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Integrated use of organic and synthetic fertilizers improves soil functioning, growth, yield and quality attributes of maize

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Abstract
The combined use of organic and chemical fertilizers may prove to be an excellent option for maintaining soil health, increasing fertilizer use efficiency, and improving crop quality and yield of maize. So, with this objective in mind a pot experiment was conducted to find out the effectiveness of organic and synthetic fertilizers on the yield and quality of maize crop. Two organic fertilizer levels (FYM and Press-mud @ 2% and 4% of each) were tested separately and in combination with recommended NPK fertilizers (160: 80: 60 kg ha^{-1}). The experimental units were laid out in a completely randomized design (CRD) with five replications. The results showed that the combined use of NPK + press mud @ 4% caused a significant increase in grain numbers per cob (8.6%), weight of grains per cob (11.5%), grain yield, biological yield, harvest index, stalk yield, and oil and protein contents up to 11.5%, 6.3%, 19.7%, 12.6% and 12.0%, respectively as compared to the control treatment. The oil and protein contents were also improved significantly over the sole application of NPK fertilizer. Soil health was also improved due to the combined use of synthetic fertilizer with 4% press mud and 2% FYM. It is concluded that integrated use of NPK fertilizers and press mud @ 4% not only increased the quality and yield of maize, but it also improved soil health.

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Introduction

Maize is an important grain and fodder crop all over the world and is produced in large areas, since it is used as a staple food in some areas of the world (Kandil, 2013; Zamir et al., 2013; Bright et al., 2019; Shekhar and Singh, 2022). In Pakistan, total cultivated area under maize is about 1.41 million ha with 8.46 million tones estimated yield. Its average yield of grain is 5970 kg ha^{-1} (Anonymous, 2021). The demand for maize crop is increasing because of its nutritive value as food for humans and feed for domestic animals and poultry (Rehman and Ishaque, 2011). Maize production in Pakistan is low, which is because of a multitude of factors such as low soil organic matter, inadequate nutrient supply, low plant density, weed infestation, insect pest attack, water scarcity, fluctuations in temperature, etc. (Oad et al., 2004). Of all these factors, low organic matter and inadequate nutrient supply are the problems that deteriorate our soil resources and decrease the yield of crops. So, to avoid this situation, an integrated plant nutrient management system has gained a substantial position, and it may play a critical role in maintaining soil fertility via natural substances like the usage of organic fertilizers along with inorganic fertilizers. Organic manures have various additive effects on soil productivity and crop yield such as

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supply of macro- and micro-nutrients, improvement and maintenance of soil health, build-up of soil organic matter. and restoration of beneficial microbes (Ali et al., 2017). Therefore, the combined application of organic manures along with chemical fertilizers is needed to enhance and maintain the fertility of soil for specific cropping systems (Bending et al., 2002; Kannan et al., 2013; Roba, 2018).

Although utilization of organic and synthetic fertilizers in combination may prove to be a successful way of obtaining high fertilizer use efficiency, increased water holding capacity of soil and decreased bulk density of soil, soil fertility ultimately improves maize growth, quality and yield of grain; for instance, 63% more dry matter yield was observed over the control in maize due to press mud application (Rong et al., 2001; Sharif et al., 2002; Sing et al. (2008). Similarly, Sing et al. (2007) documented that the use of press mud and FYM has proved to be more effective in maintaining nutrient levels in soil than that by inorganic fertilizers. Therefore, the combined nutrient management such as with compost, FYM, crop residues and green manure, is an alternate and highly beneficial technique to be used to reduce the input cost of fertilizers.

Keeping in view the importance of organic and synthetic fertilizers, an experiment was conducted to evaluate the efficacy of the combined application of inorganic and organic fertilizers for improving quality, growth and yield of a maize (*Zea mays* L.) hybrid under the agro-climatic conditions of Sargodha, Pakistan.

Materials and Methods

Experimental area and soil

An experiment was carried out to assess the effectiveness of sole and combined use of organic along with inorganic fertilizer to improve growth, yield and quality of maize (*Zea mays* L.). The experiment was laid out at the Research area of the College of Agriculture, University of Sargodha, Pakistan, during spring under the agro-climatic conditions of Sargodha. Before crop sowing, the samples from the experimental soil used to fill the pots was collected and analyzed for physicochemical properties (**Table 1**).

