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Evaluation of egg-based fermented liquid formulation for improving organic rice yield

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Abstract

The productivity of organic farming relies on locally available or prepared organic inputs. One such locally organic formulation, fermented egg extract (FEE), requires scientific evaluation of its physicochemical and biological properties, as well as optimisation of its foliar spray concentration for enhanced rice growth and productivity. Preparation of FEE was carried out by immersing 10 eggs in lemon juice (extracted from 20-25 lemons) for 10 days. Then, eggs were crushed well and 250 g of jaggery was supplemented and set aside for another 10 days. The filtered liquid formulation was taken for laboratory analysis. The results on physicochemical and biological properties showed that the pH was acidic (4.8) and EC was 9.85 dSm^{-1}. It contains a considerable amount of plant nutrients such as N (0.93%), P (0.13%), K (0.15%), Ca (292 ppm), Mg (112 ppm), S (7.93 ppm), Cu (0.3 ppm), Zn (7.9 ppm), Mn (0.17 ppm) and Fe (8.4 ppm). Microbial populations namely total fungi, bacteria and actinobacteria found in FEE were 15 × 10^4, 197 × 10^6 and 3.23 × 10^2 (CFU ml^{-1}), respectively. A field study was carried out for 2 consecutive years to evaluate the impact of foliar spray of different FEE concentrations at panicle initiation and flowering stages of rice. The findings indicate that foliar spray of 2.0% FEE twice significantly improved growth, yield attributes, grain productivity (4897 kg/ha) and benefit-cost ratio (2.27) compared to untreated control. Thus, 2.0% fermented egg extract foliar application is identified as a potential nutrient formulation to enhance the productivity of organic rice.

Keywords

fermented egg extract; foliar spray; liquid formulation; organic rice; productivity

Introduction

Rice (*Oryza sativa* L.) serves as the primary source of nutrition for more than 50% of the world's population and is cultivated in over 100 countries (1). China, India and Indonesia are the highest rice producers in the world (2). Organic farming is practised in over 186 countries, covering 71.5 million ha, with the number of organic farms increasing annually (3). India occupies the 8th spot globally in organic land area, with a total certified area of 4.3 million ha, comprising 2.65 million ha of arable land and 1.68 million ha of wild harvest (4). The demand for organically produced rice has been steadily increasing both domestically and internationally, mainly driven by raising awareness of the positive environmental and health benefits of organic foods. Globally, the organic rice market is growing at a rate of 8% annually from 2019 to 2025 (5). Moreover, some research highlights that consumers are ready to purchase organic foods by paying up to 40% more in comparison to conventional foods (6). Organic rice cultivation is traditionally followed in many North Eastern states and Uttarakhand, especially before the widespread use of chemical fertilizers (7). Eventually, farmers moved to input-intensive conventional rice cultivation because of the low productivity of organically grown rice.

As organic cultivation of rice completely avoids inorganic fertilizers, it is crucial to supply necessary nutrients through organic sources throughout the cultivation period. Ensuring a continuous supply of nutrients during the entire growth period is important in organic rice production to avoid any nutrient deficiencies and stress. Presently, various organic manures such as farmyard manure, vermicompost, fly ash, pressmud and crop residues are commonly used in organic rice cultivation. In addition, numerous indigenous organic preparations, such as panchakavya, amirthakaraisal, amrithpani, vermiwash, fish waste extract etc., are applied to boost the performance of crops grown organically (8). Many studies found that these organic formulations had substantial quantities of macro and micronutrients, growth hormones and essential amino acids to boost crop growth and improve the immune system (9). Organic liquid formulations influence crop production in many ways, such as growth and development, disease and insect control, increased yield attributes, yield and quality. Further, foliar application of fermented organic products enriched with beneficial microorganisms in crop production not only improves plant health and productivity but also provides good-quality, non-residue food.

The success of organic farming depends on locally available or prepared organic inputs, which are economically sound. One such locally prepared organic formulation is fermented egg extract, which was originally conceived as medicine for asthma in Theni district of Tamil Nadu, India and later many farmers used it as foliar nutrition for plant growth (10). It is made from eggs, which have a wide range of essential amino acids, act as a potential organic fertilizer and plant growth stimulant. Supply of fermented egg extract through a drip system at 5 L/ha combined with the recommended NPK during vegetative and peak flowering stages, improved the performance of rice fallow greengram (11). Scientific evidence on the impact of fermented egg extract on crop growth and yields is very meagre. This study aimed to characterise the physicochemical and biological properties of FEE and to determine the optimal foliar spray concentration for enhanced rice growth and productivity.

