{a}
75	0.41 ± 0.01^{a}	0.86 ± 0.10^{a}	0.02 ± 0.16^{a}	0.05 ± 0.12^{a}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

a,b,c,d,e denote the significant differences within the values provided in the table, which were studied through Duncan's Multiple Range test. Each value is Mean ± SD of three replicates, means with different superscript letters within each column arestatistically significant (P < 0.05). '-' represents the non availability of plant specimen for analysis.

Table 4
Effect of TRME (pH- 5 ± 0.5 and 7 ± 0.5) on the total carbohydrate content of broad bean

Treatment %	10days				15days				20days				25days				30days			
	Root		Shoot		Root		Shoot		Root		Shoot		Root		Shoot		Root		Shoot	
	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7
Control	2.10 ± 0.08^{e}	2.32 ± 0.08^{d}	3.12 ± 0.15^{e}	3.21 ± 0.36^{d}	2.16 ± 0.02^{c}	2.45 ± 0.14^{d}	3.21 ± 0.14^{d}	3.28 ± 0.22^{c}	2.21 ± 0.05^{c}	2.51 ± 0.10^{d}	3.45 ± 0.08^{c}	3.58 ± 0.28^{d}	2.36 ± 0.03^{c}	2.54 ± 0.05^{c}	3.47 ± 0.05^{d}	3.61 ± 0.25^{d}	2.39 ± 0.02^{c}	2.62 ± 0.12^{c}	3.56 ± 0.05^{d}	3.66 ± 0.11^{d}
10	2.19 ± 0.14^{e}	2.46 ± 0.12^{d}	3.16 ± 0.22^{e}	3.23 ± 0.09^{d}	2.27 ± 0.15^{c}	2.52 ± 0.16^{d}	3.22 ± 0.32^{d}	3.31 ± 0.11^{c}	2.32 ± 0.18^{d}	2.59 ± 0.02^{e}	3.42 ± 0.18^{c}	3.61 ± 0.26^{d}	2.48 ± 0.45^{d}	2.59 ± 0.02^{d}	3.48 ± 0.04^{d}	3.66 ± 0.02^{d}	2.51 ± 0.38^{d}	2.69 ± 0.21^{d}	3.58 ± 0.27^{d}	3.71 ± 0.39^{e}
15	2.01 ± 0.19^{d}	2.08 ± 0.05^{c}	2.81 ± 0.14^{d}	3.04 ± 0.36^{c}	2.12 ± 0.26^{c}	2.12 ± 0.11^{c}	2.84 ± 0.06^{c}	3.17 ± 0.09^{b}	2.29 ± 0.26^{c}	2.14 ± 0.05^{c}	2.88 ± 0.35^{b}	3.21 ± 0.32^{c}	2.36 ± 0.15^{c}	2.19 ± 0.16^{bc}	2.96 ± 0.17^{c}	3.26 ± 0.06^{c}	2.38 ± 0.15^{c}	2.21 ± 0.22^{bc}	2.95 ± 0.11^{c}	3.31 ± 0.26^{c}
25	1.61 ± 0.12^{c}	1.72 ± 0.09^{bc}	2.42 ± 0.24^{c}	2.09 ± 0.02^{c}	1.71 ± 0.15^{ab}	1.79 ± 0.10^{b}	2.46 ± 0.14^{b}	2.17 ± 0.02^{ab}	1.77 ± 0.08^{b}	1.82 ± 0.08^{b}	2.53 ± 0.37^{ab}	2.20 ± 0.29^{b}	1.82 ± 0.46^{b}	1.89 ± 0.18^{b}	2.56 ± 0.41^{b}	2.29 ± 0.18^{b}	1.87 ± 0.12^{ab}	1.97 ± 0.36^{b}	2.61 ± 0.09^{b}	2.34 ± 0.18^{b}
50	1.02 ± 0.23^{b}	1.12 ± 0.16^{b}	2.01 ± 0.25^{b}	1.42 ± 0.06^{b}	1.11 ± 0.09^{a}	1.22 ± 0.24^{a}	2.08 ± 0.05^{a}	1.48 ± 0.21^{a}	1.19 ± 0.54^{a}	1.29 ± 0.10^{a}	2.12 ± 0.27^{a}	1.57 ± 0.04^{a}	1.27 ± 0.51^{a}	1.37 ± 0.05^{a}	2.18 ± 0.05^{a}	1.61 ± 0.05^{a}	1.31 ± 0.18^{a}	1.42 ± 0.04^{a}	2.21 ± 0.04^{a}	1.65 ± 0.05^{a}
75	0.71 ± 0.05^{a}	0.92 ± 0.02^{a}	1.86 ± 0.06^{a}	1.20 ± 0.05^{a}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

