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Original article

Zinc-biochemical co-fertilization improves rice performance and reduces nutrient surplus under semi-arid environmental conditions

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ABSTRACT

Biofertilizers are a promising approach to substantially improve nutrient recovery and crop production. Moreover, zinc (Zn) deficiency is one of the key abiotic factors limiting global rice production. However, the effect of Zn-biochemical co-fertilization on rice production and nutrients recovery and surplus under semi-arid environmental conditions is not fully obvious. Two years field experiment was conducted to evaluate the effect of Zn-biochemical (nitrogen “N”, phosphorus “P”, and potassium “K”) co-fertilization on yield and yield components, physico-chemical characteristics, and nutrient recovery and surplus as well as farm profitability of four rice (*Oryza sativa* L.) cultivars treated with two Zn levels (no Zn application, and 600 mg chelated Zn L⁻¹ as a foliar application) and six fertilization regimes (no fertilizers application, biofertilizers, 25% NPK plus biofertilizers, 50% NPK plus biofertilizers, 75% NPK plus biofertilizers, and 100% NPK). Biofertilizers mixture (cerealain, phosphorine, and potassiomage) were used. The results revealed that chemical constituents, growth attributes, yield, yield components, nutrients uptake (N, P, K, and Zn), and nutrients recovery (N, P, and K) significantly increased due to Zn foliar application. Biofertilizers replacement for 25% of inorganic NPK combined with Zn provides the highest nutrients uptake through increasing N, P, and K recovery by 57–94%, 61–128%, and 45–69%, respectively in the four rice cultivars compared with 100% NPK treatment. This improvement in nutrients uptake and recovery was attributed to decrease nutrients surplus by 64–78%, 46–53%, and 50–59%, respectively. Additionally, Zn-biochemical co-fertilization improves growth attributes, yield, and yield components of rice cultivars through producing more contents of chlorophyll *a* and *b*, carotenoids, total carbohydrates, and total amino acids than using 100% NPK alone. All previous characteristics significantly affected by the cultivated rice variety. The net return under the treatment of 75% NPK plus biofertilizers plus Zn foliar application was 21.5–27.5% higher than the treatment of 100% NPK. Therefore, our findings suggest that biofertilizers replacement for 25% of inorganic NPK combined with Zn foliar application supplies a financially attractive choice to substantially enhance nutrient recovery and production of rice, while effectively reducing nutrients loss.

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1. Introduction

Rice (*Oryza sativa* L.) is one of the major staples, feeding more than 50% of the universal population. Rice supplies the world's population with 15% and 21% of their protein and energy needs, respectively (Rehman et al., 2012). To feed ever-rising world population, which is expected to reach 10 billion by the end of this century, an increase in rice production per unit area is direly needed (Von Grebmer et al., 2017). However, the production of rice is constantly decreasing in Egypt because Egyptian government is reducing the cultivation of water-consuming crops, such as rice,

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raising concern about a deterioration of the quality of the country's most fertile farmland. For example, rice production in Egypt decreased by 35%, 41%, and 37% in 2018 as compared to 2015, 2016, and 2017, respectively (FAOSTAT, 2021). Consequently, the Egyptian rice production per unit area must be greatly increased to compensate for this decrease in cropped area, especially in light of the population increase, which is expected to reach approximately 50% and 100% by 2050 and 2100, respectively (Worldometers, 2017). On other side, several authors reported significant increase of physiochemical attributes, grain yield, and yield attributes as well as nutrient recovery and surplus due to rice varietal differences (Abd El-Wahab, 1998; Moonmoon et al., 2017). Varietal improvement plays an important role in increasing rice yields. Increases in rice production depend on the availability of high yielding varieties (Abd El-Wahab, 1998). Varieties differ in their ability to impact productivity and such differences may be due to genetically variation in used genotypes and their interaction with environmental conditions (Khattab, 2019).

Expensive inorganic fertilizers have been utilized by farmers to higher the crop production (Ahmed et al., 2020). This practice, however, has led to an elevate in production costs and in many cases caused environmental issues such as soil fertility loss, eutrophication, groundwater nitrate contamination, soil acidification, and greenhouse gas emissions (Penuelas et al., 2013; Galloway & Cowling, 2021). Egypt is the largest consumer of fertilizers (average of 500 kg ha⁻¹) in Africa (El Gharous & Boulal, 2016). It uses, for instance, approximately 35% of all the inorganic fertilizer nitrogen (N) used in Africa during 2010–2015 (Elrys et al., 2019b). In Egypt, out of the total inorganic fertilizers used in agriculture, rice accounts 12.3% (Heffer et al., 2009). Moreover, more than 60% of the inorganic N fertilizer in Egypt is applied to cereal crops, and 97.5% of that is applied to maize, rice, and wheat only (Elrys et al., 2019b). Furthermore, the rice N footprint (the amount of reactive N emitted into the environment by people consumption and the related production of food and energy) in Egypt increased by 50% during the last five decades (Elrys et al., 2019b; Elrys et al., 2021), while the average rice N recovery was approximately 24% (Elrys et al., 2021). Thus, massive using of inorganic fertilizers is obviously a major limiting factor. It is imperative to develop integrated approaches to using inorganic fertilizers reasonably with the use of environmentally friendly alternative resources.

Fertilizers other than inorganic fertilizers such as compost, organic manure, and biofertilizer shall be utilized as environmentally friendly alternative. Among them, biofertilizers are the most important because of their positive influences to both plant production and the environment (Kantachote et al., 2016). For instance, biofertilizers from *Rhodospseudomonas palustris* enhanced rice and reduced methane emissions (Kantachote et al., 2016). Mixing *Rhodobacter capsulatus* with 50% inorganic N fertilizer resulted in a rice grain yield statistically equivalent to 100% inorganic N fertilizer (Gamal-Eldin & Elbanna, 2011). Azolla's biofertilizer replacement for 25% of inorganic N supplies a financially attractive choice to substantially enhance N use efficiency (NUE) and production in intensive rice cropping systems, while effectively decreasing N loss (Yao et al., 2018). Moreover, a number of various microorganisms have been isolated and characterized for their phosphorus (P)-mobilizing ability (Khan et al., 2007). Furthermore, highest K uptake was observed in plants fertilized with nutrients containing silicate-dissolving bacteria (Jadid Soleimandarabi et al., 2017). Hence, biofertilizers are proven powerful agents and significant alternative supplement for sustainable rice cultivation. However, the effect of biofertilizers mixture (N fixing bacteria, P solubilizing bacteria, and K solubilizing bacteria) as alternatives for inorganic fertilizers on rice production and nutrient surplus in intensive cropping systems is not fully obvious.

Zinc (Zn) deficiency is one of the key abiotic factors limiting global rice production (Rehman et al., 2012). Zinc deficiency happens mainly in alkaline and calcareous soils (e.g. in Egypt) due to high bicarbonate (HCO₃⁻) concentration or high soil pH (Qadar, 2002). Rice grown on soils with Zn deficiency produces low yield quantity and quality (Welch and Graham, 1999). Zn deficiency in rice grains considers widespread nutritional disorder influencing human health. Zn -deficiency in plants reduces leaf chlorophyll content and chlorophyll *a*: *b*, indicating damage to intrinsic quantum efficiency of the photosystem-II (PSII) units (Chen et al., 2008). Enzyme and protein synthesis Zn is important for activity of a number of plant proteins. Moreover, rice cultivation is in transition in many rice growing regions of the world including Egypt, from flooding to alternate wetting and drying, aerobic culture, raised beds or other systems of rice intensification. This transition towards water-saving rice cultivation may decrease soil moisture content, and soil variables controlling Zn availability of rice are likely to change (Gao et al., 2006) and have a main influence on rice production in various systems. Consequently, Zn as a key abiotic factors limiting rice production suffers from complex problems in Egypt, which are the actual deficiency of Zn availability due to high soil pH in addition to the potential deficiency due to the inevitable shift towards water-saving rice cultivation. However, the effect of Zn-biochemical (NPK) co-fertilization on rice production and nutrients recovery is still largely unknown.

To our knowledge, this is one of the first study to evaluate the effect of Zn-biochemical (NPK) co-fertilization on yield and yield components, physicochemical characteristics, nutrient uptake, nutrient recovery, nutrients surplus, and farm profitability of four rice cultivars under semi-arid environmental conditions. Two years field experiment was conducted to tested the following hypotheses: 1) Zn fertilizers would improve rice performance and reduce nutrient surplus under semi-arid environmental conditions, 2) substituting biofertilizers NPK mixture for 25% of inorganic fertilizers NPK would improve yield and physiochemical attributes as well as nutrient recovery and surplus of various rice cultivars, 3) the effect of Zn-biochemical co-fertilization would be cultivar dependent, and 4) Zinc foliar application with substituting mixture biofertilizers for 25% of inorganic fertilizers would be the best treatment for increasing rice production and farm profitability, while reducing nutrients loss.

2. Materials and methods

2.1. Experiment site

The present study was conducted in El-Ibrahimiya district, Sharkia Governorate (30.72 °N, 31.56 °E), Egypt during two successive summer seasons of 2019 and 2020. Soil samples were collected from the experimental sites at the depth of 0–30 cm before planting to determine soil physical and chemical properties according to (Black, 1968). The soil is classified as a clay with organic matter content of 24 g kg⁻¹, C:N of 11:1, total N of 1.3 g kg⁻¹, available P of 14 mg kg⁻¹, available K of 156 mg kg⁻¹, available Zn of 1.0 mg kg⁻¹, pH of 7.9 (Moderately alkaline), and electric conductivity (EC) of 1.88 dS m⁻¹ as averages of both seasons.