Table 1. Physico-chemical properties of the soil used in the study

Soil properties	Values	Soil properties	Values
Soil texture	Loam	Soil organic matter (mg kg^{-1})	7.80
Saturation percentage	38.00	Soil organic carbon (mg kg^{-1})	4.53
Soil pH	7.70	Available phosphorus (mg kg^{-1})	8.42
ECe (dS m^{-1})	1.50	Extractable potassium (mg kg^{-1})	210.00

Experimental design and treatments

The experiment was performed in earthen pots with dimensions 24 × 24 × 24 cm using a completely randomized design (CRD) with five replicates, and each pot had 20 kg of soil. The experiment consisted of treatments: T_1, Recommended NPK (R-NPK) fertilizers @ 160: 80: 60 kg ha^{-1}; T_2, Farm yard manure (FYM) @ 2%; T_3, FYM @ 4%; T_4, Press mud (PM) @ 2%; T_5, PM @ 4%; T_6, R-NPK + 2% FYM; T_7, R-NPK + 4% FYM; T_8, R-NPK + 2% PM; and T_9, R-NPK + 4% PM. The crop was sown in pots using the dibbling method.

The seed of a maize hybrid (DK-6103) was used in this study. Before sowing, the maize seeds were treated with “Confidor” @ 5 g per kg of seed to avoid any seed contamination and disease attack. Three seeds per pot were sown manually and thinning was carried out 15 days after germination, thereby maintaining one plant per pot at early stages.

At maturity of the crop, data regarding yield, yield determining parameters (i.e. plant height, cob number $plant^{-1}$, number of grains cob^{-1}, grain weight cob^{-1}, 100-grain weight, grain yield, biological yield, stalk yield, harvest index) and quality parameters (grain oil content and grain protein content) were recorded using standard protocols. Plant height of the crop was recorded at maturity from the soil surface to the tip of plant with a meter rod. The number of cobs was counted from each plant. Total grains per cob were counted from each pot and average grains were calculated. All the grains of a cob

were weighed using an electric balance for the determination of grain weight cob^{-1}. For the determination of 100-grain weight, a 100-grain sample was obtained from each pot, then the samples were oven-dried in an electrical oven. After drying the samples, 100-grain weight was recorded. Stalk, biological and grain yields from each pot were recorded at maturity after harvesting of the crop. After grain and biological yield determination, the crop harvest index (HI) was determined by the following formula:

$$Harvest\ index\ (\%) = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

Grain oil content was determined using the Soxhlet method as described by A.O.A.C (1999). For this, 5 g of dried sample of maize grains from each treatment were taken for oil extraction and placed in a thimble. These samples were prepared by oven drying. The thimble was placed in the Soxhlet extractor. A cleaned 150 mL round bottom flask was filled with 90 mL petroleum ether and the whole setting was placed on a mantle to boil the petroleum ether. Then oil extraction was carried out for almost 6 h to ensure complete oil recovery from the sample. Then the condensing unit was removed from the extraction unit and the sample was cooled. Finally, the oil was removed from the sample. The oil percentage of the sample was determined using the below given formula:

$$Oil\ content\ (\%) = \frac{(\mathrm{W1} - \mathrm{W2})}{\mathrm{P}} \times 100$$

W_1 = Empty thimble; W_2 = Thimble with sample; P = sample weight

Grain nitrogen contents were determined through the micro-Kjeldahl distillation method (Okalebo et al., 2002). For this determination, a sample of one g of grain was digested in 15 mL concentrated sulfuric acid. After digestion, the distillation was done through the addition of sodium hydroxide that converted NH_4^+ to NH_3. Ammonia was then captured by aqueous boric acid solution in a receiving vessel. At the end, titration of NH_3 was done using acid-base reaction between sulfuric acid and indicator mixtures. Nitrogen percentage was determined using the following formula:

$$Nitrogen\ (\%) = \frac{(\text{mL of standard acid} - \text{mL of blank}) \times \text{Normality}\ (N) \times 1.4007}{\text{Sample weight}}$$

The amount of total nitrogen in the raw material was multiplied with 6.25 and protein content in grains was calculated through the following formula:

$$Protein\ content = \text{Nitrogen}(\%) \times 6.25$$

Statistical analysis

The results for quality and yield parameters were collected and then statistically analyzed through the Fisher's analysis of variance procedure, and the means of treatments obtained were compared through the Tukey's HSD test where the probability level was set at 5%.