a,b,c,d,e denote the significant differences within the values provided in the table, which were studied through Duncan's Multiple Range test. Each value is Mean ± SD of three replicates, means with different superscript letters within each column are statistically significant (P < 0.05). '-' represents the non availability of plant specimen for analysis.

Table 5
Effect of TRME (pH- 5 ± 0.5 and 7 ± 0.5) on the total protein content of broad bean

Treatment %	10days				15days				20days				25days				30days			
	Root		Shoot		Root		Shoot		Root		Shoot		Root		Shoot		Root		Shoot	
	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7
Control	2.16 ± 0.11^{d}	2.22 ± 0.17^{e}	2.24 ± 0.14^{c}	2.34 ± 0.16^{d}	2.28 ± 0.18^{bc}	2.31 ± 0.12^{c}	2.36 ± 0.21^{c}	2.41 ± 0.52^{d}	2.33 ± 0.25^{c}	2.38 ± 0.15^{c}	2.39 ± 0.21^{c}	2.48 ± 0.25^{d}	2.43 ± 0.10^{c}	2.47 ± 0.41^{c}	2.49 ± 0.09^{c}	2.52 ± 0.11^{d}	2.47 ± 0.15^{d}	2.51 ± 0.12^{c}	2.55 ± 0.35^{bc}	2.65 ± 0.41^{d}
10	2.18 ± 0.16^{d}	2.28 ± 0.06^{e}	2.36 ± 0.22^{d}	2.46 ± 0.16^{e}	2.31 ± 0.12^{c}	2.38 ± 0.05^{c}	2.42 ± 0.58^{d}	2.49 ± 0.45^{d}	2.36 ± 0.23^{d}	2.41 ± 0.32^{c}	2.45 ± 0.09^{d}	2.57 ± 0.18^{e}	2.42 ± 0.05^{c}	2.48 ± 0.12^{c}	2.51 ± 0.11^{d}	2.69 ± 0.21^{e}	2.52 ± 0.25^{e}	2.58 ± 0.10^{c}	2.59 ± 0.36^{c}	2.71 ± 0.47^{e}
15	1.96 ± 0.22^{c}	2.05 ± 0.05^{d}	2.26 ± 0.31^{c}	2.21 ± 0.15^{c}	2.02 ± 0.52^{b}	2.12 ± 0.12^{bc}	2.31 ± 0.27^{c}	2.25 ± 0.25^{c}	2.09 ± 0.24^{bc}	2.14 ± 0.09^{bc}	2.37 ± 0.13^{c}	2.31 ± 0.06^{c}	2.16 ± 0.07^{bc}	2.19 ± 0.18^{b}	2.42 ± 0.08^{c}	2.35 ± 0.11^{c}	2.24 ± 0.05^{c}	2.24 ± 0.09^{bc}	2.57 ± 0.16^{c}	2.44 ± 0.15^{c}
25	1.52 ± 0.08^{bc}	1.85 ± 0.22^{c}	2.07 ± 0.09^{bc}	2.01 ± 0.14^{bc}	1.63 ± 0.08^{ab}	1.99 ± 0.12^{b}	2.12 ± 0.12^{b}	2.12 ± 0.22^{b}	1.67 ± 0.34^{b}	2.09 ± 0.05^{b}	2.19 ± 0.10^{b}	2.19 ± 0.07^{b}	1.72 ± 0.05^{b}	2.17 ± 0.25^{b}	2.24 ± 0.23^{b}	2.21 ± 0.41^{b}	1.78 ± 0.35^{b}	2.21 ± 0.37^{b}	2.29 ± 0.12^{b}	2.27 ± 0.54^{b}
50	1.07 ± 0.18^{b}	1.27 ± 0.02^{b}	1.86 ± 0.05^{b}	1.87 ± 0.48^{b}	1.10 ± 0.24^{a}	1.31 ± 0.21^{a}	1.89 ± 0.09^{a}	1.92 ± 0.11^{a}	1.17 ± 0.02^{a}	1.39 ± 0.08^{a}	1.92 ± 0.41^{a}	1.96 ± 0.18^{a}	1.21 ± 0.21^{a}	1.45 ± 0.12^{a}	1.99 ± 0.38^{a}	2.01 ± 0.22^{a}	1.29 ± 0.14^{a}	1.49 ± 0.09^{a}	2.07 ± 0.33^{a}	2.25 ± 0.05^{a}
75	0.86 ± 0.37^{a}	1.01 ± 0.12^{a}	1.27 ± 0.02^{a}	1.31 ± 0.32^{a}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

