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ORIGINAL PAPER

Combined Vinasse and Mineral NPK Fertilizer Affect Physio-Biochemical, Root, and Yield Characters of Faba Bean (*vicia faba* l.) Genotypes Grown on Saline Soil

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Abstract

The objective of this study is to assess the effects of combined applications of recommended traditional chemical fertilization NPK 100% without Vinasse and with 100, 75, 50 NPK + Vinasse on Physio-Biochemical, root, shoot traits, yield, and its attributes for four faba bean Giza-429, Giza-843, Misr-2 and Sakha-3 genotypes which are cultivated in the soil is salt affected of eastern Egypt. The experimental design was conducted using a strip-plot design with three replicates in 2020 and 2021. Treatments of different doses of chemical fertilization were 100% NPK without Vinasse and 100%, 75%, and 50% of NPK with Vinasse as supplementary organic fertilizer were arranged in vertical strips, while horizontal strips were devoted to faba bean genotypes (*Vicia faba L.*) i.e. Giza-429, Giza-843, Misr-2, and Sakha-3. Relative to the other fertilizer combinations, the 75% NPK + Vinasse did not contribute more than 5% more yield to Sakha 3 than it did with the 100% NPK + Vinasse. Both the fertilizer and cultivar main effects had the same positive linear trend, with relatively higher total seed yield in the case of the 75% NPK + Vinasse and of cv. Sakha 3. The harvest index (HI) was different only for fertilizer levels.The 75% NPK + Vinasse differed by a very negligible margin from the 100% NPK + Vinasse, but both differed from the other two levels. Along with seed yield, both straw and biological yields were significant for the interaction effects, as well as for the main effects. Within all fertilizer combinations, cultivars Giza 843 and Saka 3 consistently, as a subgroup, outyielded the other two cultivars, not only for total seed yield but also for all other yield component characters.

Keywords Faba bean · Vinasse · Mineral NPK · Salinity · Physio-biochemical traits · Root traits · Yield traits and K^+/Na^+ ratio

1 Introduction

Legume crops are the essential pulse crop in the world. It is a vital source of proteins, calories, minerals, dietary fibers, and vitamins for millions of people (FAO 2020). Moreover, legume crops improve soil fertility through their efficient role in the fixation of biological nitrogen in soil. Faba bean (*Vicia faba L.*) is an important leguminous crop used for human nutrition in Egypt, it is considered one of the important winter season legume crops

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(Semba et al. 2021). Due to its high protein content of 28–30% and high carbohydrates 51–68 (Abdellatif et al. 2012; Martineau-Côté et al. 2022). In Egypt, its cultivated area was 0.40 M h^{-1} with a production of 0.139 million tons (FAO. 2022), the total production of this crop is still scanty to cover the local consumption. From the aforementioned data, there is a big request to overcome this space between native production and demand from here. Raising crop yield is one of the principal specific purposes of the agricultural strategy and can be executed by enlarging the cultivated field, using superior productive varieties, and providing sufficient and harmonious rates of indispensable plant nutrients and weed control. High salinity levels limit some faba bean cultivation in salt-affected soil, negatively impacting physiological traits, pigments, cell viability, and yield. Salinity hampers water absorption and nutrient uptake, leading to reduced growth and unfavorable K^+/Na^+ and Ca^{2+}/Na^+ ratios in leaves (Filipović et al. 2020; Afzal et al. 2022; Elsherpiny 2023).

At present days, there is an increasing requirement for chemical fertilizers to meet high agricultural production. Chemical fertilizer plays a significant part in increasing soil fertility and crop productivity (Hera 1995; Jiang et al. 2018). Nevertheless, continuous use of chemical fertilizers has contributed to a consequent decline in agricultural soil quality decreased soil organic matter content, and an increase in soil acidification and environmental pollution (NING et al. 2017). Hence, the utilization of organic fertilizer which has richer nutrient elements, can improve the physical properties of soil by enhancing aggregate stability and reducing soil bulk density. It can also improve the biological and biochemical properties of soil and optimize soil microbial structure (Diacono and Montemurro 2011; Ladha et al. 2022).

