Effects of Cattle Biogas Effluent Application and Irrigation Regimes on Rice Growth and Yield: A Mesocosm Experiment

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Abstract

Biogas effluent (BE) is a potential source of fertilizer for rice. Alternate wetting and drying (AWD) irrigation based on surface water level can increase rice grain yield (GY), but its effect under BE application is still unclear. To test whether AWD can increase GY under BE application, we conducted a mesocosm experiment under screen-house conditions in Can Tho, Vietnam. We measured rice growth and yield under three nitrogen (N) treatments—zero-N (Z), synthetic fertilizer (SF), and BE—and two irrigation regimes—continuous flooding (CF) and AWD with a re-irrigation threshold of 15 cm below the soil surface. Chlorophyll content (SPAD) and leaf color chart (LCC) values were higher in SF than in BE, but plant height was comparable. Although GY was not significantly different between CF and AWD, AWD increased it by 12% in BE relative to CF. All N use efficiency (NUE) indices (apparent N recovery, agronomic NUE, and internal NUE) were comparable between AWD and CF in BE and SF. The results indicate that AWD irrigation is feasible under the application of cattle BE and the combination can improve rice GY.

Discipline: Agricultural Environment Additional key words: alternate wetting and drying, leaf color chart, nitrogen use efficiency, organic fertilizer, SPAD

Introduction

Rice (*Oryza sativa* L.) is the main agricultural crop in Vietnam and is grown on 82% of the total agricultural production area or approximately 7,500 ha, with a total annual yield of 44,000 t (GSOVN 2018, Vu et al. 2018). The Mekong Delta is called the "rice bowl" of the country, with a total area of 3.9 million ha; it accounts for 45% of the total rice area and produces 55% of Vietnam's agricultural products, predominantly rice and vegetables, and aquaculture, and 57% of the country's total rice output (Shrestha et al. 2016, Quan et al. 2020). In recent years, intensive rice farming in Vietnam has brought excessive use of inorganic fertilizers and pesticides, which has led to serious environmental problems, such as water pollution (Stone & Hornberger 2016) and greenhouse gas emissions (Tran et al. 2018). Rice farmers have adopted alternate wetting and drying (AWD) irrigation to improve the use of water resources and reduce environmental issues (Yang et al. 2007, Wang et al. 2016). Rice grain yield (GY) increases with AWD irrigation in rice fields when synthetic fertilizers (SFs) are used (Ye et al. 2013, Ullah et al. 2018, Uno et al. 2021). Can it also do so with organic fertilizer, which supports sustainable agriculture and improves soil fertility (Assefa & Tadesse 2019)?

In Vietnam, anaerobic digester is a popular technology for treating livestock wastes (Ho et al. 2015, Kinyua et al. 2016) because it is inexpensive, effective,

*Corresponding author: hckhanh@ctu.edu.vn Received 21 September 2021; accepted 11 January 2022. and locally available (Yen Phi et al. 2009). Moreover, this technology can help solve environmental issues (Dhingra et al. 2011) and produce biogas (a mixture of CH₄ and CO₂) for home cooking or lighting (Zhang et al. 2013, Naik et al. 2014). Additionally, biogas effluent (BE) contains enough nitrogen (N; mainly as ammonium, NH₄-N), phosphorus (P), and potassium (K) to have agronomic benefits if used as a soil amendment (Kinyua et al. 2016). Therefore, BE is used as fertilizer (Adu-Gyamfí et al. 2012, Grima-Olmedo et al. 2014). BE was used as an N fertilizer to grow rice and upland crops (Haraldsen et al. 2011; Oritate et al. 2016; Gao et al. 2019; Huynh et al. 2019; Minamikawa et al. 2020, 2021). However, these experiments focused on continuous flooding (CF) conditions, not AWD conditions, except for Minamikawa et al. (2021). Therefore, this study was conducted to test whether AWD can increase GY when BE was used. Plant characteristics that are closely related to yield formation under mesocosm conditions were determined.