2.2. Experimental design and study factors

In total, 48 treatments repeated four times were implemented in a split-split plot design. The foliar treatments were randomly occupied in the main plots, and in the sub- and sub-sub plots, tested cultivars and fertilizers regimes were randomly applied, respectively. Three foliar sprays with Zn as a chelated Zn 14% EDTA

was added at a rate of 600 mg L⁻¹, while spraying with tap water was used as a check treatment. Foliage applied treatments were carried out using water (595 L ha⁻¹ per spray) at 10, 25 and 40 days after transplanting. Furthermore, four rice (*Oryza sativa* L.) cultivars (Giza 178, Giza 179, Sakha 104, and Egyptian hybrid rice 1"EHR1") were used. Six bio-chemical fertilization regimes (no fertilizers application, biofertilizers, 25% NPK plus biofertilizers, 50% NPK plus biofertilizers, 75% NPK plus biofertilizers, and 100% NPK) were followed. The recommended doses of NPK (100% NPK) were set up by adding 145 kg N ha⁻¹ as ammonium sulfate (21 % N); 75 kg P₂O₅ ha⁻¹ as calcium super phosphate (15.5% P₂O₅); and 67 kg K₂O ha⁻¹ as potassium sulfate (48% K₂O) to the soil. The recommended doses of inorganic fertilizers used in our study are the typical doses for the commercial rice production in the region. Before transplanting, full dose of superphosphate and potassium sulfate were added. Three equal doses of ammonium sulfate were applied at 10, 25 and 40 days after transplanting. Biofertilizers mixture (cerealin biofertilizer contains *Azospirillum brasilense* and *Azospirillum lipoferum* as N₂ fixing bacteria, phosphorine biofertilizer contains *Bacillus megaterium* var. *phosphaticum* as phosphate solubilizing bacteria, and potassiomage as K solubilizing bacteria "*Bacillus cerculance*") were applied at 10 days after transplanting. The commercial biofertilizers were produced by Agriculture Research Center, Giza, Egypt, and used at the recommended dose of 210 g ha⁻¹ for each biofertilizer. The experimental unit size was 12 m² (3 m × 4 m). Rice seeds at rate of 145 kg ha⁻¹ were soaked in water for 24 h, then drained and incubated for 24 h. Thereafter, seeds were manually broadcasted in the first week of May in both seasons. Rice seedlings were transplanted at 30 days after sowing in hills 20 × 20 cm a part (25 hills m⁻²). The preceding crop was berseem clover (*Trifolium alexandrinum* L.) in both seasons. All other agronomic practices including, irrigation, weeds, pest and disease control were applied as recommended for the commercial rice production.

2.3. Determination of physiochemical constituents

At heading stage, fresh leaves were collected from each plot to determine the photosynthetic pigments (chlorophyll *a* "Chl. a", chlorophyll *b* "Chl. b", and carotenoids) according to Fadeels (1962). The contents (mg g⁻¹ FW) of these pigments were calculated according to the modified formula of (Wettstein, 1957). Total carbohydrates were determined in the dried samples of shoots and leaves of all treatments according to (Williams, 1984). Amino acids were extracted from oven dry leaves using ethyl alcohol (80%, v/v). The quantitative amino acids determination was carried out according to (Christias et al., 1975). Grain samples were taken at harvest from each plot and dried at 70 °C and milled to fine powder. Dried samples were digested using a mixture of sulfuric acid and perchloric acid to estimate N, P, K, and Zn. Total N and K levels were determined using a microkjeldahl method and flame photometer device, respectively (Chapman & Pratt, 1982). Total P was colourmetrically determined using ascorbic acid method (Watanabe & Olsen, 1965). Zn content was estimated using atomic absorption spectrophotometry (Williams, 1984). Grain nutrient uptake was computed by the multiplication of nutrient content and dry matter. Grain protein uptake was computed by multiplying grain N uptake by 5.70 (Bishni and Hughes, 1979).

2.4. Calculation of nutrients surplus and nutrients recovery

Nutrients surplus was calculated as the difference between inorganic nutrients input through fertilization and total nutrients uptake at the final harvest (Yao et al., 2018; Elrys et al., 2019a). Fertilizer nutrients (N, P, or K) recovery, indicating the increase of

total N, P, or K uptake at harvest per unit of N, P, or K fertilizers application rate (Varvel & Peterson, 1990):

$$\text{Nutrient recovery} = \frac{\text{NF} - \text{NC}}{\text{R}} \times 100$$

where: NF, NC, and R refer to the total nutrient uptake from nutrient fertilized plot, total nutrient uptake from unfertilized plot, and rate of fertilizer nutrient applied, respectively.

2.5. Rice yield and yield attributes measurements

At harvest (end of September in the two seasons), ten guarded plants were taken at random from each experimental plot to determine plant height (cm). Also, the number of panicle m⁻² and the following yield attributes were recorded on ten panicles, i.e., panicle length, number of filled grain panicle⁻¹, number of spikelets panicle⁻¹, filled grain percentage, panicle grain weight, and 1000 grain weight. Moreover, the following final yield traits were recorded from a central area of 2 m² per plot: grain yield at grain moisture content of 14%, total yield, and harvest index (HI).

2.6. Economic analysis

Total costs of applied agricultural practices including seeds, fertilizers, irrigation, power, labor, machinery and land rent were estimated. The costs of all farm operations were estimated based on the official and the actual market prices determined by Egyptian Ministry of Agriculture. Three economic parameters were estimated; total income (USD ha⁻¹), net return (USD ha⁻¹) and return invested (USD). The total income from grain yield was calculated by multiplying grain yield by actual price which was 267 USD Mg⁻¹. Net return from production of rice was estimated as the difference between total income and total costs. Besides, return invested was calculated by dividing total income by total cost.

2.7. Statistical analysis

The data were statistically analyzed according to Gomez and Gomez (1984) by using MSTAT-C (Nissen, 1983) where statistical program Version 2.1 was used for analysis of variance (ANOVA). Combined ANOVA was performed across the two seasons after testing the homogeneity of the experimental errors by Bartlett's test (Steel, 1997). Differences among foliar application treatments, cultivars, fertilization regimes and their interactions were separated by the least significant difference (LSD) at $p \leq 0.05$ (WG, 1980).

3. Results

3.1. Zinc-biochemical co-fertilization improves physiochemical constituents of rice

Chlorophyll *a* (Chl. a), chlorophyll *b* (Chl. b), carotenoids, total carbohydrates, and total amino acids significantly (all $p < 0.05$) increased due to Zn foliar application (Table 1) in both seasons and their combined. Compared with the control, these increments were 10.7, 10.4, 6.5, 2.2, and 7.0%, respectively, as an average of two seasons. The previous characteristics significantly (all $p < 0.001$) affected by the cultivated rice variety (Table 1). The highest contents of Chl. a, Chl. b, and carotenoids were recorded under Giza 178 cultivar, followed by Sakha 104, Giza 179, and EHR1 cultivars, respectively. Whilst, the highest content of total carbohydrates and total amino acids was observed under Giza 179 and EHR1 cultivars, respectively. Furthermore, there was a significant (all $p < 0.001$) effect of the fertilization regime on the pre-

Table 1
Influence of zinc-biochemical co-fertilization on the leaf photosynthetic pigments contents, total carbohydrate and total amino acids of the four studied rice cultivars.

Study factor	Chl. a (mg g ⁻¹ FW)	Chl. b (mg g ⁻¹ FW)	Carotenoids (mg g ⁻¹ FW)	Total carbohydrate (mg g ⁻¹)	Total amino acids (%)
Zinc foliar application (Zn)					
Control	1.31B	0.48B	1.24B	22.4B	75.8B
Zn	1.45A	0.53A	1.32A	22.9A	81.1A
Cultivar (C)					
Giza 178	1.49A	0.55A	1.34A	22.6B	74.2D
Giza 179	1.34C	0.47C	1.24C	23.3A	78.6C
Sakha 104	1.47B	0.54B	1.31B	22.1C	79.5B
EHR1	1.26D	0.46D	1.23D	22.7B	81.6A
Fertilization regime (F)					
Control	0.77F	0.30F	0.71F	13.1E	42.4E
Biofertilizers	1.28E	0.43E	1.12E	20.2D	71.7D
25% NPK + biofertilizers	1.33D	0.49D	1.23D	22.3C	77.5C
50% NPK + biofertilizers	1.65B	0.61B	1.52B	26.7B	93.3A
75% NPK + biofertilizers	1.69A	0.62A	1.59A	27.3A	93.4A
100% NPK	1.62C	0.59C	1.50C	26.4B	92.4B
ANOVA					
Zn	1	0.039	0.004	0.009	0.003
C	3	<0.001	<0.001	<0.001	<0.001
F	5	<0.001	<0.001	<0.001	<0.001
Zn × C	3	<0.001	<0.001	<0.001	0.522
Zn × F	5	<0.001	0.007	0.010	0.085
C × F	15	<0.001	0.043	<0.001	0.659
Zn × C × F	15	0.155	0.704	<0.001	0.999

Means followed by different letters in the same direction differ significantly by LSD ($p \leq 0.05$).

vious attributes (Table 1). Chl. a, Chl. b, carotenoids, total carbohydrate, and total amino acids were higher in all fertilization regime treatments compared to the control treatment, where the highest values was noted in the treatment of 75% NPK + biofertilizers, followed by 50% NPK + biofertilizers, and 100% NPK, respectively, whereas the lowest values were observed in the treatment of biofertilizers alone. Compared with the treatment of 100% NPK, the previous characteristics increased by 4.32, 5.08, 6.00, 3.41, and 1.08%, respectively, as an average of two seasons under the treatment of 75% NPK + biofertilizers, and by 1.85, 3.39, 1.33, 1.14, and 0.97%, respectively under the treatment of 50% NPK + biofertilizers.

Significant (all $p < 0.05$) interaction influences were observed under the effect of Zn × cultivar, Zn × fertilization regime, and cultivar × fertilization regime for Chl. a, Chl. b, carotenoids, and total amino acids (Table 1). However, the significant (all $p < 0.05$) interaction impacts of all factors (Zn × cultivar × fertilization regime) was noted with carotenoids and total amino acids. Excluding control, maximum contents of previous attributes (Fig. 1) were noted in the treatment of 75% NPK + biofertilizers + Zn foliar application in both seasons and their combined, while minimum contents were recorded in the treatment of biofertilizers without Zn.

3.2. Zinc-biochemical co-fertilization increases rice growth, yield and yield attributes

Results in Table 2 illustrate variations among the study factors for plant height, panicle length, number of panicles m⁻², number of spikelets panicle⁻¹, number of filled grain panicle⁻¹, filled grain, 1000-grain weight, panicle grain weight, grain yield, total yield, HI, and protein content. All previous characteristics except for plant height and HI significantly (all $p < 0.05$) increased due to Zn foliar application compared to without Zn application in both seasons and their combined. The former characteristics under Zn foliar application were 3.41, 6.38, 8.04, 10.6, 7.48, 9.42, 11.7, 15.5, 18.1, 15.6, 1.44, and 25.2%, respectively higher than without Zn foliar application. Moreover, all previous characteristics significantly (all $p < 0.005$) affected by the cultivated rice variety and fertilization regime. The highest plant height was recorded under

Giza 178 cultivar, whereas the highest panicle length, number of panicles m⁻², 1000-grain weight, grain yield, total yield, and grain protein content were noticed under Sakha 104 cultivar, and the highest number of spikelets panicle⁻¹, panicle grain weight, and HI were recorded under EHR1 cultivar. Moreover, the highest number of filled grain panicle⁻¹ and the percentage of filled grain were observed under both Giza 178 and EHR1 cultivars without significant differences. Such as what was observed in the physio-biochemical constituents, the highest values of all rice growth and yield attributes were noted in the treatment of 75% NPK + biofertilizers, followed by 50% NPK + biofertilizers, and 100% NPK, respectively, whereas the lowest values were observed in the treatment of control, followed by the treatment of biofertilizers alone. Compared with the treatment of 100% NPK, rice growth and yield attributes increased by 5.88, 6.70, 4.39, 11.4, 15.2, 14.2, 17.4, 18.9, 15.4, 4.11, and 12.6%, respectively under the treatment of 75% NPK + biofertilizers. Whilst, these attributes increased by 61.5, 51.8, 37.2, 41.6, 46.4, 11.9, 71.2, 61.8, 62.8, 56.4, 4.5, and 83.4%, respectively under the treatment of biofertilizers alone compared with the control treatment.