a,b,c,d,e denote the significant differences within the values provided in the table, which were studied through Duncan's Multiple Range test. Each value is Mean ± SD of three replicates, means with different superscript letters within each column are statistically significant (P < 0.05). '-' represents the non availability of plant specimen for analysis.

Table 6
Effect of TRME (pH- 5 ± 0.5 and 7 ± 0.5) on the total amino acid content of broad bean

Treatment %	10days				15days				20days				25days				30days			
	Root		Shoot		Root		Shoot		Root		Shoot		Root		Shoot		Root		Shoot	
	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7	pH- 5	pH- 7
Control	3.42 ± 0.19^{d}	3.52 ± 0.21^{e}	4.01 ± 0.14^{e}	4.07 ± 0.14^{e}	3.46 ± 0.21^{d}	3.67 ± 0.09^{d}	4.06 ± 0.11^{e}	4.17 ± 0.15^{c}	3.52 ± 0.12^{c}	3.71 ± 0.32^{d}	4.26 ± 0.35^{e}	4.28 ± 0.15^{cd}	3.68 ± 0.18^{d}	3.77± 0.22^{d}	4.47 ± 0.38^{e}	4.50 ± 0.18^{d}	3.71 ± 0.22^{c}	3.82 ± 0.09^{d}	4.52 ± 0.24^{e}	4.62 ± 0.36^{d}
10	3.21 ± 0.21^{d}	3.66 ± 0.12^{f}	3.81 ± 0.35^{d}	4.10 ± 0.18^{e}	3.24 ± 0.41^{c}	3.78 ± 0.52^{e}	3.86 ± 0.52^{d}	4.22 ± 0.09^{c}	3.27 ± 0.05^{bc}	3.81 ± 0.11^{d}	3.91 ± 0.12^{d}	4.36 ± 0.35^{d}	3.29 ± 0.18^{c}	3.84 ± 0.38^{e}	3.95 ± 0.11^{d}	4.54 ± 0.09^{d}	3.33 ± 0.38^{bc}	3.87 ± 0.22^{d}	3.99 ± 0.09^{d}	4.68 ± 0.14^{d}
15	3.01 ± 0.12^{c}	2.81 ± 0.41^{d}	3.52 ± 0.38^{c}	3.96 ± 0.11^{d}	3.11 ± 0.29^{b}	2.96 ± 0.12^{c}	3.56 ± 0.19^{c}	4.01 ± 0.08^{bc}	3.16 ± 0.35^{b}	3.12 ± 0.14^{c}	3.58 ± 0.11^{c}	4.12 ± 0.05^{c}	3.17 ± 0.39^{b}	3.32 ± 0.11^{c}	3.61 ± 0.14^{c}	4.38 ± 0.38^{c}	3.21 ± 0.18^{b}	3.56 ± 0.21^{c}	3.66 ± 0.12^{c}	4.50 ± 0.09^{c}
25	2.62 ± 0.09^{bc}	2.40 ± 0.31^{c}	3.32 ± 0.22^{b}	3.62 ± 0.43^{c}	2.67 ± 0.12^{ab}	2.42 ± 0.15^{b}	3.35 ± 0.35^{b}	3.68 ± 0.22^{b}	2.69 ± 0.18^{sb}	2.46 ± 0.14^{b}	3.39 ± 0.36^{b}	3.71 ± 0.18^{b}	2.71 ± 0.11^{sb}	2.51 ± 0.09^{b}	3.41 ± 0.17^{b}	3.82 ± 0.17^{b}	2.73 ± 0.21^{s}	2.56 ± 0.05^{b}	3.42 ± 0.14^{b}	3.93 ± 0.19^{b}
50	2.41 ± 0.11^{b}	2.11 ± 0.38^{b}	3.02 ± 0.38^{ab}	3.12 ± 0.18^{b}	2.45 ± 0.21^{a}	2.16 ± 0.21^{a}	3.09 ± 0.18^{a}	3.19 ± 0.41^{a}	2.48 ± 0.18^{a}	2.18 ± 0.14^{a}	3.12 ± 0.05^{a}	3.22 ± 0.11^{a}	2.51 ± 0.26^{a}	2.21 ± 0.05^{a}	3.16 ± 0.09^{a}	3.26 ± 0.12^{a}	2.53 ± 0.35^{a}	2.26 ± 0.35^{a}	3.21 ± 0.12^{a}	3.28 ± 0.10^{a}
75	1.19 ± 0.41^{a}	2.01 ± 0.12^{a}	2.01 ± 0.18^{a}	2.16 ± 0.11^{a}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

a,b,c,d,e denote the significant differences within the values provided in the table, which were studied through Duncan's Multiple Range test. Each value is Mean ± SD of three replicates, means with different superscript letters within each column are statistically significant (P < 0.05). '-' represents the non availability of plant specimen for analysis.

