

## Evaluation of effluent from fish (*Labeo rohita*) scale processing as a fertilizer for paddy (*Oryza sativa*) production

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### Abstract

**Purpose** Fish scale contains two important constituents, the hydroxyapatite and collagen. The purpose of this paper was to use the dried form of the effluent, called Fish Scale Effluent (FSE), generated through simultaneous recovery of these constituents, as a fertilizer.

**Method** The FSE was obtained from *Labeo rohita* (Rohu) scale by treatment in sequence with potassium hydroxide, phosphoric acid, and ammonium sulfate. Chemical composition of FSE was analyzed. The FSE was applied as a sole fertilizer for IR36 paddy plant grown on sand in plastic pot. Various growth parameters and grain qualities were evaluated.

**Results** FSE contained most of the macro- and micro- elements required for growth, and the plant could be harvested by 126 days. Among different concentrations, 2% FSE (w/v) solution gave the best growth results, viz., plant height at maturity (98 cm), effective tillers (22.2/plant), spikelets (90/panicle), paddy production (44.2 g filled grain/plant) and chaffyness (only 2.85% of total paddy). The paddy thus produced exhibited 75.11% hulling, the de-hulled rice containing 55.18% of whole kernel having hardness of around 6 kg. The brown rice contained (sample basis) protein, fat, fibre, ash, and carbohydrate as 7.40%, 1.71%, 0.93%, 1.11% and 75.35%, respectively; morphology of the starch granule being irregular polyhedral in shape with maximum size <10  $\mu\text{m}$ .

**Conclusion** Since these plant growth and grain quality criteria are in acceptable limit, the FSE could be utilized as a potential fertilizer for paddy production.

**Keywords** *Labeo rohita* scale, Green technology, Fish scale fertilizer, Pot culture, Paddy plant, Rice starch

### Introduction

World demand for fertilizer nutrients comprising nitrogen (N), phosphate ( $\text{P}_2\text{O}_5$ ) and potash ( $\text{K}_2\text{O}$ ) was estimated to increase from  $184017 \times 10^3$  tonne in 2015 to  $201663 \times 10^3$  tonne in 2020 (FAO 2017; FAO 2015), pointing to an estimated growth rate of 1.5% per annum from 2014/15 to 2021/22 (IFA 2017). Rice, wheat, and maize together consume almost half of the total fertilizer globally, the share of each equally varying between 14% and 16% (Singh and Singh 2017; Yara 2018). India is the second-largest consumer of fertilizer in the

world, consuming about  $26500 \times 10^3$  tonne (Sharma and Thaker 2011). Out of this, rice consumes the highest share (31.8%) followed by wheat (21%) and maize (2.3%), as per the available data for the year 2003/04 (FAO 2005).

The generation, and disposal of municipal solid waste (MSW) is a major problem in the modern era resulting in ecological problem. As reported by Abdel-Shafy and Mansour (2018), MSW generated from developing countries are mainly from households (55–80%), followed by market or commercial areas (10–30%); if classified by material, food sources account for 15% of the total MSW. Recently, researchers have emphasized valorization of various organic fractions of MSW (Medina-Salas et al. 2019; Pleissner and Lin 2013). Accordingly, Chew et al. (2019); Rajeswari et al. (2018) and Bratovic et al. (2018) reported sustainable transformation of various types of biomass wastes into fertilizers. Such an approach can serve the dual purpose

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of waste minimization and agricultural enhancement (Luz et al. 2020).

The global fish consumption is increasing at 3.2% per year (FAO 2018). According to a recent review (Ahuja et al. 2020), it may be noted that several researchers documented the processing technology of using wasted/damaged whole fish or body parts thereof as a possible fertilizer for plants. Hydrolyzed fish waste using fruit wastes has been reported as an organic fertilizer for the growth of *Basella alba* (Ranasinghe et al. 2021). Aranganathan and Rajasree (2016) observed an appreciable tomato plant growth with the liquid fertilizer produced by a bacterial degradation of a mixture of marine trash fish and molasses. The production of a bio-fertilizer for anticipated use in agriculture was reported by Sahu et al. (2014) through a fermentation of Tilapia (*Oreochromis niloticus*) wastes (viscera, head, frames) with molasses. The residual solid obtained through cooking followed by separating the oil from cooked mixture of viscera, heads, bones, trimmings, tails, intestine, etc., of Nile Tilapia proved to be a satisfactory fertilizer for tomato and onion (Lema and Degebassa 2013). Similarly produced (as by Lema and Degebassa 2013) solid from mixed body parts of fish (the name not available) was further bacteriologically degraded to a liquid fertilizer by Dao and Kim (2011). They reported the height and thickness of the stem as well as the number and length of leaf for red bean and barley through a hydroponic culture; the results were comparable to that of a commercial fertilizer. The potential of germinating cress (*Lepidium sativum*) seed by a liquid fertilizer developed through autoclaving persuaded by a bacterial fermentation of a similar mixture of fish body parts was established by Kim et al. (2010).