Furthermore, significant (all $p < 0.05$) interaction influences were noted under the effect of Zn × cultivar, Zn × fertilization regime, cultivar × fertilization regime, and Zn × cultivar × fertilization regime for all growth and yield attributes. However, number of panicles m⁻², number of spikelets panicle⁻¹, 1000-grain weight, and HI did not response significantly to the interaction effect of Zn × fertilization regime. Number of panicles m⁻², and number of spikelets panicle⁻¹ also did not response significantly to the interaction effect of Zn × cultivar × fertilization regime. Likewise, maximum contents of previous attributes (Figs. 2 and 3) were observed under the treatment of 75% NPK + biofertilizers + Zn foliar application, while minimum values were recorded in the control treatment (Fig. 4). The response equations showed diminishing increase in grain yield with addition of 75% NPK + biofertilizers under fourth cultivars with foliar application of Zn. These interactions ascertain the view that a complementary effect could have been taken place between the foliar application of Zn, cultivars and fertilization regimes were the best of this combination was observed in foliar application of Zn with sowing Sakha 104 with adding of 75% NPK + biofertilizers (Fig. 4).

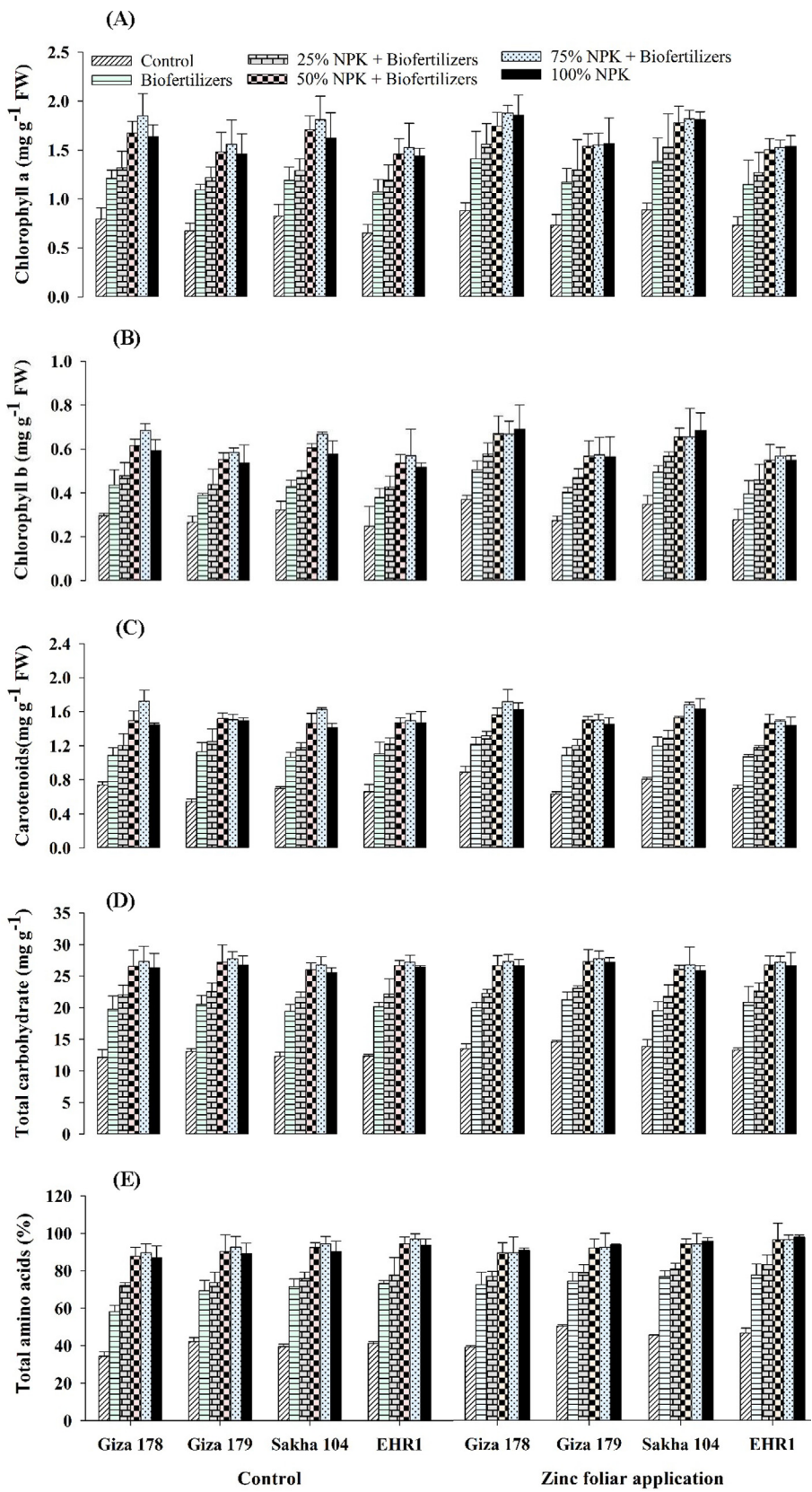


Fig. 1. Impact of zinc-biochemical co-fertilization on chl. a (A), chl. b (B), carotenoids (C), total carbohydrate (D), and total amino acids (E) of the four studied rice cultivars. Data are mean of two seasons (n = 4) ± SE.

Table 2
Influence of zinc-biochemical co-fertilization on yield, yield components and grain protein content of the four studied rice cultivars.

Study factor	Plant height (cm)	Panicle length (cm)	Number of panicles m ⁻²	Number of spikelets panicle ⁻¹	Number of filled grain panicle ⁻¹	Filled grain (%)	1000-grain weight (g)	Panicle grain weight (g)	Grain yield (Mg ha ⁻¹)	Total yield (Mg ha ⁻¹)	Harvest index (%)	Grain crude protein (kg ha ⁻¹)	
Zinc foliar application (Zn)													
Control	85.0A	18.8B	448B	113B	107B	81.7B	17.1B	2.33B	8.73B	18.0B	48.5 A	206B	
Zn	87.9A	20.0 A	484A	125 A	115A	89.4A	19.1A	2.69A	10.3A	20.8 A	49.2 A	258 A	
Cultivar (C)													
Giza 178	89.6A	19.4A	440D	123B	117A	86.2A	16.3D	2.24C	9.15C	18.2 D	48.0 BC	216C	
Giza 179	84.9C	19.5A	463B	117C	108B	85.9B	16.9C	2.34D	9.11C	18.8C	47.8C	222B	
Sakha 104	86.7B	19.7A	520A	110D	103C	83.8C	20.0A	2.70B	10.5A	21.6 A	48.6B	265 A	
EHR1	84.6C	18.9B	442C	127A	117A	86.4A	19.4B	2.79A	9.34B	19.0B	51.0 A	224B	
Fertilization regime (F)													
Control	47.0F	11.0F	266E	66.3E	63.2F	70.6E	9.11E	1.31F	4.71F	10.1 E	46.6C	96.5F	
Biofertilizers	75.9 E	16.7E	365D	93.9D	92.5E	79.0D	15.6 D	2.12E	7.67E	15.8 D	48.7B	177 E	
25% NPK + biofertilizers	85.5 D	19.2C	429C	116C	112D	88.9C	18.2C	2.61D	8.91D	18.4C	48.5B	216 D	
50% NPK + biofertilizers	101C	23.1B	574B	143B	130B	92.3B	20.7B	2.88B	11.5B	23.0B	50.0 A	294B	
75% NPK + biofertilizers	108 A	23.9A	594A	156A	144A	93.5A	24.1A	3.31A	13.2A	26.3 A	50.7 A	322 A	
100% NPK	102B	22.4C	569B	140B	125C	89.1C	21.1B	2.82C	11.1C	22.8B	48.7B	286C	
ANOVA													
	df	p-value of the main effects and their interactions											
Zn	1	0.081	0.015	0.017	0.035	0.020	<0.001	0.028	0.007	0.008	0.006	0.107	0.019
C	3	<0.001	0.004	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
F	5	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Zn × C	3	<0.001	0.011	<0.001	0.014	<0.001	<0.001	0.002	0.187	<0.001	<0.001	<0.001	<0.001
Zn × F	5	<0.001	0.005	0.239	0.920	<0.001	<0.001	0.338	<0.001	<0.001	<0.001	0.080	<0.001
C × F	15	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Zn × C × F	15	<0.001	<0.001	0.938	0.284	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means followed by different letters in the same direction differ significantly by LSD ($p \leq 0.05$).

3.3. Zinc-biochemical co-fertilization increases nutrient recovery and reduces nutrient surplus

Compared to control, Zn foliar application significantly (all $p < 0.05$) increased grain nutrients (N, P, K, and Zn) uptake and nutrients (N, P, and K) recovery, but decreased nutrients surplus (N, P, and K; Table 3). Grain N, P, K, and Zn uptake, and N, P, and K recovery under Zn foliar application treatment were 24.8, 31.5, 26.5, and 46%, and 20.7, 14.7, and 16.6, respectively higher than without Zn foliar application. Whilst N, P, and K surplus decreased by 67.1, 30.2, 36.9%, respectively. Moreover, the highest nutrient uptake and lowest nutrient surplus were recorded under Sakha 104 cultivar. The highest N and P recovery was also observed with Sakha 104 cultivar, but the highest K recovery was noted under Giza 178 cultivar. Grain nutrients uptake, nutrients recovery, and nutrients surplus significantly (all $p < 0.001$) influenced by the fertilization regime. The highest nutrients uptake was observed when biofertilizers was added with 75% NPK, while the highest nutrient recovery and lowest nutrients surplus were recorded when biofertilizers was added with 25% NPK. Compared with the treatment of 100% NPK, nutrients uptake, and nutrients recovery increased by 12.3, 15.5, 14.6, and 14.9%, and 55.0, 69.2, and 46.4%, respectively under the treatment of 75% NPK + biofertilizers, while nutrients surplus decreased by 65.4, 45.4, and 51.0%, respectively.

