Black rice cv. ‘Chakhao Amubi’ (*Oryza sativa* L.)
Response to organic and inorganic sources of nutrients on growth, yield and grain protein content

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Abstract
A field experiment was conducted at Crop Research Farm, SHUATS, Allahabad in 2016 Kharif season to assess the effect of different sources and levels of nutrients on growth, yield, and grain protein content of Black rice cv. ‘Chakhao Amubi’. The experiment was laid out in Randomized Block Design, replicated thrice and consisted of 12 treatments. The results revealed that different sources of nutrients along with different levels of RDF (Recommended Dose of Fertilizer *i.e.*, 75–40–40 N-P-K kg ha\(^{-1}\) for Uttar Pradesh Kharif season) have significant effect on growth, yield, grain protein content and economics of cultivation of black rice. Treatment T\(_5\) (FYM @ 10 tonnes + 100 % RDF through inorganic sources) performed significantly higher than the other treatments with higher number of effective tillers/hill (11.70), panicle length (25.56 cm), number of spikelets/panicle (230.40), number of filled spikelets/panicle % (87.67), test weight (24.66 g), grain protein content (13.20 %) and consequently grain yield (5016 kg ha\(^{-1}\)) and straw yield (13550 kg ha\(^{-1}\)). This research depicts that integrated use of organic and inorganic sources of nutrients to be the best method for increasing productivity of Black rice.

Keywords: Black Rice, FYM, Organic, Inorganic, Protein, Chakhao

Introduction
Black rice is a type of the rice species *Oryza sativa* L. which is glutinous, packed with high level of nutrients and mainly cultivated in Asia. Black rice is also known as purple rice, forbidden rice, heaven rice, imperial rice, king’s rice and prized rice. This rice includes several varieties with a long history of cultivation in Southeast Asian countries such as China, India and Thailand (Kong *et al*., 2008)\(^{17, 34}\). There are more than 200 types of black rice varieties in the world. Only China is responsible for 62 % of global production of black rice and it has developed more than 54 modern black rice varieties with high yield characteristics and multiple resistances. China cultivates most of the black rice followed by Sri Lanka, Indonesia, India and Philippines etc. Thailand occupies the ninth position to black rice cultivation (Ichikawa *et al*., 2001; Sompong *et al*., 2011)\(^{[17, 34]}\). The term ‘black rice’ actually refers to a variety of rice types from the species *Oryza* sativa, and is descriptive of the colour of grain, rather than other properties. Black rice also comes in a number of short grain, long grain and glutinous varieties similar to brown rice. The dark purple colour of Black rice is due to the high anthocyanin content, located in the pericarp layers (Takashi *et al*., 2001)\(^{36}\). Rice varieties with coloured pericarp (other than white and red) are usually known as ‘Black rice’. In Asian countries, Black rice is popular and mixed with white rice prior to cooking to enhance the flavour, colour and nutritional value (Yang *et al*., 2003)\(^{39}\). Black rice also contains higher levels of proteins, vitamins and minerals than common white rice (Suzuki *et al*., 2004)\(^{35}\). Compared to white rice, black rice is relatively richer in the mineral contents such as Fe, Zn, Mn and P and has higher variability in mineral content depended upon varieties and soil types of the planting area (Qiu *et al*., 1993; Liu *et al*., 1995; Zhang, 2000)\(^{[31, 23, 40]}\). Supplementation of black scented rice in the diet will have a great impact on human health (Asem *et al*., 2015)\(^{11}\). The escalating prices of chemical fertilizers and world energy crisis have diverted attention of agronomists and soil scientists to find out the other possible alternate source of nutrients. Khan *et al*., 2006\(^{20}\) stated that it has become important to use available organic manures efficiently through suitable application methods, time of application and follow integrated nutrient management practices by combining inorganic fertilizers with organics such as FYM. It also has been shown to considerably improve rice yields by minimizing nutrient losses to the environment and managing the nutrient supply, and thereby results in high nutrient use efficiency (Bezbarah *et al*., 2011; Parkinson, 2013)\(^{7, 29}\). Therefore, it is imperative to compare the production efficiency of different rice production systems under organic, inorganic
and integrated (organic + inorganic) nutrient management practices. An integrated nutrient management in which both organic manures and inorganic fertilizers are used simultaneously has been suggested as the most effective method to maintain a healthy and sustainable soil system while increasing crop productivity (Jensen, 1993; Palm et al., 1997) [18, 28]. Use of organic and inorganic nutrients alone or with their integration (Bishst et al., 2002) [9] has been found important for food security as well as soil sustainability. Therefore keeping this in view, the present study was undertaken to investigate the effect of different sources and levels of fertilizers which consists of organic, inorganic and integrated sources of nutrient.