Materials and Methods

Preparation of fermented egg extract (FEE)

The preparation of FEE is straightforward and utilises locally available inputs (Fig. 1). It was prepared by keeping 10 eggs immersed in juice extracted from 20-25 lemons and kept for 10 days in a plastic container. Then, eggs were crushed and 250 g of jaggery was added. The mixture was stirred and left for 10 days. After 10 days, the liquid portion was filtered in a separate container, collected and stored for lab analysis and field application (12).

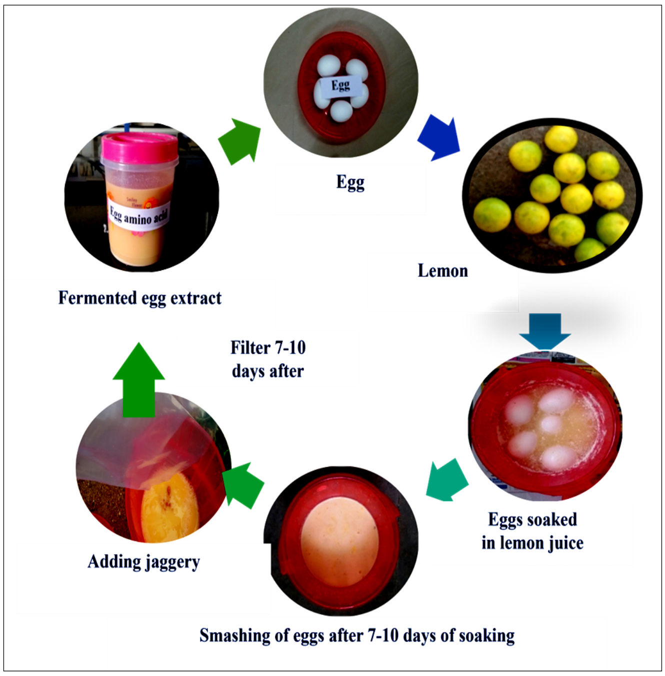

Fig. 1. Preparation of fermented egg extract.

Characterisation of FEE

The physiochemical and biological properties of the FEE were assessed after preparation. The pH and electrical conductivity (EC) of FEE were measured in triplicate. All macro and secondary nutrients were analysed by using standard methods. Total nitrogen was estimated using the Kjeldahl method (13). Total potassium was measured using flame photometry and total phosphorus was measured with colorimetry. Micronutrients (Zn, Fe, Cu and Mn) were analysed using the triple acid extract method and measured by the Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES), Model: 6500 series. The microbial population in the FEE was enumerated by the serial dilution plate count method. One milliliter of the FEE sample was taken and diluted with 9 mL of distilled water to get a 10^{-1} concentration and further dilutions were made up to 10^{-6}. Total fungi, bacteria and actinomycetes were enumerated by plating on suitable media (nutrient agar, rose bengal and Ken Knight's agar, respectively). The petriplates were incubated at 30 °C ± 1 °C, after varying incubation periods, depending on the microorganism and colonies were recorded as colony-forming units (CFU) per milliliter of sample.