Vinasse is considered as a by-product of sugar production. It represents residues from molasses fermentation processes. Nowadays, Vinasse is considered a source of organic fertilizers within the agricultural sector as an eco-friendly source of nutrient elements (Oldroyd and Dixon 2014; Hoarau et al. 2018). Liquid vinasse composition is characterized by high organic compounds and major ions (Oldroyd and Dixon 2014; Prado et al. 2013; Katakojwala et al. 2019), contains high quantities of the plant macronutrients such as nitrogen, phosphorus, potassium, iron, copper, magnesium and manganese. The use of vinasse in fertigation systems has pros; as it can improve soil fertility and crop productivity (Jiang et al. 2012). Application of vinasse on short-term crops leads to providing higher yield than crops treated with chemical fertilizer NPK (15:15:15) (Clementson et al. 2016; de Chaves et al. 2021). Vinasse can be an alternative and complementary source of nutrients; furthermore, it has a significant impact on enhanced nodules formation and N-fixation and increased legume production (Udvardi and Poole 2013; Yadav et al. 2017). Finally, the practical management of chemical fertilizer and vinasse on salt-afflicted soils and tolerant genotypes can play a significant role in increasing and sustaining the national food security of faba bean and also it can solve the environmental problem of the disposal of this agro-industrial residue (Prado et al. 2013; Soobadar and Ng Kee Kwong 2012; de Chaves et al. 2021). In the present study, field trials of different doses of recommended chemical fertilization NPK with vinasse as supplementary organic fertilizer were conducted in newly reclaimed soil which is salt-affected, to investigate the effects of combined applications for mineral fertilizer reduction and supplementation with vinasse fertilizer on chemical, physiological, yield and its attributes for four faba bean genotypes.

2 Materials and Methods

2.1 The Experimental Site, Soil, and Climatic Conditions

A field experiment was conducted at a private farm, in Ismailia Governorate, Egypt (30° 35´41.9" N and 32° 16´ 45.8" E) in 2020 and 2021. The physical and chemical properties of the experimental soil were determined according to (Cotteine et al. 1982) before sowing. The experimental soil was sandy throughout the profile depth from 0 to 60 cm (65.4% coarse sand, 28.3% fine sand, 1.9% silt, and 4.4% clay). The pH was 8.1 (Suspension 1: 2.5) and the electrical conductivity was 4.1 dS m^{-1} (saturated paste extract), the available nutrients of experimental soil of N. P and K (mg kg^{-1}) were 34.8, 11.5, and 33.2 respectively; and organic matter % was 0.6 and $CaCO_3$% was 1.62, Soluble cations (mmole l1) i.e. Ca^{++}, Mg^{++}, Na^+ and K^+ were 0.93, 0.84, 1.49 and 0.43 respectively; and soluble anions CO_3^{--}, HCO_3^- and CL were 1.37, 1.02 and 1.30, respectively, while SO_4- was nil. The experimental soil was NPK deficient. The monthly minimum and maximum temperatures and total rainfall for the two growing seasons are shown in Table 1.

2.2 Agronomic Practices and Experimental Design

The Vinasse used in this study contained 20% total free amino acid, 7% free amino acid; 4.62% total-N; 0.2% P_2O_5; 9.8% K_2O; 0.87% Ca; 0.16% Mg; 10.04% S; 8.5 B (mg L^{-1}); 5.3 Mo (mg L^{-1}); 71 Fe (mg L^{-1}); 11.3 Mn (mg L^{-1}); 483.8 Zn (mg L^{-1}); 5.3 Cu (mg L^{-1}); 762.6 Cytokinins (mg L^{-1}); 495.2 Gibberellic acid (mg L^{-1}); 59.75% organic matter, 34.66% organic carbon and 7.23 pH. Vinasse composition analysis according to Angel Yeast Factory. The vinasse 9

Table 1 Climatic conditions of the study site (wind speed, relative humidity, maximum temperature, minimum temperature, precipitation rate) of the area in the 2020/21 and 2021/22 seasons