In general, significant (all $p < 0.05$) interaction influences were recorded under the effect of Zn × cultivar, Zn × fertilization regime, cultivar × fertilization regime, and Zn × cultivar × fertilization regime for nutrients uptake, nutrients recovery, and nutrients surplus (Table 3). However, grain Zn uptake, and P and K recovery did not response significantly to the interaction between Zn application and cultivars, whereas P surplus did not response significantly to the interaction between Zn and fertilization regime. Moreover, grain K uptake did not response significantly to cultivar × fertilization regime, and Zn × cultivar × fertilization regime. The treatment of 75% NPK + biofertilizers + Zn foliar application was the best treatment for increasing nutrients uptake (Fig. 5). The treatment of 25% NPK + biofertilizers + Zn foliar application recorded the highest nutrients

recovery and lowest nutrients surplus, while, the highest nutrient surplus and minimum nutrient recovery were observed when 100% NPK was applied lone (Fig. 6).

3.4. Zinc-biochemical co-fertilization increases farm profitability

The use of biofertilizers with 50% NPK and 75% NPK increased the net return compared to the use of the recommended doses of NPK alone (Table 4). The net return under the treatment of 75% NPK + biofertilizers without Zn foliar application was 81.2, 71.2, 82.5, and 156.6% higher than 100% NPK treatment for Giza 178, Giza 179, Sakha 104, and EHR1 cultivars, respectively, whereas these increments were 27.5, 21.5, 21.8, and 24.8%, respectively when Zn foliar application was done. The use of Zn foliar application alone without fertilization reduced cash losses by 48.2, 68.5, 75.8% for Giza 178, Giza 179, and EHR1 cultivars, respectively, whereas the net return was only 104 USD when Zn foliar application was used alone for Sakha 104 cultivar. Excluding control, the highest net return (2303 USD ha⁻¹) for all experiment treatments and cultivars was recorded when 75% NPK + biofertilizers treatment was added to Sakha 104 cultivar, while the minimum net return (136 USD ha⁻¹) was noted when biofertilizers were added alone to Giza 179 cultivar. When we used the recommended dose of NPK alone without biofertilizers, the highest net return was also recorded in Sakha 104 cultivar but without the application of foliar Zn.

4. Discussion

Zinc is involved in several physiological processes including protein synthesis, enzyme activation, carbohydrates metabolism, auxins, lipids, and nucleic acids. However, Zn deficiency is widespread in alkaline soils (as in our study) because its availability is inversely associated to soil pH. Moreover, lowland rice with uninterrupted flooding is widespread practiced in world irrigated lands. Rice is grown in Egypt by transplanting seedlings to a paddy field. Land preparation involves soaking, then plowing, and harrowing of saturated soils (Farooq et al., 2007). Zinc deficiency in rice occurs

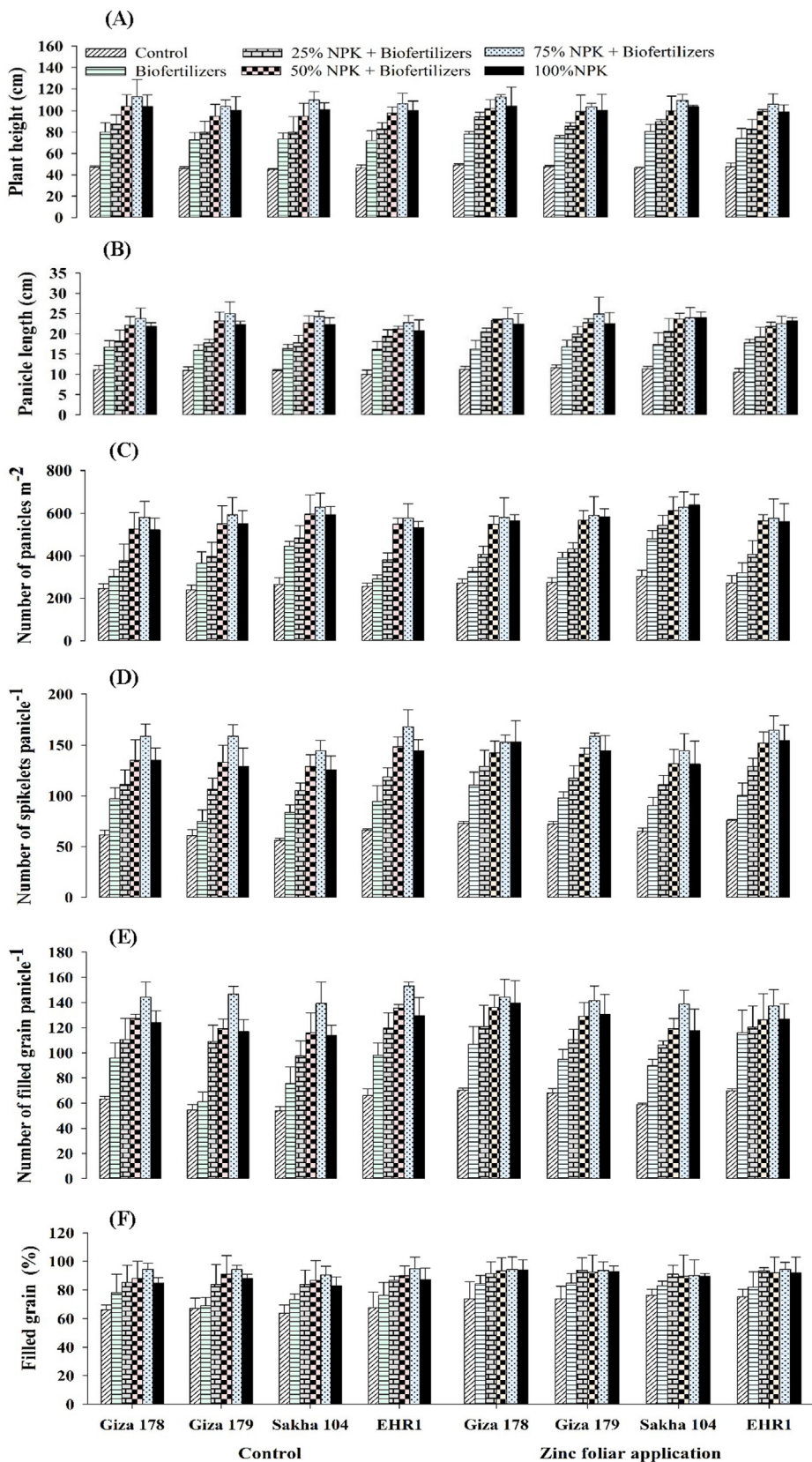


Fig. 2. Impact of zinc-biochemical co-fertilization on plant height (A), panicle length (B), number of panicles m^{-2} (C), number of spikelets $panicle^{-1}$ (D), number of filled grain $panicle^{-1}$ (E), and the percentage of filled grains (F) of the four studied rice cultivars. Data are mean of two seasons ($n = 4$) \pm SE.

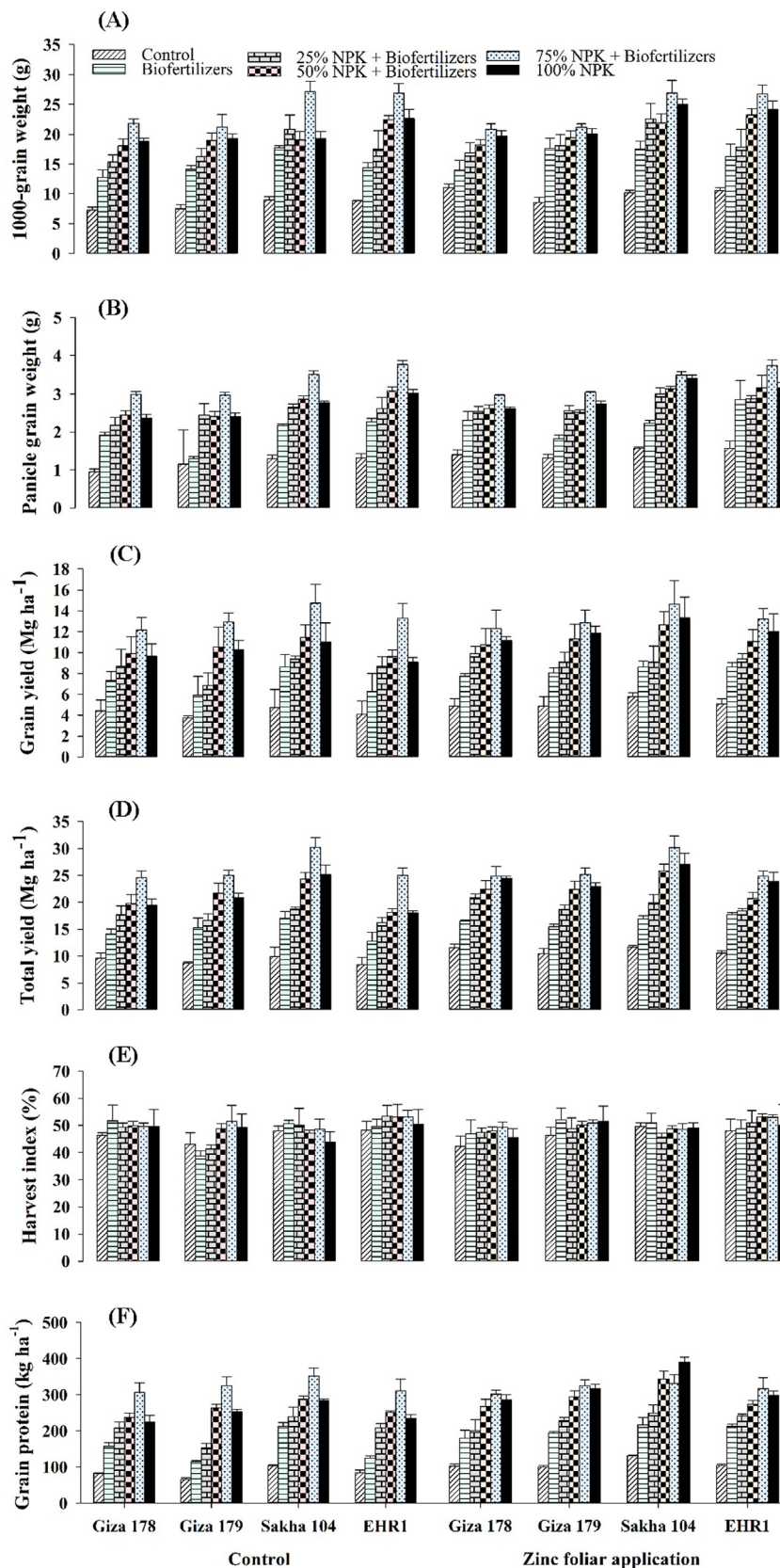


Fig. 3. Impact of zinc-biochemical co-fertilization on 1000-grain weight (A), panicle grain weight (B), grain yield (C), total yield (D), harvest index (E), and grain protein uptake (F) of the four studied rice cultivars. Data are mean of two seasons (n = 4) ± SE.