Experimental design and crop management

Field trials were conducted during *Rabi*, 2021 & 2022 at Anbil Dharmalingam Agricultural College and Research Institute, Tamil Nadu Agricultural University, Tiruchirappalli. The trial plot soil was sodic (pH 9.1) with sandy clay loam in texture and moderately drainage. The soil contained low, medium and high available N (182 kg/ha), P (14.5 kg/ha) and K (296 kg/ha), respectively. The trial was conducted in a randomised block design with 4 replications. The experimental plot size was 8 × 5 m (40 m^2). Different concentrations of fermented egg extract (0.5, 1.0, 1.5 and 2.0%) for foliar spray and control were the treatments combination. Fermented egg extract at 2.5, 5.0, 7.5 and 10 L was mixed with 500 L of water to get 0.5, 1.0, 1.5 and 2.0% concentration, respectively. The foliar spray was done at panicle initiation (70-75 days after sowing) and flowering stages (100-105 days after sowing) of rice. In the control treatment, no foliar spray was followed. The sodic soil -tolerant rice variety TRY 3 was used. Recommended seeds (40 kg ha^{-1}) were treated with liquid biofertilizers of *Azsopirillum* and *Phosphobacteria* each at 125 mL and shade-dried for 30 min before sowing. Seedlings were raised organically by applying 10 kg of neem cake and 15 kg of vermicompost during the last ploughing in the nursery area of 800 m^2 for 1 ha of planting. Before pulling out the seedlings, 20 kg of gypsum was added to the nursery to prevent damage to the rootlets. In the main field, daincha was raised as a green manure and ploughed *in-situ* at 45 DAS using a tractor-drawn cage wheel. Through green manuring, 15.5 tonnes of green biomass and 57 kg/ha of nitrogen were added to the soil. Neem seed cake at 150 kg/ha and groundnut cake at 100 kg/ha were applied during the final ploughing and levelled before planting. Biofertilizer Azophos at 2.5 kg/ha and bio-control agent *Bacillus subtilis* at 2.5 kg/ha were mixed with 25 kg of farmyard manure and applied uniformly before transplanting. Seedlings were transplanted at 30 DAS with a spacing of 20 × 15 cm. During the seedlings' establishment period, irrigation water was kept at a depth of 2.5 cm and later 5 cm of water was kept. Totally 1484.4 and 1216 mm of water were used during *Rabi*, 2021 and 2022 respectively, which includes 984.4 and 416 mm of rainfall. The number of irrigations was 11 and 18 during *Rabi*, 2021 and 2022 respectively. Weed management was carried out using rotary weeders twice, 15 and 30 days after planting.

Biometric observation

Biometric observations such as plant height (cm), dry matter production (kg/ha) and grain productivity were assessed before crop harvest. The number of productive tillers was counted using 4 randomly selected quadrats measuring 0.25 m^2 each within the net plot area and the values were expressed as numbers m^{-2}. The chlorophyll content of the uppermost fully expanded leaves was measured at the milking stage (115-120 days after sowing) using the SPAD-502 chlorophyll meter (14). The soluble protein content of physiologically active leaves was estimated at 15 days after spray. The number of filled grains per panicle was assessed using 10 randomly selected panicles, threshed individually and counted. The crop was harvested when it was fully mature. The border rows on either side were harvested in each plot and removed from the field. The net plot was harvested, threshed and winnowed, grain was subsequently sun-dried to achieve 14% moisture and the grain yield was obtained. Grain protein content was analysed by following a standard method and expressed in percentage (15). The economics of rice cultivation such as net returns, gross returns and BCR were calculated based on the prevailing market prices.

Statistical analysis

The experimental mean data recorded during both years was subjected to statistical analysis (16). This included appropriate statistical tests and calculations to determine the significance of the treatment effects. To determine the significance between treatments, a 5% probability level CD value was used.

Results and Discussion

Physicochemical properties of FEE

After the preparation, the characterisation of FEE was carried out through laboratory analysis. The physicochemical properties of FEE are given in Table 1. The low pH (4.8) is attributed to microbial fermentation. The addition of jaggery during preparation was utilised by the microorganisms to produce lactic or acetic acid which led to acidic pH. The production of acetic acid was due to the fermentation of lactate and citrate or the breakdown of amino acids by bacteria (17). The occurrence of soluble salts in the fermented formulation increased the electrical conductivity of FEE (9.85 dSm^{-1}). Besides these, it contained a substantial quantity of nutrients viz., N (0.93%), P (0.13%), K (0.15 %), Ca (292 ppm), Mg (112 ppm), S (7.93 ppm), Cu (0.3 ppm), Zn (7.9 ppm), Mn (0.17 ppm) and Fe (8.4 ppm) which can be efficiently absorbed by the crops. The micronutrients present in the FEE formulation may serve as an effective source for delivering micronutrients through foliar supplementation during critical crop periods. The total bacteria, fungi and actinobacteria in FEE were 197 × 10^6, 15 × 10^4 and 3.23 × 10^2 CFU/ml respectively.