2020/21	WS2M	RH2M	TEMP 2 M		Precipitation (mm)
Month			MIN	MAX	
NOV	2.56	58.34	15.48	27.37	0.01
DEC	2.90	63.43	10.13	20.54	0.46
JAN	3.16	68.22	7.54	17.56	1.03
FEB	2.49	66.99	8.19	19.99	0.47
MAR	3.24	61.29	10.28	23.95	2.62
APR	2.98	61.16	12.43	25.93	3.29
2021/22	WS2M	RH2M	T2M_MIN	T2M_MAX	Precipitation (mm)
Month					
NOV	2.26	64.20	14.36	24.48	0.65
DEC	2.14	64.45	10.88	22.15	0.15
JAN	2.66	63.49	9.00	20.83	0.13
FEB	2.56	65.74	8.93	21.21	0.88
MAR	2.98	60.77	10.27	23.06	0.99
APR	3.24	52.19	12.34	28.75	0.02

T2M_ MIN, MAX (Maximum and minimum temperature at 2 m high); RH2M (Relative humidity at 2 m high); WS2M (Wind speed at 2 m high), Precipitation mm (Precipitation rate mm/month)

L h^{-1} was applied after 20 DAS as fertigation. All diluted vinasse was added to plants in equal doses at a 7-d interval, until 90 days along the period of plant growth the total amount added was (90 L h^{-1}).

Two factors were laid out in a three-randomized complete strip block design. The vertical strips were: 100% NPK (T1); 50% NPK w/vinasse (T2); 75% NPK w/vinasse (T3); and 100% NPK w/vinasse (T4). Horizontal strips were faba bean (*Vicia faba L.*) cultivars: Giza429 (G1); Giza843, (G2); Misr-2 (G3), and Sakha3 (G4). These cultivars are salt tolerant (Atwa et al. 2008; Mahdi 2016; Ali et al. 2019). Seedswere obtained from the Agricultural Research Center (ARC), Giza, Egypt. Treatments were applied into 10-row 4 m × 5 m plots. Urea (46% N) was applied 21 and 45 DAS at a rate of 47.6 kg N h^{-1}. Calcium superphosphate (15.5% P_2O_5) was added at a rate of 73.8 kg P_2O_5 h^{-1} before planting. Potassium sulphate (48% K_2O) was applied at a rate of 114.24 kg K_2O h^{-1} 21 and 45 DAS. The planting date was 10 November in the 2 yrs. at a seeding rate of 120 kg h^{-1}. Three seeds were planted in 20-cm apart hills on 50-cm apart rows; at 21 DAS, seedlings were thinned to two. Harvest was on 6 April in both years. Reference crop evapotranspiration (ETo) was estimated according to the Penman–Monteith equation (Allen et al. 1998). Full ET_0, 6,666 m^3 h^{-1}, dripped watering was supplied each year crop coefficient (Kc) studied under Egyptian conditions as stated by FAO 56 (Allen et al. 2005). The seeds were inoculated with the proper strain of Rhizobium (*Rhizobium leguminosarum*). All other field management practices were carried out –weeding, disease, and pest control–if needed.

2.3 Physio-Biochemical Traits

A 10-plant simple random sample was taken from the middle rows in each plot, 60 DAS, to estimate Chlorophyll a, Chlorophyll b (mg g^{-1} fresh weight), carotenoids, total photosynthetic pigments (Lichtenthaler and Wellburn 1983), Chlorophyll a/b ratio, membrane stability index (MSI) (Premachandra et al. 1990), phenolic content (mg g^{-1} dry weight) (Shahidi and Naczk 1995), proline (mg g^{-1} dry weight) (Troll and Lindsley 1955), and osmotic potential (bars).