DISCUSSION

The effluent from the process of parboiling of paddy is very huge as massive amount of rice is being processed per day, as the effluent is let out as such in the nearby agricultural land or local water bodies causing irreplaceable environmental damage.[15] From table 1 it was evident that before the treatment of the effluent through the process of fermentation all the physico-chemical characteristics of effluent were not in a range making it accessible for agricultural purposes or were not in the acceptable range in accordance with the drinking water quality standards, but after the treatment of 96 h there was a characteristic decrease in the parameters of TRME (Table 1) thus making the effluent less harmful, helping in its usage for irrigation. This may be due to the activity of the bacteria that breaks down the organic and inorganic moieties in the effluent so as to survive in the nutrition rich environment. The amount of carbon content in the effluent governs the organisms' capability to accumulate PHA, hence the organism uptakes all the carbon and stores them as PHA inclusions, which were then extracted and used. Due to these metabolic processes, the pH of TRME was found to be 5 ± 0.5, this acidic pH may be due to the generation of volatile fatty acids (VFA's) from the excess carbon in the substrate by the bacteria for the synthesis of PHA.[16] In a way to understand the plants response towards direct usage of TRME with and without pH adjustments were studied extensively. From the results it was clear that broad bean could productively grow even at the acidic pH which was only little lesser than that of the control. Neutralized TRME showed better growth characters and biochemical parameters than control at 10%, which upon increment did not show positive effects on the plant growth. The rate of germination (Figure 1) depicts that, with lesser substitution of effluent the seed were able to germinate easily within a period of 4 to 5 days, but with increasing effluent concentration, even after 9 days only a minimal growth was observed. This may be due to the stress conditions created by the effluent, disturbing the carbohydrate and protein metabolites of the membrane of the seed. [17] As sufficient water absorption is essential for proper seed germination, without which seedling growth and development will be severely affected.[18-19] The maximum shoot and root was recorded at 10% and minimum at 75% of effluent concentrations, as compared to control. At 10% of effluent concentration, increase in root and shoot length was high (10.9 ± 0.05 and 62.5 ± 0.61 for acidic pH; 11.2 ± 0.06 and 65.6 ± 0.22 for neutral pH) at 30th day as compared to control (Table 2), whereas at 75% of effluent concentration, a sharp decrease in length of root and shoot was recorded at all the days. One of the factors responsible for the hampered germination was the influence of dehydrogenase enzyme activity caused by the increased concentration of effluent.[5] Chlorophyll estimation is one of the important plant parameters which are used as an index of production capacity of the plant. The chlorophyll content is an ecological index as well as growth parameters.