Impact of FEE on rice growth and physiological parameters

The results of the field experiment showed that a 2.0% FEE foliar spray resulted in statistically taller plants (114.9 cm) and higher DMP (12230 kg ha^{-1}) at maturity compared to both 0.5% FEE and the control (Table 2). However, the spray of 1.5% FEE (11805 kg ha^{-1}) was statistically comparable with 2.0% FEE. The application of FEE at 2% registered 9.4 and 14.3% of higher plant height and DMP over control treatment. The availability of macro- and micronutrients from FEE enhanced cell division and increased metabolic activity, leading to an increase in the height of the plants. Further, a continuous supply of nutrients via foliar spray during the panicle initiation and flowering stages enhanced photosynthetic activity, which increased the leaf area index (LAI) and light interception, ultimately boosting dry matter production. The control plot (without foliar spray) exhibited significantly lower plant height compared to FEE-treated plots in both years. Similarly, the availability of growth regulators such as IAA, GA_3, Cytokinin and important plant

Table 1. Physiochemical and biological properties of FEE

Parameters	Fermented egg extract
pH	4.80 ± 0.35
EC (dSm^{-1})	9.85 ± 0.71
Nitrogen (%)	0.93 ± 0.71
Phosphorus (%)	0.13 ± 0.01
Potassium (%)	0.15 ± 0.01
Calcium (ppm)	292 ± 21.08
Magnesium (ppm)	112 ± 8.09
Sulphur (ppm)	7.93 ± 0.57
Copper (ppm)	0.30 ± 0.02
Zinc (ppm)	7.90 ± 0.57
Manganese (ppm)	0.17 ± 0.01
Iron (ppm)	8.40 ± 0.61
Bacteria (10^6 CFU ml^{-1})	197 ± 18.80
Fungi (10^4 CFU ml^{-1})	15.00 ± 1.43
Actinobacteria (10^2 CFU ml^{-1})	3.23 ± 3.08

Data are the mean values of three replications with ± standard error (n=3).

nutrients from the organically fermented product panchakavya showed significant improvement in the rate of growth in *Alium cepa* (18). Similar effects of spraying fermented formulations like Panchakavya promoted fast cell division and multiplication, owing to the availability of growth enzymes, which contributed to increased plant height in *Phyllanthus* and higher plant height, green forage and dry matter yield in fodder cowpea (19, 20).

Foliar application of 2.0% FEE resulted in significantly higher mean leaf chlorophyll (SPAD reading 40.0) and soluble protein content (48.1 mg g^{-1}) compared to the control, 15 days after application (Fig. 2). There was 9.0% and 12.9% higher leaf chlorophyll and soluble protein in leaves respectively, when the foliar spray of 2.0% FEE was compared to control. The increase in chlorophyll and soluble protein content in leaves was mainly due to the enhanced release of nitrogen and micronutrients from the fermented egg formulation, which promoted their synthesis in growing tissues. Similarly, increased chlorophyll content in tomato leaves was observed primarily due to the availability of growth-promoting substances and substantial plant nutrients in the egg-lime mix with Panchakavya (18). The lower soluble protein content in leaves was observed in the control, primarily due to the limited availability of nitrogen in the soil. Similarly, a foliar spray of 1.0% FEE increased the leaf -soluble protein content in paddy (21).

Impact of FEE on yield parameters of rice

Rice yield parameters improved significantly with the foliar spray of FEE. Foliar spray of 2.0% FEE exhibited significantly more productive tillers (293 m^{-2}) and filled grains/panicle (132.4) than other treatments (Table 2). The 1.5% FEE treatment also showed a comparable effect, with an average of 281 productive tillers per square meter and 126.1 filled grains per panicle. The control plot recorded lesser productive tillers (242 m^{-2}) and filled grains/panicle (106.2). Applying a foliar spray of 2.0% FEE twice during the reproductive stage produced 21.2 and 24.7% more productive tillers and filled grains over control. This increase was primarily due to the macro and micronutrients supplied by the foliar spray, which enhanced nutrient assimilation, boosted photosynthetic activity and improved the source-sink relationship, ultimately leading to higher filled grain production per panicle. Similarly, more florets were noticed in orchids as compared to control under foliar spray of fermented organic fertilizers (22). A similar effect of the FEE on yield contributing characters and yield of rice fallow green gram was previously reported when it was supplied by a drip system critical growing period (vegetative and flowering) at a rate of 5 L/ha (23). The test weight of rice was not significantly affected by the foliar spray of FEE.