2.4 Root Traits

A 10-plant simple random sample was taken from the middle rows in each plot, 80 DAS, to estimate the mean value of root traits. Plants were uprooted and washed with tap water, then roots were cleaned with distilled water and blotted with tissue paper. Both shoot and root dry weights (g) were measured following oven-drying samples at 70◦C for 48 h. Per plant, the total nodule number was counted on the main and lateral roots. The active nodules were separated from the roots, cut into two pieces, and observed for the inside color. Only pink/red nodules were recorded as healthy and active. The active nodules were oven-dried at 70°C for 48 h and then weighed. The nitrogenous enzyme activity (μmol C_2H_4 g^{-1} dry-weighed nodule h^{-1}) was estimated using an acetylene reduction assay as described by (Burns and Holsten 1973).

2.5 Seed Yield Component and Yield

At harvest (147 DAS), a 10-plant simple random sample was collected from each plot's inner rows to estimate plant height (cm), branches $plant^{-1}$, number of pods $plant^{-1}$, $seedpod^{-1}$, 100- seed weight (g), seed $plant^{-1}$, seed weight (g) $plant^{-1}$. Plots were harvested to determine seed, straw, and biological yields (kg h^{-1}), and harvest index (HI). Both seeds and straw were dried for 48 h at 70°C to spectrophotometrically estimate Na and K ion concentration, following nitric-perchloric acid digestion(Kacar 1972).

2.6 Measurements of Nitrogen, Phosphorous, and Potassium

Total N, P, and K were determined as described by (AOAC 1975).

N uptake, N recovery efficiency (NRE), and N use efficiency (NUE) were calculated as follows;

N uptake (kg h^{-1}) = N in seed (kg h^{-1}) + N in straw (kg h^{-1}), whereas N in seeds $(\text{kg h}^{-1}) = \frac{\text{Seed N \%}\times\text{seed yield (kg/h)}}{100}$ and N in straw $(\text{kg h.}^{-1}) = \frac{\text{Straw N\%}\times\text{straw yield (kg/h)}}{100}$

$$NRE = \frac{\text{Total N uptake(kg/h)} \times 100}{\text{N applied (kg/h)}}$$

$$NUE = \frac{\text{Seed yield (kg/h)}}{\text{N applied (kg/h)}}$$

The accumulated total P in seeds and straw was used to calculate P uptake (kg h^{-1}), P recovery efficiency (PRE) and P use efficiency (PUE), where:

P uptake (kg h^{-1}) = P in seed (kg h^{-1}) + P in straw (kg h^{-1}), whereas P in seeds $(\text{kg h}^{-1}) = \frac{\text{Seed P \%×seed yield (kg/h)}}{100}$ and P in straw (kg h.$^{-1}$) = $\frac{\text{Straw P \%× straw yield (kg/h)}}{100}$

$$PRE = \frac{\text{Total P uptake (kg/h)} \times 100}{\text{P applied (kg/h)}}$$

$$PUE = \frac{\text{Seed yield (kg/h)}}{\text{P applied (kg/h)}}$$

The accumulated total K in seeds and straw was used to calculate K uptake, K recovery efficiency (KRE), and K use efficiency (KUE), where:

K uptake (kg h^{-1}) = K in seed (kg h^{-1}) + K in straw (kg h^{-1}), whereas K in seeds (kg h^{-1}) = $\frac{\text{Seed K \%×seed yield (kg/h)}}{100}$ and K in straw (kg h.$^{-1}$) = $\frac{\text{Straw K \%× straw yield (kg/h)}}{100}$

$$KRE = \frac{\text{Total K uptake (kg/h)} \times 100}{\text{K applied (kg/h)}}$$

PUE = $\frac{\text{Seed yield (kg/h)}}{\text{K applied (kg/h)}}$. was calculated according to (Keuter et al. 2013).