The scales obtained from edible fish (approximately 2% of the body weight), being nonedible, are dumped as a waste (Harikrishna et al. 2017; Panda et al. 2014; Huang et al. 2011). Fish scales, in general, are composed of a mineral (hydroxyapatite, HAP) and a fibrous protein (collagen). Because of slow biodegradation due to its calcified tissue, fish scale creates a disposal problem. Harikrishna et al. (2017) reported the production of an alkaline protease enzyme and an amino acid rich hydrolyzate through a bacterial fermentation of fish scales. However, both HAP and collagen, individually, possess numerous medical and technical applications, and in the recent past, several attempts have been taken to isolate these constituents from scales of different fishes, e.g., tilapia (*Tilapia nilotica*) (Kongsri et al. 2013)

and rohu (*Labeo rohita*) (Das and Kumar 2020; Das and Kamble 2019; Pati et al. 2010). The basic principle in these approaches was the disintegration/fractionation of the scale through a treatment with an aqueous solution of HCL and/or NaOH and high-temperature exposure, in different permutation-combination. In most of these reports, the aim was the isolation of either the mineral or the collagen; but there was no mention regarding the effect on the environment through disposal of the liquid effluent, which ought to be generated in any chemical processing.

In our laboratory, we had developed a green technology through which along with the simultaneous recovery of both the HAP and collagen from a fish scale, the generated effluent could be utilized as a fertilizer for various plant growth and vegetation (Sarkar et al. 2019; Singh Deb 2016). The objective of the present paper was to evaluate the efficacy of the fish scale effluent as the fertilizer for growth of paddy plant, and to characterize the produced paddy concerning de-hulling, proximate composition and hardness of brown rice, and morphology of the starch isolated from the endosperm.

## Materials and methods

### Materials

Scales of fish from a carp family, *Labeo rohita* (called rohu), was collected from the Technology Market, Indian Institute of Technology (IIT) Kharagpur, West Bengal, India. It was washed thoroughly with potable water until all the useless material got removed, and dried at 55 °C-60 °C for 24 h in hot air oven (Electronics & Electrical Engineering Co., India). The dried scale, i.e., the raw material was stored in zip-lock polythene pouch at ambient condition.

Commercial grade different synthetic fertilizers, such as urea, muriate of potash (MOP), and single super phosphate (SSP), paddy seeds (IR 36) (treated with 2 g dithane M45 per kg of the seed for 24 h in a polythene bag) (Ibiam et al. 2008) for germination, and field-grown matured paddy of the same variety for a comparison of the de-hulling and hardness, were donated by the agricultural farm of Agricultural and Food Engineering department of IIT Kharagpur. The chemicals used throughout the experiment were of analar grade and indigenously available. The water used in different steps was either potable water or distilled water, as mentioned hereunder.

To keep away any nutrition leaching from soil and an earthen pot, the plant was grown on sand (a medium grain size) taken in plastic pots of about 0.25 m<sup>3</sup> (internal capacity), both supplied by a local supplier.

### Fractionation of fish scale

Fish scale (100 g) with 6 g KOH was soaked overnight at room temperature (28 °C-30 °C) in distilled water (dw), so that total volume of the mixture was 1000 ml. The solution was decanted, and the soaked scale was re-soaked for the same time in the same way in fresh 0.6% (w/v) KOH solution. The decanted solution from the initial step was added to the re-soaking system, and the mixture was boiled till the scale disintegrated into flaky solid, i.e., crude HAP that was separated and purified. The liquid part was neutralized with phosphoric acid, to which then ammonium sulfate was added to precipitate 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 (Plate 1), hereunder called Fish Scale Effluent (FSE), and was used as fertilizer. The whole procedure was described earlier (Sarkar et al. 2018).

### Analysis of FSE

For determination of C, H, N and S, analysis was done by CHNS analyzer (Elementar, Vario macro cube, Germany) (Fadeeva et al. 2008). K was analysed by Ion Chromatography using 883 Basic IC plus (Metrohm Herishau, Switzerland) instrument (Michalski 2006). Other components, i.e., Mg, Ca, Cd, Mn, Pb, Cr, Zn and Fe were analysed using PinAAcle 900H Atomic Absorption Spectrometer (Perkin Elmer, Singapore) (Karpiuk et al. 2016). P was estimated through the chemical method (Akenga et al. 2014) using molybdate and with the help of a UV-vis spectrophotometer (ELICO Double Beam SL 210, Hyderabad, India).

### Use of FSE for paddy plant growth

#### Preparation of sand bed in pot

Firstly, the sand was sieved out to discard undesirables including large pieces of stones, brick and mud, and fine sand. Only medium grade size that passed through

32 mesh screen and retained over 42 mesh screen was taken to provide desired porosity in the sand mass for good aeration at the root zone and proper water percolation. The sand was then washed several times (10-12 times) using potable water to remove all the clay material, followed by further washing twice with dw. Washed sand was partially dried in the sun, and finally by heating at 100 °C for sterilization (Bernhardt and Swiecki 2015) in a hot air oven, followed by ambient cooling to attain room temperature. Pots were also cleaned from any materials physically adhered, washed twice with potable water and twice with dw. These were then dried in ambient condition, finally filling with 8.5 kg of the prepared sand in each pot.