Impact of FEE on grain and straw productivity and HI of rice

Foliar application of 2.0% FEE in rice resulted in significantly higher mean grain weight (4897 kg/ha), straw yield (6874 kg/ ha) and harvest index (0.42) compared to 0.5% FEE and control treatments (Table 3). However, it was statistically significant with a 1.5% FEE. The control plot recorded a reduced grain yield of 4083 kg/ha. Fermented egg extract at 1.5% and 2.0% registered 15.6 and 19.9% of higher grain yields than control. This is primarily due to the improved uptake and assimilation of macro and micronutrients available in the FEE through foliar application during the panicle initiation and flowering stages. This enhanced nutrient uptake likely boosted metabolic activity and cell division, resulting in increased height in plants, more leaves and enhanced chlorophyll content. As a result, the photosynthetic activity increased, leading to improved yield attributes and higher grain yield in rice. Similarly, egg lime mix + panchakavya sprayed plants exhibited maximum fruit yield over control in tomato (24, 25). The presence of growth hormones, such as auxins, gibberellins and cytokinin, in the liquid organic formulations, plays a key role in boosting crop yields by enhancing the plant's metabolic functions (26). Similarly, the effects of another fermented formulation called fermented fish waste extract on the grain yield of amaranthus and grain yield of green gram were reported (7, 27).

Table 2. Impact of foliar spray of FEE on growth and yield parameters of rice

Treatments	Plant height (cm)			DMP (kg ha^{-1})			Productive tillers m^{-2}			Filled grains $panicle^{-1}$		
	2021	2022	Mean	2021	2022	Mean	2021	2022	Mean	2021	2022	Mean
T_1- 0.5% FEE	107.5	109.3	108.4	9573	12622	11098	249	256	253	108.0	121.1	114.6
T_2- 1.0% FEE	108.4	115.0	111.7	10172	13162	11667	265	264	265	115.3	129.3	122.3
T_3- 1.5% FEE	111.8	114.7	113.3	10083	13526	11805	271	290	281	119.0	133.2	126.1
T_4- 2.0% FEE	113.4	116.3	114.9	10673	13787	12230	281	304	293	126.7	138.1	132.4
T_5- Control	103.0	107.0	105.0	9425	11978	10702	228	256	242	100.3	112.1	106.2
SEd	2.1	2.4	2.3	195	311	253	7.0	7.9	7.5	3.2	3.0	3.1
CD (P=0.05)	4.4	5.0	4.7	410	653	532	14.0	16.6	15.3	6.8	6.3	6.6

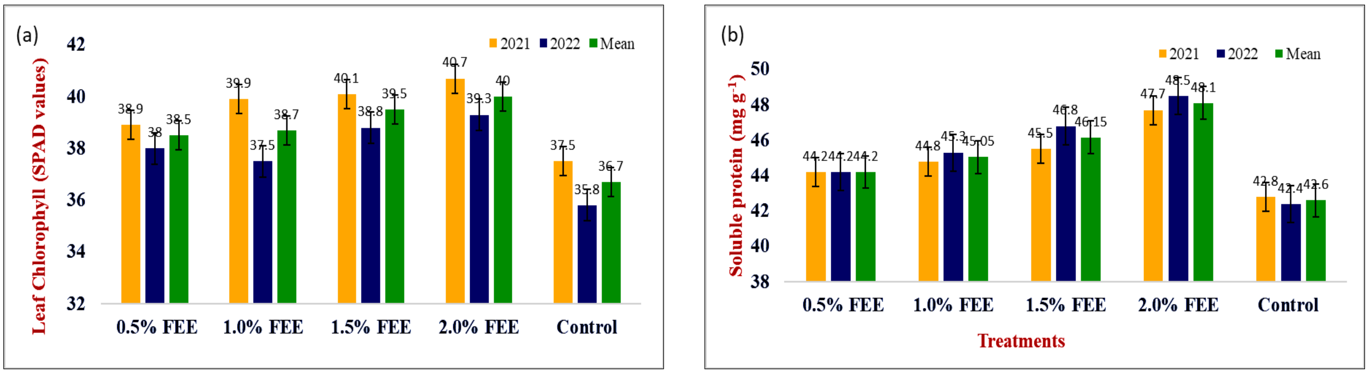

Fig. 2. (a) Leaf chlorophyll (SPAD value) and (b) soluble protein content of rice as significantly influenced by foliar spray of fermented egg extract.

Impact of FEE on grain protein

Grain protein content (8.51%) was significantly higher in foliar application of 2% FEE over control treatments (8.11%) (Table 3). Enhanced grain protein content was mainly due to the supply of nitrogen from FEE, which promoted the synthesis and accumulation of more protein in grains. Additional N application enhanced the total rice grain protein content as reported in earlier studies (28).