Materials and methods

1. Plant materials and study site

A mesocosm experiment was conducted in a screen-house at Can Tho University, Can Tho, Vietnam (10°01'41"N, 105°45'33"E). The experiment lasted 91 days, from September 20 to December 22, 2018. The mean outside air temperature and mean outside daily solar radiation during the experiment measured at Can Tho Meteorological Station (0.5 km east of the screen-house) were 27.7°C and 381 W m⁻² d⁻¹, respectively. The seed of paddy rice cultivar "OM5451" was purchased from Cuu Long Delta Rice Research Institute in Can Tho.

2. Soil properties and cattle BE characteristics

The soil was collected from the plow layer, 1 week before the experiment started, (0 cm-20 cm) of a farmer's rice field in Can Tho ($10^{\circ}10'37''N$, $105^{\circ}32'49''E$) during the summer–autumn rice season. The soil was a silty-clay Fluvisol (clay 52%, slit 48%, sand 0.3%) (Van et al. 2014) with the following initial properties: pH(H₂O), 5.36; organic matter, 7.82%; total N, 3.4 g N kg⁻¹; CEC, 17.07 meq 100 g⁻¹; exchangeable Mg, 4.79 meq 100 g⁻¹; exchangeable Ca, 9.12 meq 100 g⁻¹; and exchangeable K, 0.54 meq 100 g⁻¹.

BE was collected from a biogas digester operating for 3 years on a cattle smallholder farm in Can Tho and stored in a PVC tank with a lid inside the screen-house, which was well mixed by hand before each application. Its chemical characteristics are shown in Table 1.

3. Experimental design

The experiment was arranged in a completely randomized design with two irrigation regimes (CF and AWD) and three N levels (BE, SF, and a control treatment (zero-N, Z)). The experiment was conducted with six treatments, each in triplicate, as follows: Z-CF, Z-AWD, BE-CF, BE-AWD, SF-CF, and SF-AWD. Each treatment was applied in a plastic container with an area of 0.24 m² (60 cm \times 40 cm \times 30 cm deep) and filled with approximately 62 kg of unsieved wet soil to a depth of approximately 20 cm. A perforated PVC pipe 10-cm wide and 30-cm long was installed vertically in each container to a depth of 20 cm to measure the water level. In the experiment, 18 containers were closely laid out in an array of 3×6 . The array was surrounded closely by 22 extra containers with conventional rice cultivation (i.e., SF-CF treatment). In each container, pregerminated rice seeds were sown at the equivalent of 212 kg ha⁻¹ (i.e., 848 seeds m⁻²) on the leveled saturated soil. The height of rice plant canopy was adjusted by using wooden boards under containers to ensure that all treatments had the same growing conditions and applied adequate pest control.

4. Fertilizer types

In the control (Z) treatment, no N fertilizer was applied, but single superphosphate (16% P_2O_5) and potassium chloride (60% K_2O) were applied at 10, 25, and 40 days after sowing (DAS) in flooded conditions. They were split-applied at 40 kg P_2O_5 ha⁻¹ (13.3–13.3–13.3) and 60 kg K_2O ha⁻¹ (0–30–30).

In the SF treatment, urea (46% N) was applied three times at the same timing as in the control treatment at a total of 150 kg N ha⁻¹ (30–70–50). P and K fertilizers were applied as in the control treatment.

In the BE treatment, BE was applied at the same timing and the same total N rate as in the SF treatment. Based on the total Kjeldahl-N concentration (N-TKN; organic N + NH_4 -N), the amount of effluent applied was determined. The effluent was carefully hand-poured into each container: 6.01 L at 10 DAS, 12.22 L at 25 DAS,

 Table 1. Chemical characteristics of biogas effluent

 with loading from cattle manure

Variable	10 DAS †	25 DAS	40 DAS
pH (H ₂ O)	7.58	7.73	7.68
NH_{4}^{+} (mg N L ⁻¹)	98.7	98.2	90.4
Total Kjeldahl-N (mg N L ⁻¹)	117.8	116.1	98.6
$NO_3 + NO_2 (mg N L^{-1})$	1.17	5.83	4.08
Total P (mg P L^{-1})	49.8	-	_
Total K (mg K L ⁻¹)	775	_	_
1 20 1			

† Days after sowing

- Not determined

and 14.39 L at 40 DAS. The P content in BE was low, so inorganic P fertilizer was supplemented to the same total rate as in SF. K was applied as BE at a rate dependent on the K concentration in the effluent.