### Germination, preparation of seedling and growth of plant

For germination, the healthy uniform paddy seeds (IR36), after washing with sterile dw were placed evenly for 2 days on sterile filter paper that was lined in 9 cm sterile glass Petri dish and made wet with sterile dw (Vibhuti et al. 2015). Germinated seeds (4-5 in numbers) were sown in each of the pots (Kumar et al. 2017); before sowing, the sand was wetted with dw. For the next 7-10 days after sowing (DAS), as the nutrition was obtained from seeds, only distilled water was applied at regular intervals for watering. Following this, only two healthy seedlings were retained per pot after thinning. A fertilizer was then applied for further growth of the plant, as described below.

The growth study was based on completely randomized design with equal (four) replications. FSE was dissolved in dw to make different concentrations (treatments), viz., 0.5%, 1%, 2%, 5%, and 10% (w/v). These were applied (two pots for each treatment, two plants/pot) on every Monday, Wednesday and Friday in variable quantity to study the growth response of plant. Initially, the dose (ml/pot) was 7.5. About 15-20 DAS, it was increased to 15. Flowering started around 80 DAS, and onward this, the amount was increased to 22.5 ml/pot till the plant was matured. The growth kinetics (plant height and number of tillers) (Yoshida 1981) of FSE supplemented plant was measured, and also compared with that of similarly grown plant, but with synthetic fertilizer (SF). Plant height during vegetative growth period was the distance between the sand surface and the tip of the highest leaf, whereas at maturity it was between sand surface and tip of the high-

est panicle. A shoot with at least one visible leaf was counted as tiller (Yang et al. 2006). To study with synthetic fertilizer (SF), a mixture of urea, MOP and SSP in the mass ratio of 1:1:2 (w/w) was prepared; finally, its solution was made by dissolving 32 g of the mix in dw to make 120 ml. This was applied in the same dose as of FSE, but only once (Wednesday) in a week. As and when needed, dw was used for watering the plants. In general, plants remained submerged under about  $5\pm 2$  cm of water for most of the growth duration.

At the time of harvest, the number and length of panicles (the length from the panicle neck to the panicle tip) (Ghasal et al. 2015; Liu et al. 2016) were measured for the plant(s) showing appreciable paddy yield. The panicles from these plants were cleaned, and the number of spikelets, including the weight for both filled and chaffy (unfertilized and partially filled spikelets) grains (Yoshida 1981) were noted. Filled grains from the replicated plants for these treatments were pooled, thoroughly mixed, dried in sun for two days, manually cleaned from unwanted materials, if any, and was stored at the room temperature in zip-lock pouch as the paddy-stock (PS) for further analysis.

### De-hulling of paddy and hardness of brown rice

The paddy was de-hulled in two replications taking the samples randomly from two locations of the PS. Prior to de-hulling, moisture content of paddy was determined by oven drying method at 130 °C for 16 h (Rice knowledge bank 2019). The de-hulling was done in a single pass using laboratory rubber roll Sheller (Satake Corporation, Model THU35A, Japan) with roll clearance of 0.8 mm (Siebenmorgen et al. 2006). The parameters measured were the amount of whole kernel brown rice (WKBR), broken rice, the husk and unhulled paddy (Gautam et al. 2008; Badi 2013). The hulling% was calculated as the percentage of de-hulled rice (including brokens) obtained from a sample of paddy, whereas the WKBR recovery was the percentage of whole kernel (excluding brokens) obtained after de-hulling from a sample of paddy (Gautam et al. 2008; Badi 2013; Kale et al. 2017). WKBR from the two replications were pooled and thoroughly mixed, and called as brown kernel stock (BKS). Hardness of the WKBR was estimated using Texture Analyser (TA-XT2i, Stable Micro System Ltd., UK; 25 kg load cell, 25 mm probe diameter) in five replications with the samples randomly taken from BKS. A single compression force-versus time

program was adopted to compress a single grain along its thickness with pre-test, test and post-test speed of 5, 2, and 10 mm.s<sup>-1</sup>, respectively, as followed by Patel et al. (2013) and Mir and Don Bosco (2013). The peak force in the profile was considered as the hardness of grain. For comparison, these parameters were measured similarly for the paddy collected from the agricultural farm of the department.