2.7 Seed Yield Response Index (SYRI)

Seed yield response index (SYRI) was calculated using formula 2:

$$SYRI = \frac{\text{SY at a high nutrient rate} - \text{SY at the low nutrient rate}}{\text{high nutrient rate} - \text{low nutrient rate}} \left(\text{kg seeds kg nutrient}^{-1}\right).$$

where:

SY	seed yield kg h^{-1}
Low nutrient rate	23.8 N; 36.9 P and 56.5 K (kg h^{-1})
High nutrient rate	51.76 N; 73.98 P 123.06 K (kg h^{-1})

Based on the SYRI, genotypes are classified into four groups: (i) efficient and responsive (ER) that produce high seed yield at low and high rates of nutrient fertilizer; (ii) efficient and not responsive (ENR) that produce high seed yield at a low nutrient rate with lower response to increasing nutrient fertilizer than ER; (iii) not efficient but responsive (NER) that has low seed yield with response to increasing nutrient fertilizer; and (iv).

2.8 Statistical Analysis

The obtained data were subjected to testing normality (Shapiro–Wilk test) and homogeneity of variances (Bartlett's test) of the residuals prior to ANOVA and (Bartlett 1937; Shapiro and Wilk 1965). The combined data from the two seasons were subjected to ANOVA using R statistical software version 4.4.1. Differences among the treatments were separated by Tukey's HSD test ($p \leq 0.05$). The results obtained were expressed as the mean value ± standard deviation.

3 Results

3.1 Physio-Biochemical Traits

Chlorophyll pigments of the 4 faba bean cultivars were affected by the 4 levels of fertilizers. Interaction effects differed ($p < 0.05$) for each of Ch A, Ch A:B, and total pigments (Table 2). Among all interaction effects, Ch A content ranged from 0.833–1.766, from 2.08–2.76 for Ch A:B, and from 1.40–2.79 for total pigments. These increments were twice as much for both Ch A and total pigments, but was by only 32% for Ch A:B, for the (Vinasse + 75% NPK) x Sakha 3 interaction effect. The marginal main effect for each of fertilizer levels and cultivars greatly varied ($p = 0.000$) for Ch A and total pigments, where Vinasse + 75% NPK and cv. Sakha 3 had relatively higher mean values, whereas for Ch A:B, Sakha 3 did not differ ($p = 0.421$). These relatively higher main effects explain, in part, the differential magnitude of these interaction effects. However, Ch B and carotenoid varied for just the marginal main effect in response to each of fertilizer and cultivars ($p = 0.000$ and $p = 0.002$). Still both trait means were 0.545 and 0.409 for (Vinasse + 75% NPK) and 0.515 and 0.359 for cv. Sakha 3.

Means of proline, phenolic content, osmotic potential, and percentage MSI greatly differed for fertilizer x cultivar interaction effect, as well as for both main effects ($p = 0.000$) (Table 3). They all had the same trend, since the 25% reduction of NPK rate, supplemented by Vinasse, applied to Sakha 3, caused their having high means relative to other interactions. Means ranged from: 1.16–1.71, 16.94–22.73, 1.63–2.99, and 49.30–62.80, for the four traits. These ranges represent percentage increments of: 47%, 34%, 83%, and 27% in favor of Vinasse + 75% NPK rate.

Table 2 Effect of vinasse and mineral fertilization on photosynthetic pigments (mg g^{-1} fresh weight) of faba bean Genotypes

