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Fertilizer from Fish Scale for Production of Paddy: A Green Technology for Waste Utilization

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Abstract—*Fish scales, in general, are composed of collagen protein and hydroxyapatite (HAP) mineral, both having several biomedical and technological applications. Fish production and consumption are gradually increasing throughout the world. Fish scale is inedible and is totally considered as waste material. Since it is calcified tissue, its biodegradation in soil is slow and thus, creates environmental pollution. Researchers have attempted separation of either the protein part or the mineral part mostly using solutions of NaOH, HCl, and heat treatment in several permutation and combination; in these reports, no mention of the disposal of the liquid effluent generated therefrom is available. Indian fish market is mainly dominated by carp. In our laboratory we developed a process for simultaneous recovery of both collagen and HAP from Labeo rohita scale using a green technology. The liquid effluent generated in the process could be used as fertilizer for plant growth and vegetation. The objective of the present paper was to evaluate the efficacy of the fertilizer (in dry form, FSE) for growth of paddy plant. Paddy plant using 0.5-10% (w/v) of FSE solution was grown in sand bed taken in plastic pot. Height and number of tillers were measured time to time. These were compared with similarly grown plant using synthetic fertilizer. Milling quality of paddy and rice hardness was measured for the FSE grown plant showing promising growth, and compared with actual field grown rice. FSE contained all the macro and micro-elements required for paddy plant. Among the different concentrations, 2% FSE solution gave the best result with respect to height and tillers; these were better compared to that of synthetic fertilizer. Average yield of filled grain/plant with 2% FSE was 44 g, with chaffy grain being only 2.85% of total grain; its milling quality and rice hardness was comparable to field rice.*

1. INTRODUCTION

World per capita fish consumption has increased from an average of 9.9 kg in the 1960s to 14.4 kg in the 1990s and 19.7 kg in 2013, with estimates for 2014 and 2015 pointing towards further growth beyond 20 kg [3]. The scales of fishes being inedible part of the body are discarded as waste material [4, 7]. Fish scales, in general, are composed of mineral (hydroxyapatite, HAP) and fibrous protein (collagen). Being calcified tissues, biodegradation of the discarded scale is slow, and thus, creates a disposal problem.

Both HAP and collagen have various biomedical and technological applications [6, 8]. Several research works have been documented in the last decade to isolate HAP and collagen from scales of different fishes, e.g., tilapia (*Tilapia nilotica*) [5], and rohu (*Labeo rohita*) [8], respectively. In most of the reported methodologies, sequential treatment with HCL and NaOH in aqueous medium was used for disintegration/fractionation of scale to isolate collagen. Fractionation was followed by incineration at high temperature for removal of residual collagen in case of HAP. This may lead to global warming if the amount of organic material is high.

From literature review, it is seen that the mentioned methodologies targeted isolation and purification of either the protein part or the mineral part; with no mention of utilization of the generated liquid effluent, inevitable in any chemical processing. In our laboratory we developed a green technology through which along with recovery of both the HAP and collagen from fish scale, the generated effluent could be utilized as fertilizer for plant growth and vegetation [9]. The objective of the present paper was to evaluate the efficacy of the fertilizer for growth of paddy plant.

2. MATERIALS AND METHODS

2.1 Materials

Scales of fresh water fish rohu (*Labeo rohita*) was procured from Technology Market, IIT Kharagpur, West Bengal, India. After cleaning and washing, the scale was dried at 55°C-60°C for 24 h in incubator and stored in zip-lock polythene pouch at ambient condition. The dried scale was used as raw material for fertilizer production. The chemicals used throughout the experiment were laboratory grade.

A variety paddy seeds (IR 36) was collected from the agricultural farm of Agricultural and Food Engineering department of IIT Kharagpur. To avoid infiltration of any extraneous nutrients, the plant was cultivated in plastic pots and the material for filling the pot was medium grain size sand, both supplied by local supplier.

2.2 Fractionation of fish scale

Fish scale was soaked overnight in 0.6% KOH solution at room temperature. The soaked solution was collected by draining, and the scale was re-soaked similarly. The soaked scale was boiled in the pooled soaked solutions till the scale got disintegrated. The disintegrated part was the mineral part. It was separated from the liquid part, purified and converted into HAP. The liquid part was neutralized with phosphoric acid solution and then a chemical (ammonium salt) was added to it to isolate the protein (collagen) part. After collection of the protein, the remaining liquid effluent was first concentrated by boiling and then dried in hot air oven (Electronics & Electrical Engg, Kolkata) at 60 °C. The dried mass was ground to powder (Figure.1), here under called Fish Scale Effluent (FSE), and was used as fertilizer. Details of the methodology were described earlier (Singh Deb, 2016).

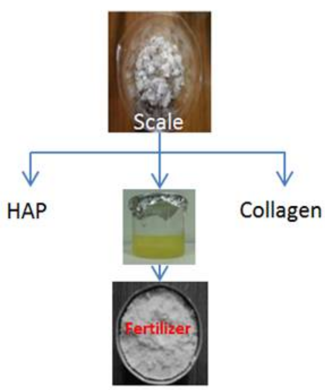

Figure 1: Schematic of fertilizer production from scale of *Labeo rohita* .