### Proximate composition

A portion of the de-hulled rice was ground to flour by mixer-grinder (Sumeet Research and Holdings Limited, Chennai, India); the rice flour was estimated (in three replications) for its proximate composition following standard methodologies (AOAC 1990). Moisture content was determined by overnight heating at 105 °C (Talpur et al. 2011), whereas protein and fat were estimated through kjeldahl method (Kelplus- Classic DX VATS (E), Pelican Equipments, Chennai, India) using the conversion factor of 5.95 (Juliano 1985) and solvent extraction in Soxhlet apparatus (Socsplus-SCS 06 RAS DLS, Pelican Equipments, Chennai, India), respectively. The defatted sample was used for fibre determination using Fibra Plus +FES 6 (Pelican Equipments, Chennai, India). For determining ash content, rice flour was burnt at 550 °C for 3-4 h in Muffle furnace (Instrumentation India, Kolkata, India).

### Characterization of rice starch

#### Isolation of starch

Starch was isolated according to Reddy and Bhotmange (2013). Briefly, rice flour (as prepared above) was extracted sequentially with 1 N NaOH, dw, 2% NaCl, and 0.1 N NaOH. The residue obtained was the white starch. This was blended in 80% ethanol, heated on water bath at 80 °C for 1 h, and then allowed to cool and settle for 4 h at 4 °C. The settled portion, i.e., the pure rice starch was freeze-dried, and powdered manually with light pressure.

#### Morphology of starch

Morphology of starch granule was checked using Scanning electron microscope (SEM) (Jeol JXA-840A, Jeol Ltd®, Tokyo, Japan). Starch powder was applied on aluminium stub using double-sided adhesive tape, and was

coated with gold (using auto quick gold coater). The images were taken at different magnifications (1500X and 3000X) at an accelerating voltage of 20 kV.

### Statistical analysis

The mean and the standard deviation of the replicated values were evaluated for analyzing the results. Pair of means was compared using t-test,  $p < .05$ .

## Result and discussion

### FSE and its composition

Plate 1 and Table 1 present the photograph and elemental analysis of the FSE.



**Plate 1** FSE from *Labeo rohita*

Though the exact amount of fertilizer required for growth of paddy plant in a field is influenced strongly, qualitatively, by the variety, season, nature and composition of the soil, rainfed/irrigated, and the field water level, the essential elements required in macro-amount,

in general, are carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium and sulfur, while the requirement in lesser/micro- amounts comprise iron, manganese, copper, zinc, molybdenum, boron and chlorine (IRRI 2007). Of these, the plant can absorb C, H, and O from air and water, while the rest are to be supplied by the fertilizer and/or soil. Since, in the present study the plant was grown on washed sand in pot, besides C, H, and O, the rest of macro- and micro-elements was supposed to be supplied by the FSE only. It may be worth mentioning that several nutritional deficiency syndromes have been reported in literature for rice (IRRI 2007; Yoshida 1981). The following results and discussion clearly describe that depending on FSE concentration, the general health of the plants (Plate 2), plant growth profile and grain quality parameters including starch morphology were acceptable. It needs mentioning that toxic metals Cd, Cr and Pb of FSE (Table 1) are below/within the allowable limits reported for fertilizers (Międażys et al. 2019; Fertiliser Association 2014). Thus, in line with the present objective, it can be stated that FSE does not possess any phytotoxicity, and if appropriately applied supports growth of paddy plants.

### Plant growth

Plant growth was monitored until the paddy became almost ready for harvesting. The time required for this, under the present experimental condition of sand in the pot, was 119 DAS. According to the bulletins published by IRRI (Khush and Virk 2005) and Anonymous (2019), the days to maturity of IR36 is about 111 and 120, respectively.

Height (H) and the number of tillers (T) of the paddy plants with the different concentration of FSE and

**Table 1** Elemental analysis of FSE

Chemicals	Value, mg/g	Chemicals	Value, mg/g
Mg	0.0129	Fe	0.0268
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



(a)



(b)

**Plate 2** Paddy plant on 112 DAS for (a)1% and (b) 2% FSE treatment

SF are shown in Figs 1 and 2, respectively. The values in these Figs represent the mean of four readings (two pots with two plants per pot). Except for 0.5% FSE, height over the whole growth phase continuously increased. This type of increasing growth profile was also observed by Yang et al. (2006) for two paddy varieties grown in China, and Hasanuzzaman et al. (2010) for a variety grown in Bangladesh. A continuous increase of height indicates that the FSE contributed no detrimental effect on growth rate throughout the life cycle (Constantino et al. 2015). For the period up to about 50 days (Fig. 1), the height was almost overlapping for 1%-10% FSE concentrations, and it was even better than that of SF. Following this, the height was in the order: 2% > 1% > SF > 5% > 10%. The maximum height noted on 119 DAS (at maturity) was 98 cm for 2% FSE and 95.7 cm for 1% FSE. These values are in close similarity with the values reported for IR36, i.e., 84 cm, 90 cm, 98 cm, 100 cm, and 89 cm, respectively, quoted by Peng and Khush (2003); Khush and Virk (2005); Mondal et al. (2012); Ansari et al. (2003), and Samal et al. (2014).