5. Water management

The water management schedules for CF and AWD are shown in Figure 1. The water depth in the containers was maintained by manual monitoring three times a week. A shallow depth (1 cm-5 cm) was maintained in both water regimes for the first 10 DAS to facilitate seedling establishment. In CF, the water depth was then maintained between +1 and +10 cm until the final drainage 15 days before harvest. In AWD, the soil surface was allowed to naturally dry until the mean water depth of three replicates dropped to -15 cm, and then the containers were reirrigated to a depth of +5 cm (Bouman et al. 2007, Li & Li 2010, Chidthaisong et al. 2018). Note that flooded conditions were retained during rice heading, and fertilizer and pesticide applications even in AWD.

6. Measurements

Before each application, we analyzed the pH, total N, nitrate-N + nitrite-N (NO₃-N + NO₂-N), NH₄-N, total P, and total K of the BE. pH was measured by a handheld meter (HM-31P; TOA-DKK, Japan). The total N was determined as the sum of Kjeldahl-N and Kjeldahl-N with Devarda's Alloy (for $NO_3-N + NO_2-N$) as analyzed using the semimicro Kjeldahl method (APHA 1998). NH₄-N, total P, and total K were analyzed by the phenate method, ascorbic acid method, and flame photometric method, respectively (APHA 1998). Twice a week, plant height, leaf color chart (LCC) value, and SPAD value were recorded from the same 12 plants in each container and averaged. Plant height was measured from 7 to 70 DAS. LCC and SPAD were recorded from 21 to 81 DAS. The color of the topmost fully expanded leaf was determined twice a week from 21 to 81 DAS against the LCC developed by the International Rice Research Institute, Philippines. The resolution for reading the LCC value by eye was set at 0.25. SPAD was measured with a chlorophyll meter (SPAD-502; Konica Minolta, Japan) at



Fig. 1. Schematic diagram of the water management plan in CF and AWD

Values indicate the days after sowing.

the same times as LCC was measured.

All aboveground parts of the plants in each container were collected after the experiment and separated into straw and panicles to determine the yield components (number of panicles, spikelets per panicle, filled grains, and 1,000-grain weight), total straw biomass, GY, and harvest index (HI). The dry weights of the straw biomass and GY were determined after oven-drying at 70°C to a constant value. The GY (rough rice) was expressed as 14% moisture content. HI was calculated as total grain weight (dry weight) divided by total aboveground biomass (dry weight) (Wang et al. 2016).

As determined above, the total plant N uptake was computed as the sum of the products of the biomass and total N concentration of each plant part (grain, leaf, and stem). The three indices of N use efficiency (NUE; apparent N recovery, agronomic NUE, and internal NUE) were determined as described by Cabangon et al. (2011). Apparent N recovery (g $N_{uptake} g^{-1} N_{applied}$) was calculated as the difference in the mean total N uptake of the three replications between fertilized and unfertilized treatments (control treatment) divided by the total N application rate. As the difference in the mean GY of the three replications between fertilized and unfertilized treatments was divided by the total N application rate in the fertilized treatment, agronomic NUE $(g_{grain} g^{-1} N_{applied})$ was calculated. Internal NUE $(g_{grain} g^{-1} N_{uptake})$ was calculated as GY divided by the total N uptake.

7. Statistical analysis

Statistical analyses were performed in SPSS v.26.0 software (SPSS Inc., Chicago, IL, USA). To test the two irrigation regime effects and N treatments, a two-way analysis of variance (ANOVA) was performed and their interactions, on the yield components, GY, straw biomass, HI, total N uptake, and internal NUE. The differences between/among treatments were analyzed by Tukey's HSD test. All differences were compared at the 5% level of significance.

Results

1. Surface water depth

The surface water depth in the CF treatments was managed as planned (Fig. 1), with the water level in all containers maintained at +2 to +10 cm throughout the experiment (7-75 DAS) (Fig. 2). In the AWD treatments, effective water control was achieved twice with the minimum water level reaching -15 cm, at 38 and 52 DAS. The water depth often dropped faster to -15 cm in the BE treatments than in the SF treatments.