Results

Maize yield and yield contributing traits

Data regarding plant height indicated that all treatments were significantly different ($P \leq 0.05$) (**Table 2**). The maximum height of plant (219 cm) was noted when R-NPK + PM @ 4% was applied, that was statistically similar to that under R-NPK + FYM @ 4% followed by the sole applications of R-NPK and R-NPK – PM @ 2%, respectively. FYM @ 2% and PM @ 2% without R-NPK also showed non-significant results for plant height. The integrated fertilizer application did not perform significant results for producing cob number per plant (**Table 2**). Data regarding grain numbers per cob showed significant differences when the synthetic and organic fertilizers were applied in combination (**Table 2**). The grain number per cob ranged from 417.33 to 479.67. The maximum grain number per cob (479.67) was recorded due to the treatment R-NPK + PM @ 4% and it was statistically similar to R-NPK + FYM @ 4% followed by R-NPK + PM @ 2%, while the lowest number of grains per cob (417.33) was obtained when the sole FYM @ 2% was applied. The results for weight of grains per cob showed that it ranged from 96.75 to 116.0 g (**Table 1**).

The highest weight of grains per cob (116.0 g) was obtained due to R-NPK + PM @ 4% application followed by the treatments, R-NPK + FYM @ 4%, R-NPK + PM @ 2%, R-NPK + PM @ 2% and sole application of R-NPK. While, the lowest weight of grains per cob was observed (96.75 g) when FYM @ 2% was applied; this was statistically at par with that observed in PM @ 2%. The 100-grain weight ranged from 24.0 g to 28.80 g (**Table 2**). The maximum100-grain weight (28.80 g) was recorded due to the application of FYM @ 2%, while all other treatments except R-NPK + FYM @ 4%, R-NPK + PM @ 2%, and R-NPK + PM @ 4% were statistically similar in effects. The lowest 100-grain weight (24.0 g) was recorded by R-NPK + PM @ 4%. The highest biological yield was observed (307.27 g per plant) in R-NPK + PM @ 4% treatment (**Table 3**) that was statistically similar to that under R-NPK + FYM @ 4%. The lowest biological yield (260 g per plant) was observed due to sole FYM @ 2% application. The results regarding maize grain

yield under various nutrient treatments (**Table 3**) were recorded as significant. Grain yield ranged from 96 g to 143.64 g per plant. The maximum maize grain yield (143.64 g per plant) was recorded in the R-NPK + PM @ 4% treatment, while this treatment was followed by R-NPK + FYM @ 4%. The minimum maize grain yield was 38.91 g per plant which had been due to the application of FYM @ 2% without R-NPK fertilizers. Data pertaining to maize stalk yield showed maximum stalk yield (16 g per plant) with R-NPK + PM @ 4% application which was statistically similar to that under R-NPK with 2%, and 4% FYM and R-NPK +2% PM (**Table 3**). However, the lowest stalk yield (12.92 g per Plant) was obtained with the application of only 2% FYM. Harvest index ranged from 38.91 to 46.74% (**Table 3**). The highest value of the harvest index was recorded up to 46.74% because of R-NPK + PM @ 4% which was at par with that obtained in R-NPK + FYM @ 4% followed by that in R-NPK + PM @ 2%.

Table 2. Integrated effect of organic and inorganic fertilizers on yield contributing parameters of maize (*Zea mays* L.)

Treatments	Plant height (cm)	Number of cobs plant^{-1}	Number of grains cob^{-1}	Grain weight cob^{-1} (g)	100-grain weight (g)
Control (NPK only)	212.00 bc	2.00	441.67 bcd	104.00 c	26.20 abc
FYM @ 2%	198.00 e	2.33	417.33 f	96.75 f	28.80 a
FYM @ 4%	209.00 cd	2.00	428.67 def	99.17 de	27.48 ab
PM @ 2%	199.00 e	1.67	419.67 ef	96.84 ef	26.60 abc
PM @ 4%	211.00 bcd	2.33	432.00 cde	100.19 d	26.37 abc
R-NPK + 2% FYM	207.00 d	2.00	444.33 bc	105.08 c	26.00 abc
R-NPK + 4% FYM	215.00 ab	1.67	469.00 a	113.21 b	24.00 c
R-NPK + 2% PM	211.00 bcd	2.33	448.33 b	105.92 c	25.58 bc
R-NPK + 4% PM	219.00 a	2.00	479.67 a	116.00 a	24.40 bc
Tukey HSD	4.26	2.27	14.15	2.35	3.11

Control: Recommended NPK fertilizers; FYM: Farm yard manure; PM: Press mud. * Means not sharing identical alphabets are different significantly at the 5% probability level

Table 3. Integrated effect of organic and inorganic fertilizers on yield and grain quality of maize (*Zea mays* L.)