The number of tillers, except for 0.5% FSE, changed with time following quadratic shape to reach respective maximum value (Moldenhauer et al. 2009) that was attained around 77 DAS for 1%-5% FSE, 84 DAS for 10% FSE, and 91 DAS for SF (Fig. 2). Following this, no newer tillers were produced; rather, some late tillers started dyeing with maturity of plants. As explained by Fageria (2014), such decrease might be due to the in-

ability of the young (late-developing) tillers to compete for light and nutrients. According to Yang et al. (2006), the late tillers are not efficient in producing panicle, waste both nutrients and energy. From the Fig. 2, the increase in tillers/day was comparable within 1%-10% FSE for up to around 42 days, following which it was in the order: 5% > 2% > 1%  $\approx$  10%. In general, the rate of increase in tiller was much better for FSE than that of SF, suggesting applicability of the effluent as fertilizer for plant growth. At the harvesting time (126 DAS), irrespective of concentration and nature, the tiller number was in the range of 16-25, manifesting a survival within 35% (for 5% FSE) - 53% (for SF) of the respective maximum number. However, the tiller with panicle was appreciable with 1% and 2% FSE, indicating 41% and 43% of the maximum as effective tiller. Around 49% and 45% of the tiller at maximum tiller stage produced panicle of IR36, as mentioned by Nuruzzaman et al. (2000a) and Fukushima (2019), respectively.

Among all the treatments, 1% and 2% FSE produced appreciable plant growth. This is apparent from Plate 2. However, Table 2 presents the average (4 replicated plants) of the growth parameters at the harvesting time for these treatments. The number of panicles/plant from Table 2 is 16 for 1% and 22 for 2%. In a close similarity, Nuruzzaman et al. (2000b) reported the value to be 21 for IR36. The average panicle length for both 1% and 2% FSE plants was approximately 26 cm. A comparable panicle length of 27.12 cm was mentioned

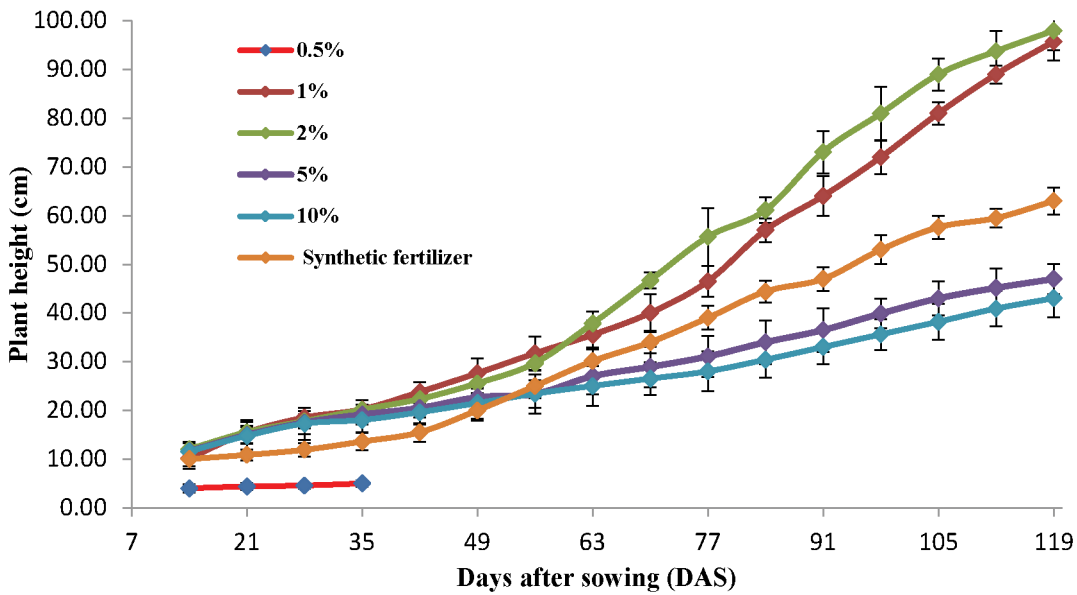


Fig. 1 Growth curve of paddy plant for different FSE concentration and synthetic fertilizer