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2. Plant height, SPAD value, and LCC value

Generally, rice growth was normal under the mesocosm conditions. Rice heading (i.e., the time to 50% panicle emergence) was observed at 62-63 DAS. Plant height was slightly different between BE and SF in the N-fertilizer treatments (P > 0.05) and was higher in those than in the control (Z) from 15 to 70 DAS (Fig. 3A). The final plant height was 64.9 cm-67.6 cm in Z, 84.5 cm-87.0 cm in BE, and 85.7 cm-87.1 cm in SF.

The SPAD and LCC values could be divided into three groups (Fig. 3B, C): control, with the lowest value (Z-CF and Z-AWD); BE (BE-CF and BE-AWD); and SF (SF-CF and SF-AWD). Both were lower in BE than in SF almost through the experimental period. Because of the parallel seasonal variations, LCC and SPAD values were positively correlated [SPAD = $9.33 \times LCC + 2.61$ ($R^2 = 0.87$), data not shown].

3. GY and its components

The number of panicles, filled grains, and 1,000-grain weight did not differ significantly among the six treatments (Table 2). However, filled grains and 1,000-grain weight were marginally higher in AWD than in CF (P < 0.1). Spikelets per panicle were significantly higher in CF than in AWD and in N-fertilizer than in no-N-fertilizer treatments.

GY differed slightly between CF and AWD, but they were slightly higher in AWD than in CF (P = 0.136, Table 2). Under CF, GY was highest in SF-CF, but under AWD, it was highest in BE-AWD. GY differed significantly between no-N-fertilizer and N-fertilizer treatments. Straw biomass showed similar results as GY, and HI was significantly affected by N treatment. GY had positive correlations with both SPAD and LCC values [GY = 63.89 × SPAD-1,427 (R^2 = 0.81) and GY = 644.2 × LCC-1,418 (R^2 = 0.82), data not shown].



Fig. 2. Surface water levels as affected by N treatment and irrigation regimes

4. Plant N uptake and NUE

Total plant N uptake was significantly affected by N treatment and irrigation regime (Table 3). AWD increased the uptake by 2.5% compared to CF. Although the statistical test was not applied, apparent N recovery and agronomic NUE showed the opposite response to AWD between SF and BE. Internal NUE was significantly affected by only N treatment; however, AWD always, slightly increased it (P = 0.239).

Discussion

1. Rice response to BE application

In the biogas digester, organic matter is decomposed, and the nutrients are mineralized into directly plantavailable forms, mainly NH_4 (45%-80% of the total N), NO_3^- , and PO_4^{3-} (Möller & Müller 2012). Rice takes up NH_4 directly (Bonten et al. 2014). This explains the normal growth with BE. The results are consistent with the previous studies, which reported that cattle BE could replace SFs in rice cultivation under CF conditions, and the plants were not adversely affected by BE (Huynh et al. 2019, Minamikawa et al. 2020).



Fig. 3. (A) Plant height, (B) SPAD value, and (C) LCC value as affected by N treatment and irrigation regimes Vertical bars indicate SEM (n = 3).

Plant height did not differ significantly between BE and SF, but SPAD and LCC values were higher in SF (Fig. 3). Total N uptake also tended to be higher in SF than in BE (Table 3), thus this is consistent with the higher SPAD and LCC values in SF. Our previous studies also observed higher SPAD and LCC values in SF than in BE (Minamikawa et al. 2020, 2021). This may have enhanced the loss of applied N by the denitrification process (Hou et al. 2007, Lindorfer et al. 2007) because BE included organic matter. However, the differences in SPAD and LCC values between SF and BE shrank to harvest (Fig. 3). This would be partly explained by the non-negligible proportion of organic N in the total applied Kjeldahl-N in BE, which gradually decomposed into plant-available N. Ammonium (NH_3) volatilization from BE application is limited under low pH conditions in soil and surface water (Nakamura et al. 2018). Because this study used acidic soil, NH₃ volatilization from the application of BE with a neutral pH would have also been limited.