2.3 Analysis of FSE

Mineral and organic matter content including Mg, Ca, Cd, Mn, Cr, Pb, Zn, Fe, P, Na, K, P, C, H, N, and S were estimated following standard methodologies.

2.4 Use of FSE on paddy plant growth

2.4.1 Preparation of sand bed

The sand was screened, washed several times using potable water followed by distilled water (dw) and then heated at a 100 °C. Pots were also cleaned and similarly ashed. Then 8.5 kg dried sand was filled in respective plastic pots (0.2566 m^3).

2.4.2 Preparation of seedling and growth of plant

Seeds were kept for 2 days on wet filter paper in a Petri dish for germination. Sand in pots was wetted with dw. Germinated seeds (4-5) were sown in each of the pots. After 7-10 days (dw for watering at regular intervals; with nutrition supplied from seeds), only two healthy seedling were retained. Fertilizer was then applied for further growth of plant, as described below.

Aqueous solutions (in dw) of FSE of different concentrations (0.5%, 1%, 2%, 5%, 10% w/v) were applied (two pots for each treatment) on every Monday, Wednesday and Friday in different amounts to study the growth response of plant. Initially, the dose was 7.5 ml. After 15-20 days, it was increased to 15 ml. After 80 days of plantation, flowering started and onwards this, the dose was increased to 22.5 ml till the paddy was matured. The parameters measured time to time were plant height and number of tillers. These were also compared with that of synthetic fertilizer. Synthetic fertilizer was prepared by dry mixing of commercial grade urea, muriate of potash (MOP) and single super phosphate (SSP) in the mass ratio of 1:1:2 (w/w). Solution of synthetic fertilizer was prepared by mixing 32 g dry mix with 120 ml of dw. This was applied in same amount but only once (Wednesday) in a week. Distilled water, as and when needed, was used for watering the plants.

Besides height and tillers, some other characteristics including number and length of panicles, number of spikelets, grain yield, etc., were also measured for the plant(s) showing appreciable growth result.

2.4.3 Milling quality and hardness of paddy

After harvesting, milling quality (at 13% moisture content, sample basis) and hardness (TA-XT2i, Stable Microsystem Texture Analyser, UK; 25 kg load cell, 25 mm probe diameter) of the grains were estimated for best treatments. These qualities were also measured for paddy collected from the field of AgFE department of IIT Kharagpur, for comparison.

3. RESULTS AND DISCUSSION

3.1 Mineral and organic matter content

Mineral and organic matter content of FSE is shown in Table 1.

Table 1: Chemical composition of FSE.

Chemicals	Amount, mg/g	Chemicals	Amount, mg/g
Mg	0.0129	Na	239.9
Zn	0.009	K	101.165
Ca	0.1871	C	14.35
Cd	0.00375	H	58.575
Mn	0.00185	N	193.9
Cr	0.00465	S	218.74
Pb	0.0064	P	15.099
Fe	0.0268	-	-

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It is seen from Table 1 that besides N, P and K, the fertilizer from fish scale contains most of the macro- and micro-elements required for growth of rice [1].

3.2 Plant growth

Height (H) and tiller (T) of respective paddy plants with different concentration of FSE and synthetic fertilizer are shown in Figure.2 and Figure.3, respectively. The values in these figures represent the mean of four readings (two pots with two plants per pot). Except 0.5% FSE, plant height is found to increase continuously till harvesting, whereas the no. of tillers increases up to around 80 days and then starts dyeing with maturity of plants.

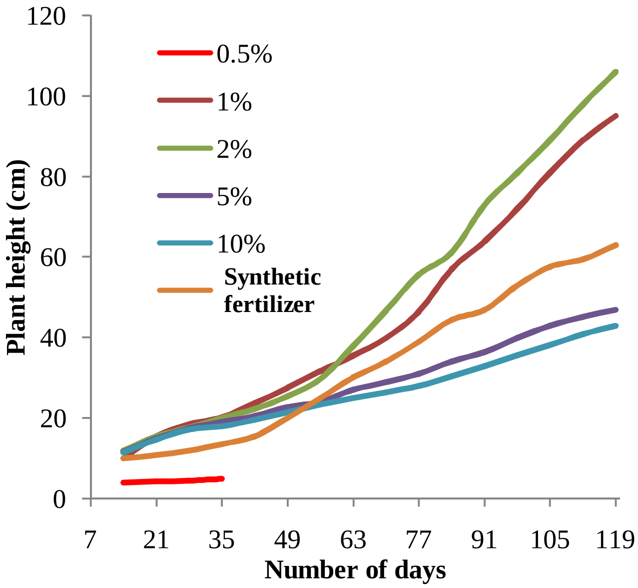

Figure 2: Growth curve of paddy plant for different FSE concentration and synthetic fertilizer.