Materials and methods
The experiment was carried out during Kharif season of 2016 at Crop Research Farm, Department of Agronomy, Naini Agricultural Institute, SHUATS, Allahabad (U.P.) which is located at 25° 24' 42" N latitude, 81° 50’ 56" E longitude and 98 meters altitude above the mean sea level. The climate is characterized by the alternate hot rainy season from late June to early September with mean temperature of 38°C. The experiment was conducted in Randomized Block Design consisting of 12 treatment combinations with 3 replications and was laid out with the different treatments allocated randomly in each replication. The treatment combinations were T1 (FYM @ 5 tonnes/ha), T2 (FYM @ 10 tonnes/ha), T3 (50 % RDF), T4 (75 % RDF), T5 (100 % RDF), T6 (FYM @ 5 tonnes/ha + 50 % RDF), T7 (FYM @ 5 tonnes/ha + 75 % RDF), T8 (FYM @ 5 tonnes/ha + 100 % RDF), T9 (FYM @ 10 tonnes/ha + 50 % RDF), T10 (FYM @ 10 tonnes/ha + 75 % RDF), T11 (FYM @ 10 tonnes/ha + 100 % RDF), T12 (No manure and fertilizer). The soil samples were collected randomly from 0 to 15 cm depth from 5 spots of the experimental field just before layout of experiment. A representative homogenous composite sample was drawn by mixing all these soil samples together, which was analyzed to determine the physico-chemical properties of the soil are sand (59.60 %), silt (25.27 %), clay (15.13 %). The available nitrogen, phosphorus, potassium are 150 kg ha⁻¹, 13.50 kg ha⁻¹, 237.04 kg ha⁻¹ respectively. The organic carbon was 0.42 %, pH 8.2 and EC 0.13 (ds m⁻¹).

Rice cultivar ‘Chakhao Amubi’ which is an open pollinated cultivar from Manipur was taken as test variety in this investigation. One hundred seeds were tested before nursery sowing or direct seeding to ascertain the germinability of rice seeds. Germination test was done using filter paper and Petri dish under laboratory conditions. The overall germination percentage was 92% so the seed quantity was adjusted accordingly. The experimental field was ploughed with the help of tractor drawn plough followed by two harrowing and planking followed by flooding and puddling operations done manually in experimental blocks. Seeds were sown in the nursery following the recommended package and practices. 20 days old rice seedlings were transplanted manually at a spacing of 20 cm x 15 cm in the experimental field on 17 July 2016. Gaps caused by mortality were filled by re-transplanting after 7 days of transplanting.

The FYM used contained 0.5-0.35-0.9 N-P-K in terms of percentage which was obtained from the Warner School of Food and Dairy Technology, SHUATS. The recommended dose of fertilizers was 75-40-40 N-P-K kg/ha for open pollinated varieties in Uttar Pradesh Kharif season. At the time of transplanting, full dose of FYM, Phosphorus through SSP and Potassium through MOP were applied as basal but half dose of Nitrogen was applied through urea. The remaining 50 % Nitrogen was top dressed through Urea at active tillering stage and panicle initiation stage. Weeding was done twice during the critical crop weed competition period. The field was maintained in a moist condition and for this, irrigation was given to maintain 2-3 inch of standing water during the whole growing period except at harvesting. Observations were recorded from 10 tagged hills in running meter from each plot by leaving the 3 border rows at 15, 30, 45, 60, 75 and 90 DAT (days after transplanting). Data was recorded from these tagged hills.