[10] The estimation of chlorophyll content showed an increasing trend up to 25% after which it declined. The variation in the chlorophyll content at higher TRME indicate that the chlorophyll synthesizing capacity of the plant gets reduced which in turn deters the overall photosynthetic process, this was in accordance with the observations made by Krupa *et al*.[20] and Gouia *et al*.[21] The reduction might have also occurred due to senescence in the plants with good growth as the chlorophyll content is higher in younger leaves.[22] The increase on the total carbohydrate content corresponding to the fresh weight of the shoot and root varied with different concentrations of the effluent, due to the stimulation or inhibition in the carbohydrate metabolism created by the effluent the above mentioned phenomenon would have occurred.[23] Early reports on the cultivation of tomato with rice mill effluent[10] and sugar mill effluent used for groundnut and paddy growth[24] were in corroboration with the present results. As seedling growth is mainly dependent on the level of protein present in the different parts of the plant, the test plant showed notable amount of protein and amino acids in the plant. Compared to root, shoot showed higher amount of protein synthesis up to 25% of TRME (Table 5 and 6), this was in accordance with the reports given by Lakshmi and Sundaramoorthy[25] on the ragi plant and the treatment of *Lycopersicum esculentum* with tannery effluent.[26] From all the results obtained, it was validated that the neutral pH was found to be a promising alternative for the cultivation of plants as they showed good improvement in both morphometric and the biochemical parameters comparable and even better than control at lower concentrations of TRME. The germination percentage was higher at 10 and 15 % in both acidic and neutral pH, with increase in the effluent concentration, there was a decrease in the germination percentage which initiated from 25 %, at 75 % the germination percentage was very low (Table 2) and at 100% no germination was noted. After 10 days, the plants grown with 75% concentration putrefied, showing poor development of root and leaves, this case was observed in both the pH conditions. These results were rationale to the findings of Subramani *et al*.[27] stating the a progressive decrease in seedling growth with the increasing concentration of fertilizer factory effluent and the marked growth promoting outcome of tannery effluent at lower concentration and at higher concentration reduction in seed germination, seedling growth and chlorophyll content in some crops were also reported.[28]

CONCLUSIONS

After the fermentative utilization of the rice mill effluent for PHA production, the left out effluent shall be effectively used for the growth of plants. Up to 50 % dilution of the effluent was acceptable for the growth of broad bean above which did not show a positive trend on the growth of the plant. 10 % dilution of the effluent with pH 7 ± 0.5 had a positive influence on the growth of the plant than that of the normal water used for agricultural purpose. Thus the study concludes that the treated rice mill effluent shall be an eminent alternative for the cultivation of broad bean.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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