N is the most important element for plant growth and the most important nutrient limiting crop yield formation. The three NUE indices tended to be higher in BE-AWD than in BE-CF (Table 3). In contrast, in SF, the apparent N recovery and agronomic NUE were lower in SF-AWD than in SF-CF. Yao et al. (2012) reported that apparent N recovery and agronomic NUE from SF were better under CF irrigation than under AWD irrigation, as SF of our study. Our three NUE indices were comparable to the

 Table 2. Yield components, straw biomass yield, grain yield, and harvest index in two irrigation regimes and three types of fertilizers in the mesocosm experiment

Treatment	Panicle	Spikelets per	Filled grains	1,000-grain	Straw biomass	Grain yield †	Harvest
Treatment	number (m ⁻²)	panicle	(%)	weight (g)	(g m ⁻²)	(g m ⁻²)	index ‡
Z-CF	606	44.7 ^b	85.3	26.9	447 ^b	359 ^b	0.407 ^b
Z-AWD	617	41.1 ^b	87.9	28.5	454 ^ь	420 ^b	0.453 ^{ab}
SF-CF	626	84.8 ^a	85.9	25.6	785 a	815 a	0.490 ab
SF-AWD	615	69.9 ª	87.1	29.1	831 a	830 a	0.480 ^{ab}
BE-CF	603	79.7 ª	83.2	26.3	731 a	768 a	0.483 ^{ab}
BE-AWD	610	76.8 ^a	90.2	27.3	807 a	863 a	0.500 a
P-value							
N treatment (N)	0.64	***	0.993	0.718	***	***	*
Irrigation regimes (IR)	0.948	*	0.057	0.074	0.169	0.136	0.302
N imes IR	0.184	0.194	0.367	0.55	0.633	0.663	0.365
⁺ Γ 1 140/	:						

[†] Expressed as 14% moisture content

[‡] Calculated on a dry-weight basis

Asterisks indicate significant differences at ***P < 0.001 and *P < 0.05 by two-way ANOVA

Different letters within the same column indicate significant difference at P < 0.05 by Tukey's HSD

	Table 3.	Nitrogen	use efficiency	(NUE)	indices in	the mesocosm	experiment
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Treatment	Total plant N uptake	Apparent N recovery †	Agronomic NUE [‡]	Internal NUE [¶]
Heatment	(g N)	$(gN_{uptake} g^{-1}N_{applied})$	$(g_{grain} g^{-1} N_{applied})$	$(g_{grain} g^{-1} N_{uptake})$
Z-CF	28.1 ^b	_	_	28.7 ^b
Z-AWD	28.9 ^ь	_	_	30.3 ^b
SF-CF	33.5 ª	0.684	13.9	47.8 ^a
SF-AWD	33.9 ^a	0.641	12.5	48.9 ª
BE-CF	32.0 ª	0.494	12.4	46. 9ª
BE-AWD	33.1 ª	0.532	13.4	50.5 ª
P-value				
N treatment (N)	***	_	_	***
Irrigation regimes (IR)	*	_	_	0.239
N×IR	0.754	_	_	0.813

[†] Ratio of difference in total N uptake between fertilized and control treatments to the total N application rate in the fertilized treatment

[‡] Ratio of difference in grain yields between fertilized and control treatments to the total N application rate in the fertilized treatment

[¶]Ratio of grain yield to total N uptake

- Not determined

Asterisks indicate significant differences at ***P < 0.001 and *P < 0.05 by two-way ANOVA.

Different letters within the same column indicate significant difference at P < 0.05 by Tukey's HSD.

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ranges recorded in the previous studies in Asia (Cabangon et al. 2011, Minamikawa et al. 2020). AWD in BE may have ameliorated the reductive soil conditions developed by organic matter input from BE (Minamikawa et al. 2021). Future studies should elucidate the underlying mechanisms of the higher tendency of NUE indices in AWD than in CF under BE application.

2. Effects of AWD irrigation on GY and its components

The water depth in BE-AWD dropped faster to -15 cm than that in SF-AWD at 38 and 52 DAS (Fig. 2). Although the underlying mechanism of this phenomenon is an open question in this study, we suggest one possible reason. That is, the used BE may have contained high solid volume, as reported in the previous studies (e.g., Kobayashi et al. 1989, Nakamura et al. 2017), though this study did not measure it. The greater the solid content is, the smaller the water volume in BE (surface water) is. This can also promote crack formation on the soil surface, thereby accelerating the water depth drop. It is necessary to elucidate the effect of BE application along with AWD on water depth.