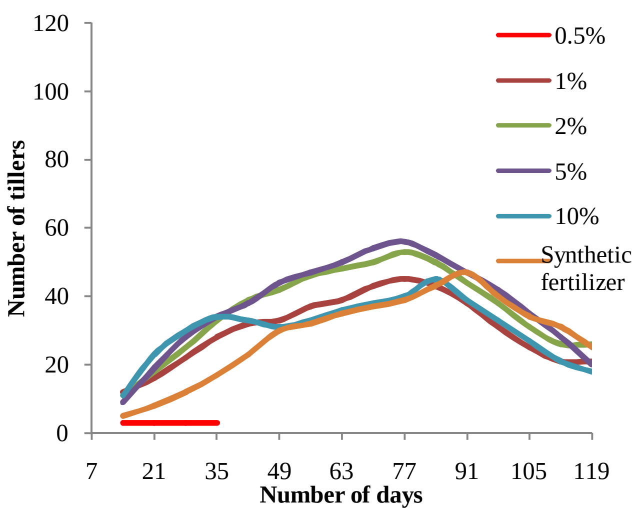

Figure 3: Number of tillers of paddy plant for different FSE concentration and synthetic fertilizer.

Continuous increase of height indicates that the FSE did not impart any detrimental effect on growth rate through the life cycle [2]. Remarkable effect of concentration on height is apparent after 56 days. Height of the plant was maximum for 2% FSE treatment followed by 1% FSE treatment, these were even better than synthetic fertilizer. Tillers are the grain bearing part of rice crop. In this study, though the plant height was not so effective for 5% FSE treated plants, number of tillers was maximum, and it was in close proximity with that of 2% FSE. Overall, it was observed that 2% FSE treated plant showed adequate growth followed by 1%. However, higher concentration of FSE appeared to be toxic with overdose of nutrient.

Table 2 presents the description of the plants in more detail as well as the grain yield at the harvesting time for 1% and 2% FSE treatments. Thus, irrespective of concentration, in matured condition all the tillers are effective tillers and all the panicles are of equal length. The average grain yield per plant is found to be 44 g for 2% FSE and 32 g for 1% FSE; chaffy grain being 2.85% and 4% of total yield, in that order. Hence, 2% FSE produced better results. It is also evident from Figure. 4 showing the picture of matured plants for these concentrations.

1% FSE 2% FSE

Figure 4: Matured paddy plant of 1% and 2% FSE treatments.

3.3 Milling quality and hardness of paddy

It is seen (Table 3) that the milling percentage of treated rice and field rice was nearly the same. For 2% FSE treated rice, hardness was followed by 1% FSE treated rice, and these were little less from field rice (Figure5).

Table 2: Plant description and grain yield of 1% and 2% FSE treatment.

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Parameter	1% FSE				2% FSE			
	Replication 1		Replication 2		Replication 1		Replication 2	
	Plant 1	Plant 2	Plant 1	Plant 2	Plant 1	Plant 2	Plant 1	Plant 2
Number of tillers	21	10	17	16	26	19	22	22
Number of effective tillers	21	10	17	16	26	19	22	22
Number of panicles	21	10	17	16	26	19	22	22
Average Length of panicles (cm)	25.5	25	27.5	26.5	24.5	25	26.5	27.5
Number of spikelets	11	10	10	11	11	12	12	12
Average plant height (cm)	95	94	96	98	86	106	92	89
Weight of filled grains (g)	48.3	21.6	31	28.6	46.4	37.7	50.6	42.4
Weight of chaffy grains (g)	0.8	0.4	1.6	2.6	1.9	0.6	0.9	1.8

Table 3: Milling characteristics of paddy obtained by 1% and 2% FSE.

Treatments	Weight, g						Milling percentage (%)
	Initial paddy	Rice after milling	Husk	Components of Rice after milling			
				Broken rice	Unbroken rice	Paddy unhusked	[(rice after milling/paddy taken) * 100]
1% FSE	38.28	31.48	6.8	8.1	20.28	3.1	82.24
2% FSE	33.63	27.86	5.77	6.7	18.56	2.6	82.84
Field rice	40	33.3	6.7	5.8	22.54	4.96	83.25

Figure 5: Hardness of FSE treated and field rice.

4. CONCLUSION

The effluent, FSE, generated from *Labeo rohita* scale during separation of the contained hydroxyapatite and collagen could be used as fertilizer to support growth of paddy plant in sand bed in plastic pot. FSE contained the macro- and micro-elements essential for paddy plant. Tested within 0.5-10% (w/v) of FSE solutions, the 2% solution produced the best result. Using this concentration, height of the plant and no. of tillers were better than that of synthetic fertilizer. The grain produced per plant was 44 g, with chaffy grain being only 2.85% of total yield, for this concentration; the milling quality and rice hardness was also comparable to that of paddy collected from agriculture field.

5. ACKNOWLEDGEMENTS

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