Treatments	Biological yield (g plant^{-1})	Stalk yield (g plant^{-1})	Grain yield (g plant^{-1})	Harvest index (%)	Oil content in grain (%)	Grain protein content (%)
Control (R-NPK)	289.90b	14.40bcd	120.00d	41.50 cde	3.94 d	8.60 e
FYM @ 2%	260.00f	12.91d	96.36g	38.91 e	3.39 h	8.14 h
FYM @ 4%	280.00d	14.13cd	112.73e	38.98 e	3.69 f	8.28 g
PM @ 2%	264.85e	13.40cd	103.64f	39.69 de	3.59 g	7.81 i
PM @ 4%	285.45c	14.13cd	105.45f	39.49 de	3.83 e	8.45 f
R-NPK+ 2% FYM	292.13b	14.78abc	125.34c	42.81 bcd	4.06 c	8.68 d
R-NPK+ 4% FYM	303.64a	15.70ab	138.18b	45.51 ab	4.18 b	8.78 c
R-NPK + 2% PM	292.73b	14.91abc	127.82c	43.68 abc	3.46 h	8.92 b
R-NPK + 4% PM	307.27a	16.00a	143.64a	46.74 a	4.54 a	8.99 a
Tukey HSD	**3.64**	**1.512**	**3.89**	**3.62**	**0.064**	**0.069**

Control: Recommended NPK fertilizers; FYM: Farm yard manure; PM: Press mud, * Means not sharing identical alphabets are different significantly at the 5% probability level

Maize quality parameters

The integrated nutrient management treatments were performed significantly in terms of oil and protein contents of maize grain (**Table 3**). The oil contents ranged from 3.39% to 4.54% (**Table 3**). The maximum oil content (4.54%) was recorded in R-NPK + PM @ 4% treatment followed by that in R-NPK + FYM @ 4%. The lowest oil contents (3.39%) were recorded by the sole application of FYM @ 2% without NPK fertilizers, while R-NPK only produced 3.94% oil content. In the case of protein contents in grains, all treatments performed differently (**Table 3**). The protein content ranged from 7.81% to 9.0%. The maximum protein content (9.0%) was recorded due to R-NPK + 4% PM @ 4% followed by that due to R-NPK + PM @ 2%, whereas the minimum protein content (7.8%1) was observed in the PM @ 2% treatment.

Chemical properties of soil after crop harvest

Soil chemical properties after crop harvest are presented in **Table 4**. The maximum EC (2.81 dS m^{-1}) was observed due to application FYM @ 4% followed by R-NPK (2.79 dS m^{-1}) and the lowest EC was observed in the treatment PM @ 2% that was up to 1.98 dS m^{-1}. The influence of the treatments on soil pH was also prominent. The maximum soil pH was observed in the treatment R-NPK without organic fertilizer and the lowest pH of 7.6 was observed in the R-NPK + FYM @ 4% treatment followed by that in

R-NPK + PM @ 2% and R-NPK + PM @ 4%. Total N content in soil increased because of nutrient management treatments. The maximum N content (403.80 mg kg^{-1}) was recorded in the NPK + PM @ 4% treatment followed by that in NPK + FYM @ 4% (392.27 mg kg^{-1}). While, the minimum N content was recorded due to the sole application of PM @ 2% (335.80 mg kg^{-1}). The maximum soil OM (0.91%) was recorded in the treatment NPK + PM @ 4% followed by that in the NPK + FYM @ 4% FYM treatment. The minimum OM was recorded when the sole R-NPK was applied (0.77%). Soil organic contents (SOC) had also shown a similar trend as the OM. The maximum SOC (0.53%) was observed in R-NPK + PM @ 4% application followed by that in R-NPK + FYM @ 4% (0.52%). The minimum SOC was observed in the case of sole application of R-NPK that was up to 0.45%. The maximum available phosphorus (9.10 mg kg^{-1}) was observed in R-NPK + PM @ 4% followed by that in the treatment R-NPK + FYM @ 4% (9.00 mg kg^{-1}). Whereas, the lowest available P was recorded due to the sole application of PM @ 2% (up to 8.38 mg kg^{-1}). The maximum extractable potassium (221.62 mg kg^{-1}) was found in the treatment R-NPK + PM @ 4% followed by that in R-NPK + PM @ 2% and the lowest K content (208 mg kg^{-1}) was observed in the sole application of PM @ 4%.