The qualitative parameter, viz., protein (%) in grains was evaluated. Nitrogen in grain was estimated by Kjeldahl method by using the KjelTRON equipment in the department’s laboratory and converted into protein (%) by multiplying with the power factor 5.95. The data collected were analyzed statistically using analysis of variance technique. Treatment means were compared by Least Significant Difference (LSD) method at 95 % confidence level.

Results and Discussion

Growth parameters

Plant height (cm): Plant height was significantly affected by various treatment combinations and increased with the advancement of crop growth up to 90 DAT (Figure 1). Significant and highest plant height was observed in T11 (FYM @ 10 tonnes ha⁻¹ + 100 % RDF) and it is statistically at par with T10 (FYM @ 10 tonnes ha⁻¹ + 75 % RDF) in all the plant growth, development and reproductive stages in which both organic and inorganic nutrients were applied in combination. However treatments receiving no manure and fertilizer (T12) and only organic source of nutrients (T1 and T2) show poor performance as compared to the ones receiving inorganic source of fertilizer (T3, T4, T5). Lodging of plants was observed in treatments which received high dose of nutrients (T11, T10, T8, T7). However, the plants that lodged early recovered within 7-10 days. Significant and higher performance of inorganic fertilizers with FYM might be due to reduced loss of nutrients and its increased availability to crop by fixation of NH₄⁺ ion with humus present in FYM (Bellaki et al., 1998) [9]. The superiority of using organic and inorganic sources of nutrients together results in availability of N, P and K which increased significantly with application of organic manures as compared to RDF and control (Meena et al., 2015) [24].

Number of tillers hill⁻¹: The number of tillers/hill gradually increases up to 45 DAT and then reduces from 60th DAT (Figure 2). The maximum number of tillers/hill was observed at 45 DAT with treatment T11 (FYM @ 10 tonnes ha⁻¹ + 100 % RDF) which is statistically at par with T10 (FYM @ 10 tonnes ha⁻¹ + 75 % RDF). Integrated use of both organic and inorganic sources of nutrient (T6, T7, T8, T9) registered appreciably more number of tillers hill⁻¹ in comparison to organic nutrient source (T1, T2) and inorganic nutrient source (T3, T4, T5) during the experimentation. Maximum tillering observed in treatment where application of FYM and inorganic fertilizers in combination would be attributed to the more availability of nitrogen which played a vital role in plant growth and the more solubility of phosphorus (Dobermann and Fairhurst, 2000) [13] which promoted root development and tillering. The reduction in number of tillers after 60 DAT is resulted due to ageing and senescence which causes death of secondary and tertiary tillers which is a genetic constitution
in which plants have a definite tillering period after which they enter into the shoot elongation and developmental stage and the young tillers do not get time to develop. Tillering is the product of expansion of auxiliary buds which is closely related to the nutritional conditions of the mother culm because a tiller receives carbohydrate and nutrients from the mother culm during its growth period which gets improved by the application of nutrients (Tisdale and Nelson, 1975) [37]. Oo et al., 2010 [27] reported that application of inorganic fertilizer alone and using combination of FYM and inorganic fertilizers significantly increased tiller number per hill when compared to the treatments of without fertilizer application and using FYM alone in black rice.