Fertilizer Treatment (T)		Chlorophyll A	Chlorophyll B	Chlorophyll A:B	Carotenoid	Total Pigments
100% NPK without Vinasse (T1)		0.940^{C} ±0.02	0.430^{B} ±0.053	2.199^{B} ±0.053	0.264^{C} ±0.01	1.64^{C} ±0.039
50% NPK with Vinasse (T2)		0.898^{D} ±0.02	0.394^{C} ±0.052	2.295^{B} ±0.052	0.243^{C} ±0.011	1.54^{D} ±0.04
75% NPK with Vinasse (T3)		1.456^{A} ±0.057	0.545^{A} ±0.047	2.664^{A} ±0.047	0.409^{A} ±0.014	2.41^{A} ±0.086
100% NPK with Vinasse (T4)		1.021^{B} ±0.029	0.460^{B} ±0.033	2.224^{B} ±0.033	0.324^{B} ±0.014	1.81^{B} ±0.053
Genotypes (G)						
Giza-429 (G1)		0.961^{D} ±0.048	0.401^{C} ±0.053	2.39^{A} ±0.053	0.271^{C} ±0.018	1.63^{D} ±0.078
Giza-843 (G2)		1.111^{B} ±0.074	0.473^{B} ±0.086	2.34^{A} ±0.086	0.326^{B} ±0.021	1.91^{B} ±0.11
Misr-2 (G3)		1.034^{C} ±0.062	0.442^{B} ±0.056	2.33^{A} ±0.056	0.283^{C} ±0.02	1.76^{C} ±0.099
Sakha-3 (G4)		1.209^{A} ±0.09	0.5150^{A} ±0.089	2.32^{A} ±0.089	0.359^{A} ±0.022	2.08^{A} ±0.131
Interaction of T * G						
100% NPK without Vinasse	Giza-429	0.866^{fg} ±0.867		2.30^{cd} ±0.045		1.47^{hij} ±0.018
	Giza-843	0.966^{ef} ±0.967		2.18^{d} ±0.087		1.68^{efg} ±0.015
	Misr-2	0.916^{efg} ±0.917		2.22^{d} ±0.172		1.57^{ghi} ±0.022
	Sakha-3	1.013^{e} ±1.013		2.08^{d} ±0.107		1.81^{e} ±0.047
50% NPK with Vinasse	Giza-429	0.833^{g} ±0.833		2.34^{bcd} ±0.101		1.40^{j} ±0.046
	Giza-843	0.940^{efg} ±0.94		2.32^{cd} ±0.17		1.60^{fgh} ±0.064
	Misr-2	0.853^{fg} ±0.853		2.30^{cd} ±0.05		1.44^{ij} ±0.044
	Sakha-3	0.963^{ef} ±0.963		2.20^{d} ±0.103		1.69^{efg} ±0.025
75% NPK with Vinasse	Giza-429	1.220^{d} ±1.22		2.61^{ab} ±0.072		2.05^{d} ±0.075
	Giza-843	1.526^{b} ±1.527		2.73^{a} ±0.101		2.51^{b} ±0.038
	Misr-2	1.370^{c} ±1.37		2.55^{abc} ±0.044		2.28^{c} ±0.058
	Sakha-3	1.706^{a} ±1.707		2.76^{a} ±0.13		2.79^{a} ±0.035
100% NPK with Vinasse	Giza-429	0.923^{efg} ±0.923		2.30^{cd} ±0.102		1.61^{fgh} ±0.015
	Giza-843	1.010^{e} ±1.01		2.10^{d} ±0.046		1.83^{e} ±0.058
	Misr-2	0.996^{e} ±0.997		2.25^{d} ±0.021		1.73^{ef} ±0.055
	Sakha-3	1.153^{d} ±1.153		2.24^{d} ±0.019		2.04^{d} ±0.069
P fertilizer		0.000	0.000	0.015	0.000	0.000
P genotypes		0.000	0.002	0.421	0.002	0.000
P T * G		0.003	0.836	0.024	0.930	0.010

Values are the mean of 3 replicates ± standard errors. Different letters within columns indicate that there are significant differences at 0.05 level of probability; where uppercase letters indicate the averages of the main effects, while lowercase letters indicate the interaction

Table 3 Effect of vinasse and mineral fertilization on physiochemical traits of faba bean Genotypes