Table 4. Chemical properties of soil after crop harvest

Treatment	EC (dS m^{-1})	pH	Nitrogen (mg kg^{-1})	OM (mg kg^{-1})	Organic-C (mg kg^{-1})	Available P (mg kg^{-1})	Extractable K (mg kg^{-1})
Control (NPK only)	2.79	8.00	368.70	7.7	4.47	8.80	211.22
FYM @ 2%	2.67	7.90	337.72	8.5	4.94	8.43	208.14
FYM @ 4%	2.81	7.80	345.80	8.8	5.11	8.59	211.16
PM @ 2%	1.98	7.90	335.80	8.4	4.88	8.38	207.94
PM @ 4%	2.63	7.90	344.65	8.9	5.17	8.45	212.67
R-NPK + 2% FYM	1.99	7.90	343.05	8.6	5.0	8.55	214.04
R-NPK + 4% FYM	2.00	7.60	392.27	9.0	5.23	9.00	219.52
R-NPK + 2% PM	2.18	7.70	370.74	8.5	4.94	8.59	219.64
R-NPK + 4% PM	2.43	7.70	403.80	9.1	5.29	9.10	221.62

Control: Recommended NPK fertilizers; FYM: Farm yard manure; PM: Press mud; OM: Organic matter

Discussion

Most of the research studies about the effect of the use of NPK fertilizers (macronutrients) are primarily focused on testing application rate, application timing, responses of crop varieties and the effect of their integration with different agronomic practices. While, most of the studies associated to the effects of micronutrient application have concentrated on quality of yield attributes. So, in the present study, the optimized integrated application of inorganic and organic fertilizers can be used for judicious availability of nutrients to crop plants, thereby maintaining ecofriendly environment. To achieve optimal growth, a balanced nutrient supply is necessary. Integrated application of nutrients from chemical fertilizers and farmyard manure sustains crop production and soil health. Integrated use of recommended NPK and press mud at the rate of 4% improved plant height of maize which might have been due to more macro- and micro-nutrients availability from the soil. These findings are parallel to those of Chandrashekara et al. (2000) showing enhanced maize growth under recommended fertilizer levels along with poultry manure. Ayub et al. (2002) observed that maximum number of maize plants was produced with increasing fertilizer levels. In the present study, cob number per plant, grain number per cob, weight of grains per cob, 100-grains weight, and ultimately grain yield per hectare were also improved by applying recommended NPK and press mud at the rate of 4%. The reason for this improvement could have been due to the integrated use of inorganic fertilizers with those of organic, because both types of fertilizers can provide to plants all essential nutrients required for optimum growth. It can also be considered a pattern that has better residual effects on growth, yield and quality of crops compared to the sole application of chemical fertilizers (Solaimam and Rabbani, 2006; Zhang et al., 2011; Ye et al., 2020). However, the increase in yield and yield contributing attributes might have been due to favorable soil health which ultimately increased the availability of macro- and micro-nutrients from mineral fertilizers and then mineralization of the press mud, thereby regulating the physicochemical properties of soil that might have made it favorable for increasing grain number per cob. However, the maximum harvest index of the crop due to the recommended level of NPK application along with 4 % press mud was effective in promoting the grain yield over biological yield. These findings are in line with the observations of different researchers on maize (Chandrashekara et al., 2000; Donatus et al., 2011; Ryan et al., 2012; Kannan et al., 2013; Garai et al., 2014). Similarly, Chandrashekara et al. (2000) and Kannan et al. (2013) also concluded that the application of recommended NPK with vermicompost had shown improvement in grain number per cob, grain yield and 100-grain weight. Mumtaz et al. (1998) also showed improvement in yield due to the application of K at the rate of 125 kg ha^{-1}. Donatus et al. (2011)