GY was 12% greater in BE-AWD than in BE-CF although not significant (Table 2). The observed GY level in BE (768-863 g m^{-2}) is comparable to those in the previous studies, in which BE-CF gave rice yields of 521-764 g m⁻² (Minamikawa et al. 2020) and >1,090 g m⁻² (Huynh et al. 2019). In SF, GY was only 2% greater in AWD than in CF (though not significant). According to Uno et al. (2021), AWD increased GY by 22% relative to CF under field conditions with a triple rice cropping system in the Mekong Delta. As per our results under mesocosm conditions, there is no negative effect of AWD on GY, and the combination of BE and AWD can increase GY. Therefore, BE is a potential source of fertilizer for rice in both CF and AWD conditions. However, a longterm continuous application of BE should take care of the accumulation of its ingredients in soil. Minamikawa et al. (2021) reported that exchangeable Mg and available P increase in paddy soil when BE is continuously applied as organic fertilizer, but exchangeable Ca and K do not. Nkoa (2014) reported the accumulation of metal elements (Cu, Zn, and Mn) by BE application in paddy field. High salinity of many liquid digestates may make them unsuitable as organic fertilizer in a long term (Alburquerque et al. 2012, Wang et al. 2019).

The results of yield component revealed that AWD recovered (rather improved) GY by increasing filled grain and 1,000-grain weight despite significantly lower spikelets per panicle (Table 2). Spikelets per panicle is affected by N uptake and dry matter production during the reproductive growth stage. The drainage in AWD,

excluding rice heading stage, may have adversely affected the spikelet formation. Weather conditions and agronomic practices during the heading and maturing stages affect filled grain. The 1,000-grain weight is generally stable but slightly affected by plant nutrient status and weather conditions before flowering. Because the weather conditions were identical, the drainage in AWD may have contributed to improving filled grain and 1,000-grain weight, relative to CF. As mentioned above, the amelioration of reductive soil conditions by AWD would have provided some preferable effects on grain formation. It is necessary to study the detailed mechanisms of improved or worsened yield components by AWD. The panicle number in Z comparable to those in SF and BE (Table 2) would be mainly explained by plantavailable N remaining in the used soil. Due to the soil collection from the farmer's field was conducted during the summer-autumn rice season, some of the applied urea (at >200 kg N ha⁻¹ season⁻¹) would have remained as plant-available N in the soil.

3. Feasibility of cattle BE for rice production

BE application achieved conventional grain and straw yields under both irrigation regimes (Table 2). The results reconfirm that cattle BE is a practical organic fertilizer for rice cultivation, as previously reported for cattle BE (Huynh et al. 2019, Minamikawa et al. 2020) and for pig BE (Vu et al. 2015, Oritate et al. 2016). The concentrations of N and P were lower in the tested cattle BE than in pig BE used in Vietnam (Thao et al. 2017b); however, they were similar to those in cattle BE used in other studies (Huynh et al. 2019, Hai et al. 2020, Minamikawa et al. 2020). Household anaerobic digesters take in livestock manure for biogas (CH₄) production, but barn-washing wastewater inevitably reduces the N and P concentrations in BE, as reported by Hai et al. (2020). Here, BE provided sufficient N and K for rice plant growth, but P was supplemented from SF. Although BE could be applied as an NPK fertilizer source, it also contains high concentrations of pathogens, which can harm soil quality and human health (Huong et al. 2014, Hai et al. 2020). Therefore, further research should be conducted on the pathogen reduction in BE before it is applied to paddy fields.

In Vietnam, other studies reported the application of pig BE to upland crops such as corn (Thao et al. 2017a) and chili peppers (Nu et al. 2015). However, these studies used only a small amount of BE, whereas huge volumes of BE are discharged into the open every day: by the majority of farmers in Hanoi (79%) and Hue (56%) into household gardens, canals, lakes, and occasionally the public sewer system (Huong et al. 2014). Thus, the use of BE in rice cultivation not only offers optimum use of available BE but also helps reduce the concentrations of pollutants in water bodies. Additionally, soil quality can be improved, as the NH_4 + concentration in paddy soil was increased when BE was applied to paddy fields (Thao et al. 2017b). Garg et al. (2005) also reported that soil physical properties are improved after anaerobic digester slurry is applied by reducing bulk density and increasing moisture retention capacity. Therefore, BE offers an alternative organic fertilizer for rice cultivation.

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