Economics of FEE spray on rice

The economic impact of foliar spray of FEE on organic rice production was significant (Table 4). The foliar application of 2.0% FEE resulted in the highest economic returns, with gross returns of Rs. 102008/ha, net returns of Rs. 57303/ha and a benefit-cost ratio (BCR) of 2.27, outperforming lower FEE concentrations. The second most effective treatment was foliar application of 1.5% FEE. The lower cost involved in egg extract preparation and the increased grain yield under this treatment were the reasons for the higher economics. Lower concentrations of egg extract gave reduced net returns and BCR than higher concentrations. Similar results were observed in previous studies, where foliar sprays of fermented panchagavya formulation led to increased net returns and BCR in fodder cowpea (20). The control treatment resulted in the lowest economic returns for rice production.

Conclusion

The study provides valuable insights into the egg-based formulation as a source of nutrients for organic rice cultivation. The increasing demand for pesticide-residue-free food and environmentally safe production practices has heightened interest in organically grown rice. The success of organic rice cultivation relies on the effective nutrient management practices implemented. A continuous and balanced supply of nutrients through organic formulation is essential in maintaining rice productivity. Currently, the use of organic liquid formulations made from locally available resources is gaining popularity among organic farmers. The present study fascinated the characterisation and effect of fermented egg extract on rice productivity. The preparation of fermented egg extract requires very little basic skill with minimum cost involved. It contains both macronutrients and essential micronutrients, besides beneficial microorganisms, which are absolutely required for rice growth and yield. Foliar application of 2.0% FEE twice during panicle initiation and flowering stages produced statistically more grain yields of rice (4897 kg ha^{-1}) and BCR (2.27) than without spray. Thus, egg-based fermented liquid formulation is a potential nutrient source for improving organic rice productivity. Further, the FEE can be tested on other crops and its storability period in future lines of research.

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Authors' contributions

RT and RS did the conceptualisation and methodology of the experiment. UND did lab analysis and statistical analysis. RT conducted the field experiment, collected data and wrote the first draft of the manuscript. RS did a second revision and edited the manuscript. All authors have read and agreed to publish the manuscript.

Table 3. Impact of foliar spray of FEE on grain and straw yields, harvest index and grain protein content of rice

Treatments	Test weight (g)			Grain yield (kg ha^{-1})			Straw yield (kg ha^{-1})			Harvest Index			Grain protein (%)		
	2021	2022	Mean	2021	2022	Mean	2021	2022	Mean	2021	2022	Mean	2021	2022	Mean
T_1- 0.5% FEE	23.8	24.0	23.9	3954	4658	4306	5432	7263	6348	0.42	0.39	0.41	8.12	8.23	8.18
T_2- 1.0% FEE	24.1	24.2	24.2	4226	4920	4573	5737	7542	6640	0.42	0.39	0.41	8.23	8.34	8.29
T_3- 1.5% FEE	24.3	24.3	24.3	4332	5105	4719	5544	7722	6633	0.42	0.40	0.41	8.42	8.45	8.44
T_4- 2.0% FEE	24.4	24.5	24.5	4525	5268	4897	5929	7818	6874	0.43	0.40	0.42	8.45	8.56	8.51
T_5- Control	23.7	23.9	23.9	3726	4440	4083	5514	7038	6276	0.41	0.39	0.40	8.03	8.18	8.11
SEd	0.4	0.4	0.4	109	135	122	154	218	186	0.01	0.01	0.01	0.16	0.17	0.17
CD (P=0.05)	NS	NS	NS	229	284	257	323	458	391	0.02	0.02	0.02	0.35	0.37	0.37

NS- Non significant .

Table 4. Impact of foliar spray of FEE on economics of rice

Treatments	Gross return (Rs. ha^{-1})			Net return (Rs. ha^{-1})			Benefit Cost Ratio		
	2021	2022	Mean	2021	2022	Mean	2021	2022	Mean
T_1- 0.5% FEE	77390	103033	90212	37478	57210	47344	1.94	2.25	2.10
T_2- 1.0% FEE	82649	108563	95606	41874	61765	51820	2.03	2.32	2.18
T_3- 1.5% FEE	84379	112429	98404	41879	63681	52780	2.09	2.31	2.20
T_4- 2.0% FEE	88280	115735	102008	46643	67962	57303	2.12	2.42	2.27
T_5- Control	73320	98437	85879	36070	55437	45754	1.97	2.29	2.13
SEd	1662	2203	1932	835	1253	1044	0.04	0.05	0.04
CD (P=0.05)	3573	4736	4155	1795	2693	2244	0.09	0.10	0.10

Compliance with ethical standards

Conflict of interest: The author declares that there is no conflict of interest.

Ethical issues: None

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