also observed the highest rate of growth and yield parameters of maize crop when poultry manure was applied @ 10 or 15 t ha^{-1} and 80 or 120 kg N ha^{-1}. It is well understood that N increases the vegetative growth of plants, while the application of K and P play a significant role in the reproductive growth of crop plants (Ma et al., 2022). It was also observed that biological yield of maize was also increased due to the combined application of inorganic and organic fertilizers. These findings are in accordance with those of Jama et al. (2003) who observed a significant increase in grain yield by organic manure application. Similarly, Aildson et al. (2005) also recorded similar results while comparing maize genotypes and different N rates (0, 60, 120 and 180 kg ha^{-1}). Maize grain yield was enhanced by increasing the rates of N. Nitrogen application is essential to improve the quality of grain and grain yield of maize (Noor, 2017; Geith et al., 2022). The increase in grain yield in NPK integrated with 4% press mud could have been due to more availability of nutrients to the crop plants and favorable soil conditions for yield contributing attributes. Normally, it is observed that the use of organic fertilizers leads to restoration of soil fertility status and sustains the crop productivity for a longer period of time, while contrarily at field scales, the sole organic fertilizer application to sustain the crop yield at the economic level cannot be expected. Therefore, it must be applied in integration with other fertilizers, which are derived from inorganic sources to meet the crop nutrient requirements (Tirol-Padre et al., 2007; Zhang et al., 2011; Davari et al., 2012; Garai et al., 2014).

The increase in protein and oil contents of maize grains may have been also due to the availability of additional phosphorus, potassium, nitrogen and micronutrients from organic fertilizers which can play a major role in improving the protein and oil contents of grains (Rasouli et al., 2022). Rehman et al. (2011) also concluded that oil contents are significantly affected by the application of different rates of NPK fertilizers. Similar findings were reported by Farhad et al. (2013) who observed a marked increase in protein content due to the integrated application of NPK and poultry manure. Sanjivkumar (2014) also recorded the increase in grain protein content due to the integrated application of organic and inorganic fertilizers compared to respective control. Ahmad et al. (2018) concluded that the application of nitrogen improves protein contents of maize. Likewise, in another study, increasing nitrogen rates increased nitrogen contents and grain yield of maize (Mason and DCroz, 2002). Many studies indicate that integrated use of organic and inorganic nutrients has significant paybacks and it plays a potential role in improving crop growth, yield and quality attributes of crops (Abedi et al., 2010; Nazli et al., 2015; Selim, 2018; Mamuye et al., 2021).

Increase in soil EC and reduction in pH after the application of 4% FYM may have been due to the release of different ions after organic manure mineralization, while a reduction of pH may be coupled with the release of different types of organic acids during the mineralization process (Adeleke et al., 2017). The increase in total nitrogen content in soil was also affected positively by nutrient management treatments. Maximum nitrogen, phosphorus and soluble potassium contents were coupled due to additional nutrients supplied from mineralization of organic manure. Improvement in organic matter content under the NPK + 4% press mud application may have been due to more organic matter by the application of press mud and FYM. Many researchers also concluded that integrated organic and inorganic fertilizers is not only used as a source of nutrients, but also can be used as soil amendment to improve the soil organic matter, moisture retention capacity and stability of soil aggregates (Rathore et al., 2011; Garai et al., 2014; Kumari et al., 2017).

Conclusion

Overall, the quality and yield of maize crop were significantly improved due to the application of FYM and press mud when applied in integration with the recommended NPK. The maximum yield and quality of maize were recorded due to the combined application of NPK recommended with 4% press mud, so it is suggested that for obtaining high maize yield and improving soil characteristics, NPK mineral nutrition should be applied in combination with 4% press mud. Finally, the awareness of agricultural experts and farmers must be twisted towards a simple practice of integrated use of organic and inorganic fertilizers that is an acceptable cost-effective practice, which can be easily followed by the farmers. It is also an environment-friendly approach.

Author(s), Editor(s) and Publisher's declarations

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Contribution of authors
Conceived the idea and supervised the field experiment, MA. Performed field experiment, BR. DEVISED layout and assisted in writing-up of the manuscript, MMJ. Data collection, AA. Assisted in data analysis, NA. Assisted in data collection and analysis as well as write-up, NA, MES.

Ethical approval
This study does not involve human/animal subjects, and thus no ethical approval is needed.

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of experiment, all materials were properly discarded to minimize any types of bio-contamination(s).

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent
All contributors have critically read this manuscript and agreed for publishing in IJAaEB.

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