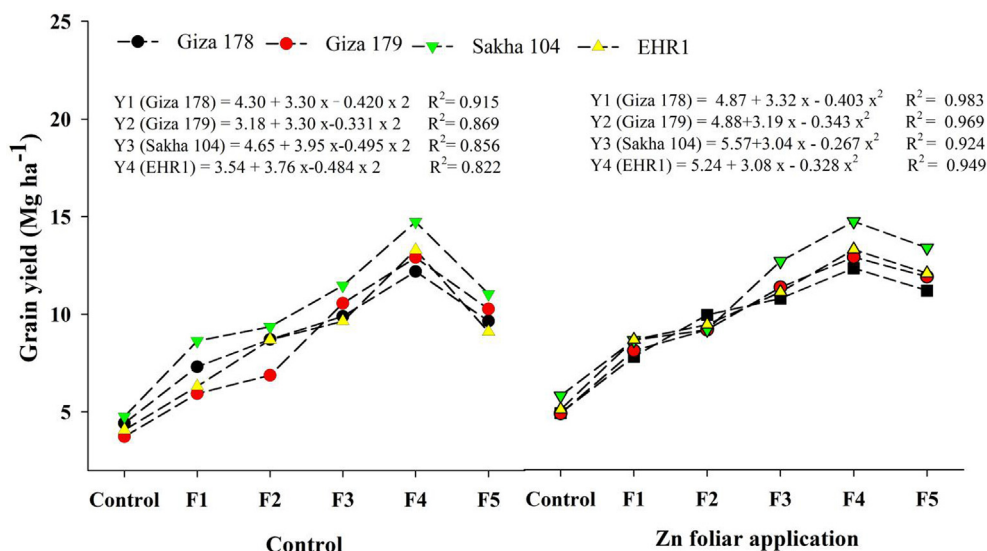


Fig. 4. Response curve of grain yield for the four studied rice cultivars under the effect of zinc-biochemical co-fertilization. F1, F2, F3, F4, and F5 refer to biofertilizers, 25% NPK + biofertilizers, 50% NPK + biofertilizers, 75% NPK + biofertilizers, and 100% NPK, respectively.

after transplanting and is a widespread phenomenon limiting productivity under saturated conditions (Quijano-Guerta et al., 2002). Significant increases in grain yield, straw and grain Zn contents were observed with foliar application of Zn as Zn-EDTA and ZnSO₄, but the highest increase was observed with Zn-EDTA application (Karak and Das, 2006). These findings were confirmed by our study where rice yield and its components significantly increased when Zn-EDTA was applied as a foliar application (Table 2). Moreover, our study revealed that Zn foliar application increased grain Zn content in different rice cultivars, which is consistent with the previous study of Rehman et al. (2012), who reported that under flooded conditions in slightly alkaline sandy clay soil, an increase by 180% in grain Zn content was recorded when foliar spray of Zn was done. This increase in grain Zn content was attributed to enhance leaf remobilization of Zn over grain filling (Rehman et al., 2012). Our study revealed improvement in number of filled grain panicle⁻¹ by 7.5% under Zn foliar application compared with no Zn application, while the filled grains percentage increased by 7.7% (Table 2). We also found that Zn foliar application significantly reduced nutrients surplus, but increased nutrients uptake and nutrients recovery (Table 3). It is possible that this increase in nutrient uptake also caused an increase in the filled grains percentage (Rana & Kashif, 2013; Fergany, 2018). Furthermore, Zn-deficient crops usually have decreased leaf chlorophyll contents (Chen et al., 2008). However, Zn foliar application significantly increased chlorophyll and carotenoids contents in rice leaves (Table 1). These increments are due to Zn acts as a structural and catalytic component of enzymes, proteins, and as co-factor for normal development of pigment biosynthesis (Balashouri & Prameeladevi, 1995).

Rice genotypes respond differently in terms of physiochemical constituents, grain yield, yield attributes, grain protein content and nutrients uptake. The genotypes; Giza 178 and Sakha 104 cultivars produced highest photosynthetic pigments. Moreover, the highest grain yield and its components, protein content as well as N and P recovery were noticed under Sakha 104 cultivar. The possible explanation for the higher grain yield, protein content and nutrients recovery by sowing Sakha 104 than other cultivars, was that the superiority genetic makeup of Sakha 104 rather than the remaining three cultivars. Also, such differences were due to

genetically variation in used genotypes and their interaction with environmental conditions (Khattab, 2019). These results are in accordance with those reported by Abd El-Wahab (1998), and Moonmoon et al. (2017).

The rice crop must uptake enough N, P, and K during its growth stage to attain optimum growth attributes and production. Inorganic fertilizers have been widely utilized to increase rice production and enhance its performance. We found that the use of recommended doses of inorganic N, P, and K fertilizers has led to increase the rice production and enhance all physiochemical attributes under the four studied cultivars. Inorganic fertilizers are readily soluble and hence can provide nutrients to rice crops within a short time after addition (Sarker et al., 2017). However, the application of large amounts of inorganic fertilizers has led to cropland degradation and declining crop yields as well as their negative impacts on the environment (Lassaletta et al., 2014). Moreover, the use of inorganic fertilizers under the current traditional agricultural methods that following in developing countries including Egypt decreases nutrients recovery while increasing nutrients surplus (Elrys et al., 2019a) (Table 3, and Fig. 5). For example, Djaman et al. (2018) revealed that NUE decreases with increasing inorganic N rates, and this might be due to increase N losses through ammonia (NH₃) volatilization, denitrification and N leaching to the environment (De Datta et al., 1989). Likewise, Lassaletta et al. (2014) reported that a further increase in inorganic N fertilizers addition would lead to a disproportionately low elevate in crop production with more environmental damage if the plants NUE is not substantially enhanced. Moreover, Oladele et al. (2019) found that moderate N rates (30–60 kg N ha⁻¹) increased rice yield, whereas high rates (90 kg N ha⁻¹) could result in increased rice height but reduced production. Furthermore, former studies have reported that decreased P-input regimes could sustain crop production while supplying adequate available P for crop growth for several years (Wang et al., 2016). When P fertilizer input increased as compared with P output in crop, P fixation in the soil increased with time due to less mobility of P nutrient (Kuo et al., 2005). Furthermore, soil potential for K supply to crops over the growing season depends on dissolved K on the one hand and K release from insoluble form to dissolved forms (Jadid Soleimandarabi et al., 2017).

Table 3
Influence of zinc-biochemical co-fertilization on grains nutrient uptake, nutrient surplus and nutrient recovery of the four studied rice cultivars.

Study factor	Grain N uptake (kg N ha ⁻¹)	Grain P uptake (kg P ha ⁻¹)	Grain K uptake (kg K ha ⁻¹)	Grain Zn uptake (kg Zn ha ⁻¹)	Nutrient surplus (kg ha ⁻¹)			Nutrient recovery (%)			
					N	P	K	N	P	K	
Zinc foliar application (Zn)											
Control	35.1B	8.44B	2.34B	0.139B	24.4A	10.5A	13.0A	64.1B	40.8B	55.5B	
Zn	43.8A	11.1A	2.96A	0.203A	8.02B	7.33B	8.20B	77.4A	46.8A	64.7A	
Cultivar (C)											
Giza 178	36.8C	8.87C	2.77A	0.171B	18.7B	9.97A	9.00C	69.8B	40.0C	62.6A	
Giza 179	37.8B	9.26B	2.45C	0.165C	16.7C	9.32C	11.6B	74.1A	43.5B	60.6B	
Sakha 104	45.0A	11.8A	2.76A	0.186A	6.65D	6.67D	8.93C	75.2A	50.7A	60.9AB	
EHR1	38.1B	9.12B	2.63B	0.161D	22.9A	9.65B	12.8A	64.0C	40.8BC	56.3C	
Fertilization regime (F)											
Control	16.4F	4.51E	1.53E	0.094F	–	–	–	–	–	–	
Biofertilizers	30.1E	7.81D	2.05D	0.129E	–	–	–	–	–	–	
25% NPK + biofertilizers	36.8D	10.8C	2.50C	0.159D	–22.6D	–2.63D	–6.5D	106A	77.1A	96.8A	
50% NPK + biofertilizers	50.0B	11.8B	3.08B	0.209B	–5.73C	4.63C	2.94C	79.8B	44.7B	64.4B	
75% NPK + biofertilizers	54.7A	12.7A	3.60A	0.231A	24.0B	11.9B	15.1B	59.2C	33.5C	47.0C	
100% NPK	48.7C	11.0C	3.14B	0.201C	69.3A	21.8A	30.8A	38.2D	19.8D	32.1D	
ANOVA											
	df	p-value of the main effects and their interactions									
Zn	1	0.018	0.016	0.034	0.018	0.037	0.020	0.008	0.041	0.045	0.010
C	3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
F	5	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Zn × C	3	<0.001	0.005	<0.001	0.088	0.001	0.002	<0.001	0.001	0.460	0.0180
Zn × F	5	<0.001	<0.001	0.022	<0.001	0.002	0.092	0.049	<0.001	0.049	<0.001
C × F	15	<0.001	<0.001	0.110	0.009	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Zn × C × F	15	<0.001	0.002	0.783	0.185	<0.001	0.025	0.006	0.004	0.046	0.009

Means followed by different letters in the same direction differ significantly by LSD ($p \leq 0.05$).