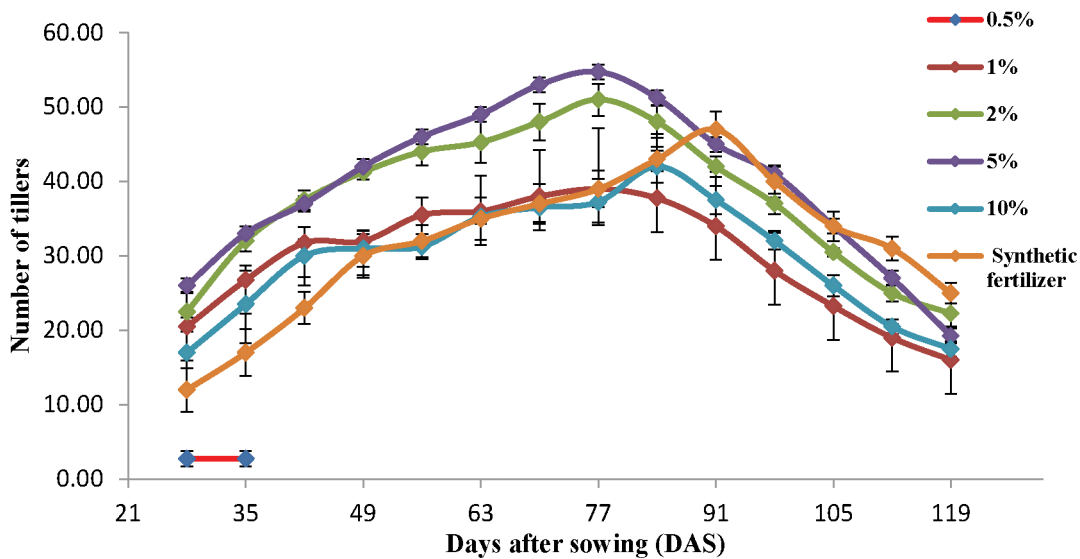


Fig. 2 Number of tillers of paddy plant for different FSE concentration and synthetic fertilizer

for IR36 by Samal et al. (2014); for the same variety, it was 19.89 cm and 19.3 cm as reported by Chandrakar (2010) and Mondal et al. (2012). Such panicle length as observed in the present study was also mentioned for two rice varieties from Bangladesh Rice Research Institute, Rajshahi (Rahman et al. 2013), and some varieties of Pusa basmati (Ghasal et al. 2015) and Sri Lankan rice (Ranawake et al. 2013) ranging between 16-35 cm, 25.4-28.1 cm and 15.1-29.0 cm, respectively.

The number of filled spikelets/panicle in Table 2 is the same (approximately 90) for both 1% and 2% FSE. An almost same value, i.e., 93 grains per panicle was quoted for IR36 by IRRI (1989). However, this is less than the value for this variety reported by Fukushima (2019); Nuruzzaman et al. (2000b); Yao et al. (2000); Ansari et al. (2003), and Mondal et al. (2012) as 107, 100, 139.9, 106.3, and 125.5, respectively. According to Horie et al. (1997), spikelet number can be increased

by increasing plant nitrogen. Such opinion on spikelet number and nitrogen fertilizer has also been expressed by Zhou et al. (2017).

From the documented agricultural field studies, the percentage of chaffy grains for IR36 varied within 18%-25% (Bodalkar and Awadhiya 2014; Mukhopadhyay et al. 2017) and 14%-26% (Ghorai and Singh 1994). Since

there was no insect infestation in the pot culture and the plants were exposed to sunlight, the percentage of chaffy grain was only within 2.85%-3.86%, i.e., almost negligible (Table 2) (Yoshida 1981). The high nutrient channeling ability from the surface to the root in sand may also be the probable cause of the low chaffyness (Marimuthu et al. 2009).

**Table 2** Average values of growth parameters and grain yield per plant at harvesting time for 1% and 2% FSE treatment

Parameter	1% FSE*	2% FSE*
Number of effective tillers	16±4.5	22.2±2.8
Number of panicles <sup>§</sup>	16	22
Length of panicles (cm)	26.1 ± 1.1	25.8 ± 1.3
Weight of filled grains (g)	32.3±4.7	44.2±4.1
Weight of chaffy grains (g) <sup>@</sup>	1.3±0.2(3.86% of total)	1.3±0.2(2.85% of total)
Number of filled spikelets/panicle <sup>†</sup>	90.5	90

\*Maximum tiller no: 39 for 1% and 51 for 2%; <sup>§</sup> all tillers had panicles; <sup>@</sup> the value in parenthesis is on total grain; <sup>†</sup> considering single grain weight as 0.0223 g (Khush and Virk 2005).

Since the effective tiller, the number of spikelet per panicle and the plant height are positively correlated to yield (Nayak et al. 2001; Ranawake et al. 2013; Ranawake et al. 2014; Zhang et al. 2017) and the plant with 1% and 2% FSE fulfilled these characters satisfactorily (Table 2, Fig. 1), the grains obtained through these concentrations were evaluated for de-hulling characteristics.

#### De-hulling of paddy and hardness of brown rice

The hulling percentage of paddy grown by 1% and 2% FSE ranged within 74%-75%, which was slightly higher than that of the field-grown paddy of the same variety (Table 3). However, these percentages are comparable to or lower than that of several varieties including traditionally cultivated scented and basmati rice of India, such as Ek-Kadi, Ghansal, Girga, Jiresal, Pusa Basmati-1 (PB-1), Pusa Sugandh-2-5 (PS-2-5), Vasumati and Mugadh Sugandh, among others, ranging within 72%-82% as mentioned by Bhonsle and Krishnan (2010). A value of 77.5%-80.5% for Pusa Rice Hybrid (PRH-10), PS-3 and PB-1 was shown by Gautam et al. (2008), while Shivay et al. (2010) reported 67.1% for PB-1 and 72.8%-75.6% for PS-2-5. For MAS-26 (an aerobic rice variety developed by Marker Assisted Selection at University of Agricultural Sciences, Bengaluru, India),

Sunil et al. (2014) quoted the hulling percent to be within 69.4% to 72.4%.