Plant dry weight hill⁻¹: The maximum plant dry weight/hill was observed in treatment T11 (FYM @ 10 tonnes/ha + 100 % RDF) in which both organic and inorganic fertilizers were applied and is statistically at par with T10 (FYM @ 10 tonnes ha⁻¹ + 75 % RDF) in the entire growing season but with T9 at 30 DAT and 75 DAT (Figure 3). Treatment receiving no manure and fertilizer (T12) and only organic source of nutrients (T1 and T2) show poor performance as compared to the ones receiving inorganic source of nutrients (T3, T4, T5, T6). FYM in combination with inorganic fertilizers significantly produced maximum plant dry matter at all growth stages and it was followed by the result of inorganic fertilizers application. Superior FYM along with inorganic fertilizers would be attributed to better physiological growth of plants as an addition of organic matter from FYM increased the soil water holding capacity and CEC, improved availability of macronutrients as well as micronutrients and improved soil physical properties (Dobermann and Fairhurst, 2000) [13]. Singh and Jain, 2000; Meena et al., 2003 [33, 25] also reported significant improvement in dry matter accumulation of rice with increasing nutrition on account of better growth and development of the plant. Furthermore, dry matter production in rice is significantly related to intercept photosynthetically active radiation (Kiniry et al., 2001) [21]. Low N concentrations in plant leaves have been described as a limiting factor for reducing radiation use efficiency and biomass productivity resulting lower dry matter production of rice radiation use efficiency and biomass productivity (Sinclair and Sheehy, 1999) [32] resulting lower dry matter production of rice.

Yield attributes

The yield attributes of rice such as number of effective tillers hill⁻¹, number of spikelets panicle⁻¹, number of filled spikelets panicle⁻¹ and test weight was significantly influenced by the different sources of nutrients. Integrated use of organic and inorganic fertilizers (T11) shows significant and highest number of effective tillers hill⁻¹, number of spikelets panicle⁻¹, number of filled spikelets panicle⁻¹ and test weight as shown in table 1. The maximum number of effective tillers hill⁻¹ was observed in treatment T11 (FYM @ 10 tonnes ha⁻¹ + 100 % RDF) followed by T10 (FYM @ 10 tonnes ha⁻¹ + 75 % RDF) where both organic and inorganic sources of nutrients were applied in combination and superior over treatments which received only organic source and inorganic source. The higher number of effective tillers/hill may be due to higher number of tillers hill⁻¹. Chang and De Datta, 1975 [10] reported that tiller number and panicle number were positively and closely correlated. who reported that the number of panicles per unit area is determined by either stand density or tiller development during vegetative growth of rice. The number of productive tillers depends on nutrient during tiller bud initiation and subsequent developmental stages (Power and Alessi, 1978) [30]. During this stage competition for nutrients is high. Supplying adequate nutrients and in readily available forms increases the number of effective tillers hill⁻¹. The lower tiller number in lower levels of nutrients supply was attributed to the failure in competition for nutrients at lower level and aggravates death of the tillers due to mutual shading (Fageria et al., 1997) [14]. The maximum number of spikelets panicle⁻¹ was observed with treatment T11 (FYM @ 10 tonnes ha⁻¹ + 100 % RDF) i.e., 230.40 which is at par with T10 (FYM @ 10 tonnes ha⁻¹ + 75 % RDF) i.e., 212.47. The lowest number of spikelets panicle⁻¹ was observed in T12 (No manure and fertilizer) i.e., 114.40. As the level of nutrient decreases, the number of spikelets decreases significantly.

The highest number of spikelets panicle⁻¹ for a combination of FYM and inorganic fertilizers (T11) was due to high available N at the panicle initiation stage may increase spikelet number per panicle (De Datta, 1981; Nachimuthu et al., 2007) [12, 26] and K increases the number of spikelets panicle⁻¹ (Dobermann and Fairhurst, 2000; Bahmaniar et al., 2007) [13, 3]. Number of spikelets is an important yield-forming attribute of rice. Number of spikelets panicle⁻¹ is associated positively with grain yield. Application of P increases the total number of spikelets panicle⁻¹ in rice (Gebrekidan and Seyoum, 2006) [16]. The potential number of grains per panicle is determined at panicle initiation and it is influenced by the plants’ nutritional status during vegetative growth.