Fertilizer Treatment (T)		Proline mg/g Dry Weight	Phenolic mg/g Dry Weight	Osmotic Potential (bars)	Membrane Stability Index%
100% NPK without Vinasse (T1)		1.42^{B} ± 0.053	18.13^{C} ± 0.252	-2.13^{B} ± 0.066	53.54^{C} ± 0.885
50% NPK with Vinasse (T2)		1.29^{C} ± 0.044	17.42^{C} ± 0.226	-1.85^{A} ± 0.094	52.17^{D} ± 0.676
75% NPK with Vinasse (T3)		1.61^{A} ± 0.029	21.11^{A} ± 0.345	-2.59^{D} ± 0.068	59.94^{A} ± 0.646
100% NPK with Vinasse (T4)		1.48^{B} ± 0.042	19.24^{B} ± 0.268	-2.40^{C} ± 0.059	56.31^{B} ± 0.52
Genotypes (G)					
Giza-429 (G1)		1.33^{C} ± 0.052	18.29^{C} ± 0.347	-2.02^{A} ± 0.095	52.90^{C} ± 0.954
Giza-843 (G2)		1.46^{B} ± 0.042	19.04^{B} ± 0.451	-2.30^{BC} ± 0.097	55.79^{B} ± 0.935
Misr-2 (G3)		1.40^{BC} ± 0.046	18.48^{C} ± 0.443	-2.17^{AB} ± 0.099	54.81^{B} ± 1.025
Sakha-3 (G4)		1.62^{A} ± 0.039	20.10^{A} ± 0.558	-2.47^{C} ± 0.107	58.46^{A} ± 0.914
Interaction of T * G					
100% NPK without Vinasse	Giza-429	1.24^{fgh} ± 0.079	17.80^{fg} ± 0.231	-1.866^{abc} ± 0.033	50.63^{jk} ± 0.606
	Giza-843	1.41^{cdefg} ± 0.025	18.06^{fg} ± 0.437	-2.166^{bcdef} ± 0.088	53.33^{ghi} ± 0.41
	Misr-2	1.36^{efgh} ± 0.083	17.43^{g} ± 0.328	-2.133^{bcde} ± 0.088	52.40^{ij} ± 1.25
	Sakha-3	1.66^{ab} ± 0.047	19.23^{de} ± 0.376	-2.366^{defg} ± 0.12	57.80^{cd} ± 1.069
50% NPK with Vinasse	Giza-429	1.16^{h} ± 0.024	16.94^{g} ± 0.442	-1.633^{a} ± 0.145	49.30^{k} ± 0.529
	Giza-843	1.35^{efgh} ± 0.132	17.63^{fg} ± 0.481	-1.933^{abcd} ± 0.219	52.86^{hi} ± 0.784
	Misr-2	1.23^{gh} ± 0.059	17.13^{g} ± 0.384	-1.800^{ab} ± 0.252	51.50^{ij} ± 0.306
	Sakha-3	1.41^{cdefg} ± 0.042	18.00^{fg} ± 0.451	-2.033^{abcde} ± 0.133	55.03^{efg} ± 0.617
75% NPK with Vinasse	Giza-429	1.53^{abcde} ± 0.082	19.83^{cd} ± 0.338	-2.366^{defg} ± 0.033	57.00^{de} ± 0.2
	Giza-843	1.61^{abc} ± 0.033	21.23^{b} ± 0.328	-2.633^{gh} ± 0.067	60.16^{b} ± 0.203
	Misr-2	1.59^{abcd} ± 0.042	20.66^{bc} ± 0.145	-2.466^{efgh} ± 0.033	59.80^{bc} ± 0.306
	Sakha-3	1.71^{a} ± 0.025	22.73^{a} ± 0.376	-2.900^{h} ± 0.115	62.80^{a} ± 0.757
100% NPK with Vinasse	Giza-429	1.37^{defgh} ± 0.082	18.60^{ef} ± 0.153	-2.233^{bcdefg} ± 0.088	54.66^{fgh} ± 0.581
	Giza-843	1.46^{bcdef} ± 0.023	19.23^{de} ± 0.233	-2.466^{efgh} ± 0.033	56.80^{de} ± 1.012
	Misr-2	1.41^{cdefg} ± 0.025	18.70^{ef} ± 0.416	-2.300^{cdefg} ± 0.153	55.56^{ef} ± 0.481
	Sakha-3	1.68^{ab} ± 0.038	20.43^{bc} ± 0.521	-2.600^{fgh} ± 0.058	58.23^{bcd} ± 0.876
P fertilizer		0.000	0.000	0.012	0.000
P genotypes		0.001	0.000	0.000	0.000
P T * G		0.000	0.000	0.000	0.000