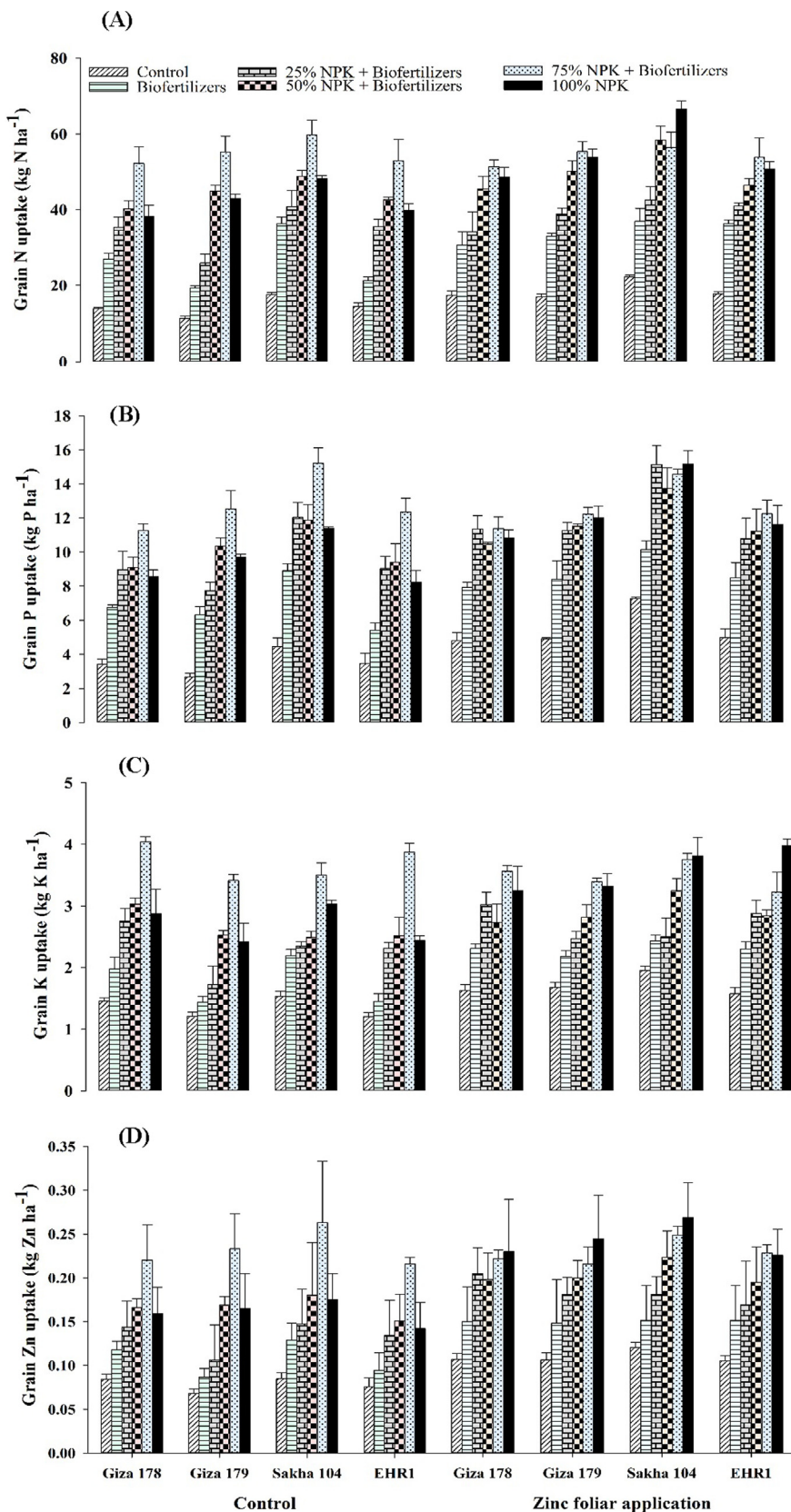


Fig. 5. Impact of zinc-biochemical co-fertilization on grain N uptake (A), grain P uptake (B), grain K uptake (C) and grain Zn uptake (D) of the four studied rice cultivars. Data are mean of two seasons (n = 4) ± SE.

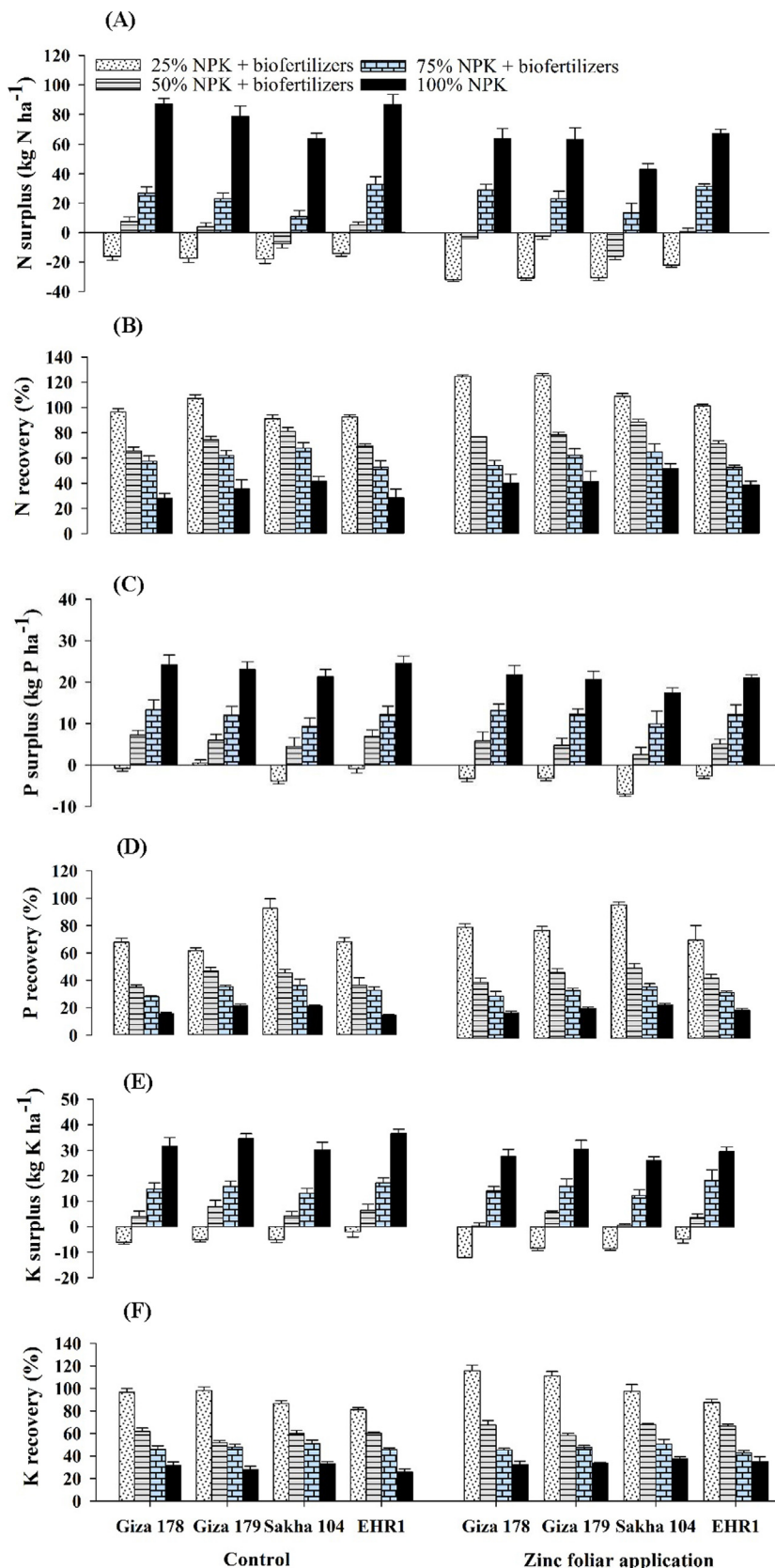


Fig. 6. Impact of zinc-biochemical co-fertilization on N surplus (A), N recovery (B), P surplus (C), P recovery (D), K surplus (E), and K recovery (F) of the four studied rice cultivars. Data are mean of two seasons (n = 4) ± SE.

Table 4
Analysis of farm operating input costs and profitability of the four studied rice cultivars under the influence of zinc-biochemical co-fertilization.

Zn foliar application	Cost and production input	Fertilization regime					
		Control	F ₁	F ₂	F ₃	F ₄	F ₅
Giza 178							
Control	Total cost (\$ ha ⁻¹)	1436	1445	1506	1567	1628	1679
	Yield (Mg ha ⁻¹)	4.42	7.31	8.71	9.89	12.18	9.65
Zn	Total income (\$ ha ⁻¹)	1179	1949	2323	2637	3248	2573
	Net return (\$ ha ⁻¹)	-257	504	817	1070	1620	894
Control	Return invested (\$)	0.82	1.35	1.54	1.68	2.00	1.53
	Total cost (\$ ha ⁻¹)	1440	1449	1510	1571	1632	1683
Zn	Yield (Mg ha ⁻¹)	4.9	7.79	9.93	10.77	12.32	11.17
	Total income (\$ ha ⁻¹)	1307	2077	2648	2872	3285	2979
Control	Net return (\$ ha ⁻¹)	-133	628	1138	1301	1653	1296
	Return invested (\$)	0.91	1.43	1.75	1.83	2.01	1.77
Giza 179							
Control	Total cost (\$ ha ⁻¹)	1436	1445	1506	1567	1628	1679
	Yield (Mg ha ⁻¹)	3.73	5.93	6.87	10.56	12.91	10.27
Zn	Total income (\$ ha ⁻¹)	995	1581	1832	2816	3443	2739
	Net return (\$ ha ⁻¹)	-441	136	326	1249	1815	1060
Control	Return invested (\$)	0.69	1.09	1.22	1.80	2.11	1.63
	Total cost (\$ ha ⁻¹)	1440	1449	1510	1571	1632	1683
Zn	Yield (Mg ha ⁻¹)	4.88	8.09	9.17	11.33	12.89	11.88
	Total income (\$ ha ⁻¹)	1301	2157	2445	3021	3437	3168
Control	Net return (\$ ha ⁻¹)	-139	708	935	1450	1805	1485
	Return invested (\$)	0.90	1.49	1.62	1.92	2.11	1.88
Sakha 104							
Control	Total cost (\$ ha ⁻¹)	1436	1445	1506	1567	1628	1679
	Yield (Mg ha ⁻¹)	4.76	8.63	9.36	11.46	14.74	11.03
Zn	Total income (\$ ha ⁻¹)	1269	2301	2496	3056	3931	2941
	Net return (\$ ha ⁻¹)	-167	856	990	1489	2303	1262
Control	Return invested (\$)	0.88	1.59	1.66	1.95	2.41	1.75
	Total cost (\$ ha ⁻¹)	1440	1449	1510	1571	1632	1683
Zn	Yield (Mg ha ⁻¹)	5.79	8.63	9.17	12.68	14.72	13.37
	Total income (\$ ha ⁻¹)	1544	2301	2445	3381	3925	3565
Control	Net return (\$ ha ⁻¹)	104	852	935	1810	2293	1882
	Return invested (\$)	1.07	1.59	1.62	2.15	2.41	2.12
EHR1							
Control	Total cost (\$ ha ⁻¹)	1436	1445	1506	1567	1628	1679
	Yield (Mg ha ⁻¹)	4.07	6.31	8.69	9.64	13.3	9.1
Zn	Total income (\$ ha ⁻¹)	1085	1683	2317	2571	3547	2427
	Net return (\$ ha ⁻¹)	-351	238	811	1004	1919	748
Control	Return invested (\$)	0.76	1.16	1.54	1.64	2.18	1.45
	Total cost (\$ ha ⁻¹)	1440	1449	1510	1571	1632	1683
Zn	Yield (Mg ha ⁻¹)	5.08	8.65	9.43	11.09	13.28	12.05
	Total income (\$ ha ⁻¹)	1355	2307	2515	2957	3541	3213
Control	Net return (\$ ha ⁻¹)	-85	858	1005	1386	1909	1530
	Return invested (\$)	0.94	1.59	1.67	1.88	2.17	1.91

F₁, F₂, F₃, F₄, and F₅ refer to biofertilizers, 25% NPK + biofertilizers, 50% NPK + biofertilizers, 75% NPK + biofertilizers, and 100% NPK, respectively.