The maximum number of filled spikelets panicle⁻¹ (%) was observed in treatment T11 (FYM @ 10 tonnes ha⁻¹ + 100 % RDF) which is at par with T10 (FYM @ 10 tonnes ha⁻¹ + 75 % RDF), T9 (FYM @ 5 tonnes ha⁻¹ + 100 % RDF), T6 (FYM @ 5 tonnes ha⁻¹ + 50 % RDF) and T7 (FYM @ 5 tonnes ha⁻¹ + 100 % RDF). The maximum test weight was also observed in treatment T11 which is at par with T10, T9 and T8. Filled spikelet panicle⁻¹ and test weight are most important yield component in which rice yield depends. Nitrogen promotes rapid growth and increased spikelet number panicle⁻¹, percentage of filled spikelets panicle⁻¹ (Fallah, 2012) [15]. The reason for maximum number of filled spikelets panicle⁻¹ (%) in treatment T11, T10, T9, T6 and T7 may be due to application of FYM and inorganic fertilizers which provide K in adequate amounts. K increases the number of filled spikelets panicle⁻¹ (Dobermann and Fairhurst, 2000; Bahmaniar et al., 2007) [13, 3]. FYM in conjunction with various levels of N, P and K supplied secondary and micronutrients and its continued use could help in avoiding the deficiencies of these nutrients by improving soil health, physical properties, biological activities and increased availability of nutrient in soil by which superior grain yield attributing characters which in turn increases the number of filled spikelets panicle⁻¹ (%). Increased rate of potassium helps to produce large amounts of starch due to K-mediated carbohydrate metabolism. Besides, it helps in efficient translocation of photo- assimilates to the developing sinks/spikelets (Beringer, 1978) [6] and enhances the number of filled spikelets panicle⁻¹ (%) and thus, K helps the rice grain and straw to gain large volume and heavier weight.

Yield: Significant and highest grain yield was reported in treatment T11, however treatments T10, T9 and T8 shows parity with T11. There was 59.47 % increase in grain yield ha⁻¹ in T11 over T12. Also addition of 10 tonnes FYM with 100 RDF (T11) increases the grain yield by 29.57 % over 100 RDF (T9) alone. Further, highest straw yield was reported in treatment T11.
which is at par with $T_{10}, T_8, T_9$ and $T_7$. The lowest straw yield $(kg\ ha^{-1})$ was observed in $T_{12}$ (No manure and fertilizer) which is also at par with $T_1, T_3$ and $T_4$.

The maximum yield attained in treatments where integrated use of FYM and inorganic fertilizers were due to the fact that inorganic fertilizers releases nutrients for the plants instantly and in readily available forms for the plants during its growth, development and reproductive phases where the nutrient demand is at its peak. Micro nutrients supplied by the FYM enhanced the macro nutrients uptake and also slowly release during the whole crop period. Slow-acting bulky organic manures like compost and farm yard manure are hard to decompose (because of wider C: N ratio) and making nitrogen available at later stages (Khan et al., 2001) [19]. Organic fertilizer with inorganic fertilizer increased the fertilizer use efficiency and improved the physical and chemical properties of soil and it would be a reason towards increased yield (Banik et al., 2006) [4]. Increasing the levels of nutrients enhances the nutrient availability thereby increases yield attributes such as number of effective tillers hill$^{-1}$ number of spikelets panicle$^{-1}$, percentage of filled grains and test weight. Bhatnagar et al., 1992 [8], Yaduvanshi, 2001 [38] also reported that long term use of inorganic fertilizer with organic manure/FYM significantly enhanced the available N, P and K along with improvement of physical properties of soil, resulting maximum grain yield of rice. Awan et al., 2000 also reported that application of nitrogen fertilizer along with organic matter significantly increased the number of tillers plant$^{-1}$, number of spikelets panicle$^{-1}$, 1000-grain weight and paddy yield than control.

Grain protein content (%): The maximum protein content in grain (%) was observed in treatment $T_{11}$ (FYM @ 10 tonnes ha$^{-1}$ + 100 % RDF) i.e., 13.20 (%) which is at par with the other treatments in which inorganic fertilizers as well as integrated application of FYM and inorganic fertilizers in different combinations were applied except in $T_{12}$ (No manure and fertilizer) i.e., 10.00 (%), $T_1$ (FYM @ 5 tonnes ha$^{-1}$) i.e., 11.07 (%) and $T_2$ (FYM @ 10 tonnes ha$^{-1}$) i.e., 11.230 %. The main effects of FYM and inorganic fertilizers significantly influenced protein concentration in the rice grain. Increasing the levels of nutrients also increases the protein content in rice. The highest protein concentration in the grains was obtained in response to the combined application of the highest rates of the FYM and inorganic fertilizers whereas the lowest was recorded for the treatment where no manure and fertilizer was applied. It may be due to higher supply of nitrogen which increases the protein (%) in rice grain. The findings are in close conformity with that of Choudhary et al., 2005 [11].