The percentage of husk of the paddy (husk/de-hulled paddy) was 19.3%, 18.6% and 19.1%, respectively, for 1% FSE, 2% FSE and field-grown samples, indicating a similar husk to kernel ratio (or grain filling) (Singh et al. 2014). The husk percentage of IR36 has been mentioned to be 21% (Khush and Virk 2005). For paddy of other varieties, husk content usually ranged within 20%-25% (Kale et al. 2017; Itagi and Singh 2015).

From Table 3, the whole kernel brown rice (WKBR) recovery was 52.97%, 55.18% and 56.35% with 1% FSE, 2% FSE and field-grown rice samples, respectively. Thus, the effluent obtained from the fish scale is an equally efficient fertilizer in producing a grain quality to withstand the de-hulling as that is produced by the farm. According to Kale et al. (2017), the variety of Pusa Basmati 1121 (PB1121) also indicated the comparable value (52.23%) of brown rice recovery. Itagi and Singh (2015), however, reported somewhat higher values within 59%-62% for IR-64 (brown), Jyothi (red) and Black Thai Jasmine (BTJ).

From above, the data on WKBR recovery (i.e., after de-hulling) is scarce and hence, the authors take the opportunity to cite some more examples, but in terms of head rice (with or without >75% of whole kernel) recovery based on paddy, which is obtained after com-



plete or partial milling/polishing, as quoted by the respective researchers. These are: 52% for IR36 (Rao 1998); 51%-53.5% for PRH-10, PS-3 and PB-1 (Gautam et al. 2008); 55.18%-64.64% for San Andrea, an Italian rice variety (Ilieva et al. 2014); and 46%-50.3% for MAS-26 (Sunil et al. 2014).

Hardness of the kernels obtained from de-hulling in the present study is not significantly different from each other and lies around 6 kg. Since hardness depends on compactness of the starch granules in the kernel, as explained by Mir and Don Bosco (2013), FSE is able to produce the same microstructure as that of field rice. If compared with other reports, such hardness is less than

that of: Koshar and K-332 (7 kg-7.5 kg) variety prevalent in the temperate region of Kashmir valley of India (Mir and Don Bosco 2013); IR-64 (brown), Jyothi (red) and Black Thai Jasmine (BTJ), all indicating approximately 12 kg -15 kg (Itagi and Singh 2015); and Jehlum rice in Kashmir (9.7 kg) (Mir et al. 2016). On the other hand, the hardness in the present study is sufficiently higher than that of PB11 indicating 0.8 kg (Kale et al. 2017). However, it needs mentioning that the criteria discussed above in this section are dependent not only on the variety, but also on the instrument and the condition used for estimation as well as moisture content of the test sample.

**Table 3** De-hulling characteristics of IR36 paddy grown by FSE and field

Treatments	Weight, g					Hulling (%)	Whole kernel brown rice recovery (%)
	Initial Paddy	Brown rice after hulling		Husk	Paddy unhusked		
		Head rice	Broken rice				
1% FSE	38.28	20.28	8.1	6.8	3.1	74.13	52.97
2% FSE	33.63	18.56	6.7	5.77	2.6	75.11	55.18
Field rice	40	22.54	5.8	6.7	4.96	70.85	56.35

### Proximate composition

Several factors including the fertilizer treatment control the composition/nutritional quality of rice (Sunil et al. 2014). The proximate composition (sample basis, sb) of rice grown with 2% FSE, as produced a higher amount of grain (vide Table 2, t-test positive), is shown in Table 4.

**Table 4** Proximate composition of brown rice obtained using 2% FSE

Ingredient	Value (% , sb)
Water	13.50±0.30
Protein	7.40±0.55
Fat	1.71±0.52
Fibre	0.93±0.10
Ash	1.11±0.16
Carbohydrate	75.35

The crude protein and fat content of the rice are 7.40% and 1.71% (vide Table 4). From the available literature, the values of crude protein and fat content (sb) of IR36 brown rice are 9.6%-11% and 1.5%-1.8%, re-

spectively (Juliano 1992; Juliano et al. 1990). Thus, the fat % is within the range, whereas that of protein is less. As explained by Mosaad (2005), biofertilizer (microorganisms) in soil helps in the accumulation of grain protein, and based on this concept, the use of sterile sand beds in the present study may be the probable reason for such low protein. The value of ash content (Table 4) is 1.11%; according to Panlasigui et al. (1991), the value is 0.65% (sb) for milled rice sample containing 11.24% (sb) moisture. Since bran is high in ash content (Juliano 2016), the value in the present work appears to be agreeable. The crude fiber and the carbohydrate (by difference) are 0.93% and 75.35%. Comprehensively, the proximate composition in the present study is in the range to that of brown rice reported by Juliano (1993); Juliano (2016), and Juliano and Tuano (2019).