The effect of individual biochemical (N, P, or K) fertilization on rice yield, yield components and nutrients uptake and recovery has been shown in several studies, and it was generally considered that these parameters increased with added biofertilizers N, P, or K with inorganic fertilizers. However, the response of these attributes to mixture biofertilizers NPK and inorganic NPK fertilizers application under different rice cultivars is still not obvious. Interaction between biofertilizers and inorganic NPK fertilizers stimulated rice plant growth and improved rice production and yield components. These results clearly indicate that the over use of 100% NPK was not in favor of rice plant and hence a certain undesirable balance between the source and the sink might have had taken place due to an excessive growth of plants causing lodging a possible mutual shading effect to lower plant leaves during grain filling. This shading effect was reported to increase photosynthates losses due an enhanced respiration by lower rice plant leaves on the expense of photosynthates portioning towards rice grain and hence grain yield. Shading to lower plant leaves in submerged rice was, also, reported to introduce soil reduction conditions which decrease the availability of nutrients and increase the toxicity of sulfur and a number of micronutrients among them mainly manganese (Tisdale & Nelson, 1966).

The synergistic influence of biofertilizers and inorganic NPK fertilizers is considered to be the result of elevated rice nutrient uptake, less nutrient losses and enhanced nutrient availability (Table 3, and Figs. 4 and 5). Nutrients recovery also influenced by nutrients loss (Table 3, and Fig. 5). Reduced nutrients loss resulted in a better use of available nutrients and thereby produced a higher nutrient recovery (Ladha et al., 2005; Lassaletta et al., 2014). In our study, the biofertilizers that partially (25, 50, or 75%) substituted the inorganic NPK fertilizers effectively decreased the total nutrient surplus (Table 3) by 53–103 kg N ha⁻¹, 11–25 kg P ha⁻¹, and 17–40 kg K ha⁻¹ under different rice cultivars compared with the 100% NPK treatment. This could be due to the mixture biofertilizers containing a consortium of N-fixing, P-solubilizing bacteria, and K-solubilizing bacteria which in addition to nutrients can provide plants with growth-promoting phytohormones, enzymes, and organic acids (Panahwar et al., 2012). These beneficial properties directly affected rice physiology and enhanced the contents of chlorophyll, carbohydrate and total amino acids in rice (Table 1, and Fig. 1). Biofertilizers are proven to increase plants nutrient uptake, and thus enhance various physiological attributes of plant performances. Higher chlorophyll contents indicate a better root system that works properly to increase plants' performance in

absorbing water and nutrients (Thakur et al., 2010). The better biofertilizers performance with medium rate of inorganic fertilizer addition was observed (Paul et al., 2013). Moreover, the effective *Azospirillum* biofertilizer application with 80% inorganic N fertilizer provides a better rice production (Islam et al., 2012) with several rice varieties (Singh, 2014). Our results are consistent with the previous results of Gamal-Eldin and Elbanna (2011), who substituted 50% of inorganic N fertilizer with biofertilizer in flooded rice field and attained grain yield statistically equivalent to 100% inorganic N fertilizer. However, our findings revealed that substituted 25% of inorganic N fertilizer with biofertilizers obtain rice yield more than 100% inorganic N fertilizer. Accordingly, the increase in rice production and yield component (including number of panicles m^{-2} , number of spikelets panicle $^{-1}$, number of filled grain panicle $^{-1}$, filled grain, 1000-grain weight, panicle grain weight, grain yield, total yield, HI, and protein content) in our study (Table 2 and Figs. 2 and 3) due to the combination of biofertilizers with inorganic fertilizers NPK might be due to the increased availability and utilization of necessary nutrients (N, P, and K). Moreover, biofertilizers plus 25% or 50% inorganic NPK resulted in an equal and higher net economic benefit (Table 4), which indicates that substituting biofertilizers for 25% or 50% of inorganic fertilizers supplies a financially attractive approach for farmers to substantially enhance nutrient recovery and rice production. Furthermore, the response of all former characteristics to biochemical fertilization regimes was cultivar dependent. In our study, four cultivars with contrasting agronomical traits were utilized to estimate the genotypic variations in rice performance and nutrient uptake response to biochemical fertilization regimes. Our findings revealed significant genotypic variations between the studied rice cultivars (Tables 1, 2, and 3). However, the cultivar of Sakha 104 achieves the highest net return under different treatments (Table 4).

In our experiment, the combined application of fertilizers and Zn also had a significant, positive effect on physiochemical constituents, yield, and yield components, grain nutrient uptake and recovery, and nutrient surplus of rice genotypes, (Tables 1–3, and Figs. 1–6), which is consistent with the previous study of Almendros et al. (2019). The positive interaction between nutrients and Zn is due to enhancements in root uptake and the translocation of Zn due to the presence of N. Several N compounds, such as amino acids, could be involved in the translocation of Zn from the root into the shoot. Speciation and localization studies conducted on cereal grain indicate that Zn interacts with proteins in the grain and therefore grain proteins constitute a physiological sink for Zn. With respect to the three-way interaction, the higher physiochemical constituents, grain yield, yield attributes, grain protein content, and nutrients uptake were produced by Giza 178 and Sakha 104 under Zn foliar spraying with adding 75% NPK + bio fertilizers. The higher net return was also recorded by sowing Sakha 104 cultivar with addition of 75% NPK + biofertilizers but without the application of Zn. This effect could be attributed to the superiority genetic makeup and varietal differences and direct or indirect positive roles of Zn as well as fertilization regimes on soil physico-chemical properties by enriching nutrients availability, and by improving soil properties.

Finally, our findings revealed that inorganic NPK fertilizers combined with NPK biofertilizers and Zn foliar application could increase rice performance by improving nutrients recovery and decreasing nutrient loss. According to these results, a complementary effect could be traced between the biofertilization and mineral fertilizers (75% NPK + biofertilizers) with foliar application of Zn with sowing Sakha 104 to maximize the grain yield and yield components as well grains nutrient uptake and recovery, while reducing nutrient losses.

5. Conclusions

Farmers in developing countries need fertilization techniques that are easier, cheaper, and more efficient than using inorganic fertilizers. The increased utilize of biofertilizers can lead to decreased inorganic fertilizers usage and thus less environmental pollution. In this study, we provide a comprehensive assessment of the effect of Zn-biochemical (NPK) co-fertilization on yield and yield components, physicochemical characteristics, nutrient uptake, nutrient recovery, nutrients surplus, and farm profitability of four rice cultivars under semi-arid environmental conditions. Our findings revealed that inorganic NPK fertilizers combined with NPK biofertilizers and Zn foliar application could increase rice performance by improving nutrients recovery and decreasing nutrient loss. According to these results, a complementary effect could be traced between the biofertilization and inorganic fertilizers (75% NPK + biofertilizers) with foliar application of Zn with sowing Sakha 104 to maximize the grain yield and yield components as well grains nutrients uptake and recovery, while reducing nutrients loss. Accordingly, NPK biofertilizers replacement for 25% of inorganic NPK with the foliar application of Zn supplies a financially attractive choice to substantially enhance nutrient use efficiency and production in intensive rice cropping systems, while effectively decreasing nutrient loss.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

Conceived and designed the experiments: E.E.A.E. and A.S.E. Performed the experiments: E.E.A.E. Contributed reagents/materials/analysis tools: E.E.A.E. Analyzed the data: E.E.A.E. AET, MES, SMS, and A.S.E. Wrote the paper: A.S.E., AET, MES, and SMS. Revised the paper: E.E.A.E. AET, MES, SMS, and A.S.E. All authors read and approved the final manuscript.

References

- Abd El-Wahab, A., 1998. Physiological behavior of some Egyptian rice cultivars under different nitrogen levels. *Egypt. J. Appl. Sci* 13, 119–129.
- Ahmed, A.K.M., Jiang, L., Wang, F., et al., 2020. Variation analysis of growth traits of four poplar clones under different water and fertilizer management [J]. *J. For. Res.* 31, 45–55.
- Almendros, P., Obrador, A., Alvarez, J.M., Gonzalez, D., 2019. Zn-DTPA-HEDTA-EDTA application: a strategy to improve the yield and plant quality of a barley crop while reducing the N application rate. *J. Soil Sci. Plant Nutrition* 19 (4), 920–934.
- Bishni, U.R., Hughes, I.L., 1979. Agronomic performance and protein content of fall-planted tritica, wheat and rye. *Agron. J.* 71, 359–360.
- Black, C., 1968. Soil plant relationships. John Wiley and Sons, of the Association of Official Analytical Chemists, 14th (ed), Published by the Association of Official Analytical Chemists, PO Box, 540. Benjamin Franklin Station, Washing Mg, dc.
- Chapman, H., Pratt, F., 1982. Determination of minerals by titration method: methods of analysis for soils, plants and water. California University, Agriculture Division, USA.
- Christias, C., Couvaraki, C., Georgopoulos, S.G., Macris, B., Vomvovanni, V., 1975. Protein content and amino acid composition of certain fungi evaluated for microbial protein production. *Appl. Microbiol.* 29 (2), 250–254.
- De Datta, S.K., Trevitt, A.C.F., Freney, J.R., Obcemea, W.N., Real, J.G., Simpson, J.R., 1989. Measuring nitrogen losses from lowland rice using bulk aerodynamic and nitrogen-15 balance methods. *Soil Sci. Soc. Am. J.* 53 (4), 1275–1281.