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Fig 1: Effect of Organic and Inorganic Sources of Nutrients on Plant Height (cm) of Black Rice

Fig 2: Effect of Organic and Inorganic Sources of Nutrients on Number of Tillers/Hill of Black Rice
Fig 3: Effect of Organic and Inorganic Sources of Nutrients on Plant Dry Weight/Hill (g) of Black Rice

Table 1: Effect of Organic and Inorganic Sources of Nutrients on No. of Effective tillers hill⁻¹, Number of spikelets panicle⁻¹, Number of filled spikelets panicle⁻¹ (%), Test weight (g), Grain Yield (kg ha⁻¹), Straw Yield (kg ha⁻¹) and grain protein content

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of effective tillers/hill</th>
<th>Number of spikelets/panicle</th>
<th>Number of filled spikelets/panicle (%)</th>
<th>Test weight (g)</th>
<th>Grain Yield (kg ha⁻¹)</th>
<th>Straw Yield (kg ha⁻¹)</th>
<th>Grain Protein (%)</th>
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<tbody>
<tr>
<td>T₁</td>
<td>FYM @ 5 tonnes ha⁻¹</td>
<td>5.73</td>
<td>123.07</td>
<td>79.54</td>
<td>22.17</td>
<td>2466.67</td>
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<td>T₂</td>
<td>FYM @ 10 tonnes ha⁻¹</td>
<td>6.30</td>
<td>135.47</td>
<td>80.48</td>
<td>22.45</td>
<td>2700.00</td>
<td>8066.67</td>
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<tr>
<td>T₃</td>
<td>50 % RDF</td>
<td>6.90</td>
<td>143.47</td>
<td>80.39</td>
<td>23.56</td>
<td>2778.33</td>
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<td>T₄</td>
<td>75 % RDF</td>
<td>7.40</td>
<td>158.07</td>
<td>82.43</td>
<td>23.74</td>
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<td>T₅</td>
<td>100 % RDF</td>
<td>8.23</td>
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<td>83.43</td>
<td>23.76</td>
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<td>164.47</td>
<td>85.50</td>
<td>23.35</td>
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<td>T₉</td>
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<td>FYM @ 10 tonnes ha⁻¹ + 75 % RDF</td>
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<td>86.98</td>
<td>24.26</td>
<td>4623.33</td>
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<td>T₁₁</td>
<td>FYM @ 10 tonnes ha⁻¹ + 100 % RDF</td>
<td>11.70</td>
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<td>87.67</td>
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<td>T₁₂</td>
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<td>5.10</td>
<td>114.40</td>
<td>77.49</td>
<td>22.00</td>
<td>2033.33</td>
<td>7016.67</td>
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<td>SEd (±)</td>
<td>0.28</td>
<td>10.95</td>
<td>1.68</td>
<td>0.73</td>
<td>297.61</td>
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<td>22.71</td>
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<td>CV (%)</td>
<td>4.18</td>
<td>7.88</td>
<td>2.47</td>
<td>3.83</td>
<td>10.18</td>
<td>8.12</td>
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*RDF- Recommended Dose of Fertilizer through inorganic fertilizers i.e., 70-40-40 kg ha⁻¹ (N-P-K) FYM: Farmyard Manure

Conclusion
From the data pertaining to the different treatments, it may be concluded that integrating organic and inorganic sources of nutrients shows to be complementary in meeting the nutrient needs and thus, proves to be the most promising production system for Black rice. Since, this study is based only on one season, further research has been conducted for confirmation and recommendation.

Acknowledgement
This research was funded by Department of Agronomy, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, 211007.

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