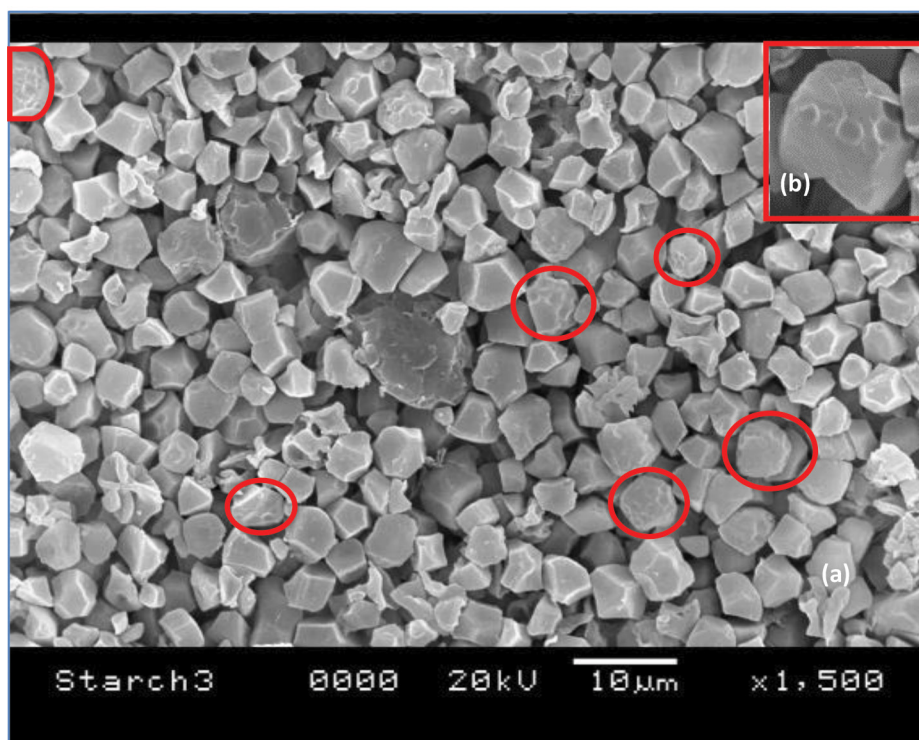
### SEM of isolated starch

Starch, a major component of rice is composed of individual granules, the morphology of which is affected, in addition to other factors, by agronomic practices (Musa et al. 2011; Wani et al. 2012). The morphological characteristics of starch granules of 2% FSE treated rice is

shown in scanning images in Plate 3(a) and inset (b). All the granules are seen to be more or less polyhedral and irregular in shapes with sharp edges (Musa et al. 2011; Wani et al. 2012; Fujita et al. 2012).

As explained by Dhital et al. (2015), starch granules, though initially generated in a rounded form, become angular as getting tightly packed with gradual accumulation in amyloplasts; during extraction, the compact assembled form gets broken and releases the individual polygonal granules. As shown in Plate 3, the FSE grown granules conform to such shape and is very much similar to that reported by Dhital et al. (2015) for IR36, however, presence of a few fused granule agglomerates is also evident. Based on size, Lindeboom et al. (2004) classified starch granules as large (>25.0  $\mu\text{m}$ ), medium (10.0 to 25.0  $\mu\text{m}$ ), small (5.0 to 10.0  $\mu\text{m}$ )

and very small (<5.0  $\mu\text{m}$ ). Thus, in the present study, the granule size is seen to be variable, the maximum size being <10  $\mu\text{m}$ . Wani et al. (2012); Juliano (1984); Li and Yeh (2001) and Yeh and Li (1996) indicated that rice starch granules are the smallest among cereals starches, and range within 2-10  $\mu\text{m}$ . On the other hand, Reddy and Bhotmange (2013) reported the diameter range of 10-150  $\mu\text{m}$  of Basmati, HMT and Swarna rice starches. Though some researchers commented on absence of naturally occurring pores on surface of rice starch granules (surface feature) (Fannon et al. 1992; Sujka and Jamroz 2007), Dhital et al. (2015), recently, evidence showed few pores on IR36 starch granules. Interestingly, some pores on the surface of the granules are also evident (enclosed by red outline) in the present study.



**Plate 3** Scanning electron micrograph of 2% FSE grown rice starch at (a) 1500X, (b) (inset) 3000X

## Conclusion

The FSE, generated from *Labeo rohita* scale during separation of its constituents, i.e., hydroxyapatite and collagen, contained several macro- and micro-elements and could be used as a fertilizer to produce paddy. Tested for IR36 on a sand bed in a plastic pot, 2% FSE solution produced the best result; the plant height at maturity, effective tillers/plant, spikelets/panicle, and yield/plant were 98 cm, 22.2, 90 and 44.2 g filled grain,

respectively. In terms of quality, the grain possessed an acceptable hulling % (75.11), nutritional quality, and starch granule morphology.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that there are no conflicts of interest associated with this study.

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