- Djaman, K., Mel, V., Ametonou, F., El-Namaky, R., Diallo, M., Koudahe, K., 2018. Effect of nitrogen fertilizer dose and application timing on yield and nitrogen use efficiency of irrigated hybrid rice under semi-arid conditions.
- Gharous, M.E., Boulal, H., 2016. IPNI North Africa challenges in nutrient management under rainfed agriculture of Morocco. *Procedia Eng.* 138, 332–336.
- Elrys, A.S., Desoky, E.-S.-M., Alnaimy, M.A., Zhang, H., Zhang, J.-B., Cai, Z.-C., et al., 2021. The food nitrogen footprint for African countries under fertilized and unfertilized farms. *J. Environ. Manage.* 111599.
- Elrys, A.S., Desoky, E.-S., Abo El-Maati, M.F., Elnahal, A.S., Abdo, A.I., Raza, S., Zhou, J., 2019a. Can secondary metabolites extracted from Moringa seeds suppress ammonia oxidizers to increase nitrogen use efficiency and reduce nitrate contamination in potato tubers? *Ecotoxicol. Environ. Saf.* 185, 109689. <https://doi.org/10.1016/j.ecoenv.2019.109689>.
- Elrys, A.S., Raza, S., Abdo, A.I., Liu, Z., Chen, Z., Zhou, J., 2019b. Budgeting nitrogen flows and the food nitrogen footprint of Egypt during the past half century: Challenges and opportunities. *Environ. Int.* 130, 104895. <https://doi.org/10.1016/j.envint.2019.06.005>.
- FAOSTAT, F., 2021. Statistical databases. Food and Agriculture Organization of the United Nations.
- Galloway, J.N., Cowling, E.B., 2021. Reflections on 200 years of Nitrogen, 20 years later. *Ambio*, 1–5.
- Gamal-Eldin, Hosny, Elbanna, Khaled, 2011. Field evidence for the potential of *Rhodobacter capsulatus* as biofertilizer for flooded rice. *Curr. Microbiol.* 62 (2), 391–395.
- Gomez, K.A., Gomez, A.A., 1984. Statistical procedures for agricultural research. John Wiley & Sons.
- Heffer, P., Gruère, A., Roberts, T., 2009. Assessment of fertilizer use by crop at the global level. International Fertilizer Industry Association, Paris, www.fertilizer.org/ifa/Home-Page/LIBRARY/Publication-database.html/Assessment-of-Fertilizer-Use-by-Crop-at-the-Global-Level-2006-07-2007-08.html.
- Islam, M.Z., Sattar, M.A., Ashrafuzzaman, M., Saud, H.M., Uddin, M., 2012. Improvement of yield potential of rice through combined application of biofertilizer and chemical nitrogen. *African J. Microbiol. Res.*, 6, 745–750.
- Jadid Soleimandarabi, M., Hashemabadi, D., Zaredost, F., 2017. The effect of potassium biofertilizer and chemical fertilizer on quantitative and qualitative traits of periwinkle (*Catharanthus roseus* cv. 'Acillata'). *J. Ornamental Plants* 7, 213–222.
- Kantachote, Duangporn, Nunkaew, Tomorn, Kantha, Thanawan, Chairapat, Sumate, 2016. Biofertilizers from *Rhodospseudomonas palustris* strains to enhance rice yields and reduce methane emissions. *Appl. Soil Ecol.* 100, 154–161.
- Khan, Mohammad Saghir, Zaidi, Almas, Wani, Parvaze A., 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture—a review. *Agron. Sustainable Dev.* 27 (1), 29–43.
- Khatab, E., 2019. Performance evaluation of some rice varieties under the system of planting in Egypt. *Asian J. Res. Crop Sci.*, 1–10.
- Kuo, S., Huang, B., Bembek, R., 2005. Effects of long-term phosphorus fertilization and winter cover cropping on soil phosphorus transformations in less weathered soil. *Biol. Fertil. Soils* 41 (2), 116–123.
- Ladha, J.K., Pathak, H., Krupnik, T.J., Six, J., van Kessel, C., 2005. Efficiency of fertilizer nitrogen in cereal production: retrospects and prospects. *Adv. Agron.* 87, 85–156.
- Lassaletta, Luis, Billen, Gilles, Grizzetti, Bruna, Anglade, Juliette, Garnier, Josette, 2014. 50 year trends in nitrogen use efficiency of world cropping systems: the relationship between yield and nitrogen input to cropland. *Environ. Res. Lett.* 9 (10), 105011. <https://doi.org/10.1088/1748-9326/9/10/105011>.
- Moonmoon, S., Fakir, M., Islam, M., 2017. Effect of drought stress on grain dry weight, photosynthesis and chlorophyll in six rice genotypes. *Sch. J. Agric. Vet. Sci* 4, 13–17.
- Nissen, O., 1983. MSTAT-C, a microcomputer program for the design, management and analysis of agronomic research experiments. Michigan State University, East Lansing, Michigan, USA.
- Oladele, S.O., Adeyemo, A.J., Awodun, M.A., 2019. Influence of rice husk biochar and inorganic fertilizer on soil nutrients availability and rain-fed rice yield in two contrasting soils. *Geoderma* 336, 1–11.
- Panhwar, Q.A., Othman, R., Rahman, Z.A., Meon, S., Ismail, M.R., 2012. Isolation and characterization of phosphate-solubilizing bacteria from aerobic rice. *Afr. J. Biotechnol.* 11, 2711–2719.
- Paul, N., Cruz, P., Aguilar, E., Badayos, R., Hafele, S., 2013. Evaluation of biofertilizers in cultured rice. *J. Biofertil Biopestici* 4, 2.
- Peñuelas, Josep, Poulter, Benjamin, Sardans, Jordi, Ciais, Philippe, van der Velde, Marijn, Bopp, Laurent, Boucher, Olivier, Godderis, Yves, Hinsinger, Philippe, Llusia, Joan, Nardin, Elise, Vicca, Sara, Obersteiner, Michael, Janssens, Ivan A., 2013. Human-induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. *Nat. Commun.* 4 (1). <https://doi.org/10.1038/ncomms3934>.
- Qadar, Ali, 2002. Selecting rice genotypes tolerant to zinc deficiency and sodicity stresses. I. Differences in zinc, iron, manganese, copper, phosphorus concentrations, and phosphorus/zinc ratio in their leaves. *J. Plant Nutr.* 25 (3), 457–473.
- Rehman, Hafeez-ur, Aziz, Tariq, Farooq, Muhammad, Wakeel, Abdul, Rengel, Zed, 2012. Zinc nutrition in rice production systems: a review. *Plant Soil* 361 (1–2), 203–226.
- Sarker, U., Uddin, M., Sarker, M., Salam, M., Khairul, A., 2017. Influence of organic and inorganic nitrogen on the growth and yield of irrigated rice. *Asian-Aus. J. Biosci. Biotech* 2, 9–23.
- Singh, M.K., 2014. Evaluation of *Azospirillum* strains as biofertilizers for rice. *Int. J. Farm Sci.* 4, 15–18.
- Steel, R.G., 1997. Principles and procedures of statistics a biometrical approach.
- Thakur, A., Uphoff, N., Antony, E., 2010. An assessment of physiological effects of system of rice intensification (SRI) practices compared with recommended rice cultivation practices in India. *Experimental Agriculture*, 46, 77.
- Tisdale, Samuel L., Nelson, Werner L., 1966. Soil fertility and fertilizers. *Soil Sci.* 101 (4), 346. <https://doi.org/10.1097/00010694-196604000-00016>.
- Varvel, G., Peterson, T.A., 1990. Nitrogen fertilizer recovery by corn in monoculture and rotation systems. *Agronomy J.*, 82, 935–938.
- Von Grebmer, K., Bernstein, J., Hossain, N., Brown, T., Prasai, N., Yohannes, Y., et al., 2017. 2017 Global Hunger Index: the inequalities of hunger. *Intl Food Policy Res Inst.*
- Wang, Yu, Zhao, Xu, Wang, Lei, Zhao, Pin-Heng, Zhu, Wen-Bin, Wang, Shen-Qiang, 2016. Phosphorus fertilization to the wheat-growing season only in a rice–wheat rotation in the Taihu Lake region of China. *Field Crops Res.* 198, 32–39.
- Watanabe, F., Olsen, S., 1965. Test of an ascorbic acid method for determining phosphorus in water and NaHCO₃ extracts from soil. *Soil Sci. Soc. Am. J.*, 29, 677–678.
- Wettstein, D., 1957. Formula of chlorophyll determination. *Exp. Cell Res.* 12, 427–489.
- WG, S.G.C., 1980. Statistical Methods 7th ed The Iowa State Univ. Press Ames, IA, 514.
- Williams, S., 1984. Official methods of analysis. Association of Official Analytical Chemists.
- Worldometers, 2017. World Population. Worldometers.info.
- Yao, Yuanlin, Zhang, Min, Tian, Yuhua, Zhao, Miao, Zeng, Ke, Zhang, Bowen, Zhao, Meng, Yin, Bin, 2018. Azolla biofertilizer for improving low nitrogen use efficiency in an intensive rice cropping system. *Field Crops Res.* 216, 158–164.
- Balashouri, P., Prameeladevi, Y., 1995. Effect of zinc on germination, growth and pigment content and phytomass of *Vigna radiata* and *Sorghum bicolor*. *J. Ecobiology* 7, 109–114.
- Fergany, M., 2018. Response of rice yield and yield components as well as grain quality to number and levels of zinc foliar spraying application. *Egypt. J. Agron.* 40 (3), 331–340.
- Rana, W.K., Kashif, S.R., 2013. Effect of different zinc sources and methods of application on rice yield and nutrients concentration in rice grain and straw. *J. Environ. Agricultural Sci. (JEAS)* ISSN 2313, 8629.
- Fadeels, A.A., 1962. Location and properties of chloroplasts and pigment determination in roots. *Physiol. Plantarum* 15, 130–147.
- Karak, T., Das, D., 2006. Effect of foliar application of different sources of Zn application on the changes in Zn content, uptake and yield of rice (*Oryza sativa* L). 18th World Congress of Soil Science, July 9–15, 2006–Philadelphia, Pennsylvania, USA.
- Chen, W., Yang, X., He, Z., Feng, Y., Hu, F., 2008. Differential changes in photosynthetic capacity, 77 K chlorophyll fluorescence and chloroplast ultrastructure between Zn-efficient and Zn-inefficient rice genotypes (*Oryza sativa*) under low zinc stress. *Physiol Plant* 132:89–101.
- Farooq, M., Basra, S.M.A., Ahmad, N., 2007. Improving the performance of transplanted rice by seed priming. *Plant Growth Regul* 51:129–137.
- Gao, Xiaopeng, Zou, Chunqin, Fan, Xiaoyun, Zhang, Fusuo, Hoffland, Ellis, 2006. From flooded to aerobic conditions in rice cultivation: consequences for zinc uptake. *Plant Soil* 280 (1–2), 41–47.
- Quijano-Guerta, C., Kirk, G.J.D., Portugal, A.M., Bartolome, V.I., McLaren, G.C., 2002. Tolerance of rice germplasm to zinc deficiency. *Field Crops Res* 76 (2–3), 123–130.
- Welch, Ross M, Graham, Robin D, 1999. A new paradigm for world agriculture: meeting human needs. Productive, sustainable, nutritious. *Field Crops Res* 60 (1–2), 1–10.