Values are the mean of 3 replicates ± standard errors. Different letters within columns indicates that there are significant differences at 0.05 level of probability; where uppercase letters indicate the averages of the main effects, while lowercase letters indicate the interaction

3.2 Root and Shoot Traits

Although both root and shoot dry weights were significant ($p = 0.002$ and $p = 0.000$) for fertilizer x cultivar interaction, as well as both their main effects; yet mean shoot-to-root ratio for interaction was not ($p = 0.08$) (Table 4). For main effects ($p = 0.00$), their ratio was relatively higher by 86%-95% for fertilizer and 81%-90% for cultivar effects, but contrary to previous traits (Table 3), neither (Vinasse + 75% NPK) combination nor cv. Sakha 3 had the relatively highest means. For each of root and shoot dry weight, however, (Vinasse + 75% NPK) x Sakha 3 interaction had high means but with trivial margins compared to 100% NPK x Saka 3 and (Vinasse + 100% NPK) x Sakha 3 interaction effects.

Four nodule traits i.e. nodule number plant^{-1}, active nodule number plant^{-1}, nodule dry weight, and nitrogenase activity are shown in Table 4. Both numbers of total and active nodules were different ($p = 0.00$ and 0.003) for fertilizer x cultivar interaction. Relative to the Control 100% (NPK), their means for 75% NPK x Sakha 3 and 100% NPK x Sakha 3 were greater by margins of 21 and 8 nodules per plant total nodule number; and were greater by 16 and 8 per plant active nodule number. With narrower margins, this trend remained quite the same for nitrogenase activity. The main effects supported these three interaction effects for these three nodule traits.

3.3 Yield and its Attributes

The three morphological traits, plant height, per plant branch, and pod numbers (Table 5) were different ($p < 0.01$) for fertilizer x cultivar interaction. These statistical interaction variations for these three traits did not have much impact on seed pod^{-1}. The latter was significant ($p = 0.01$) only for both main effects, but it had a close range between 2.7—3.3 over fertilizer and 2.9–3.4 over cultivars. The interaction effect pattern for pod plant^{-1} was performed similarly to the patterns of means of both plant height and branch plant^{-1} for each fertilizer x cultivar interaction. The simple effect of both cv. Giza 843 and cv. Sakha 3 within each fertilizer level was quite similar, both had relatively taller plants and higher per plant branch and pod number; however not necessarily were significantly different. Within 75% NPK + Vinasse, both cultivars were significantly different for plant height, yet they were not for both per plant branch and pod number.

Yield component traits –both per plant 100 seed weight and seed number—were different ($p = 0.014$ and 0.003) for fertilizer x cultivar interaction, as well as for main effects (Table 5). For per plant 100 seed weight, the simple effect means for the 4 cultivars within 100% NPK was different with a range of 72.0–78.3 g, but the range was wider for Vinasse + 75% NPK, 64.6–75.0 g. In the case of Vinasse + 50% and 100% full NPK, interaction mean ranges were quite relatively narrower and higher, 79.0–81.6 and 81.3–86.6 g, yet they were not statistically significant. Per plant total seed number interaction means performed in opposite direction with 100-seed weight means – the more weight, the less total number. This trend was consistent over all cultivar means within each of the 4 fertilizer levels despite the differential variation in the ranges. These ranges were 20, 15, 26, and 26 seeds among the 4 cultivars within fertilizer levels, but for Vinasse + 75% NPK, both minimum–maximum seed numbers (47–73 seeds) were higher compared to the other three within fertilizer levels, especially Vinasse + 100% NPK where the range was 38–64 seeds, making drops of 19% and of 12%.

A positive linear trend of total seed yield (kg h^{-1}), among the 4 cultivars, was towards cultivar Sakha 3 within each fertilizer level ($p = 0.005$) (Table 5). But all over fertilizer combinations, the 75% NPK + Vinasse did not contribute more than 5% more yield to Sakha 3 than it did with the 100% NPK + Vinasse. Both the fertilizer and cultivar main effects ($p = 0.000$ and $= 0.003$) followed the same positive linear increase, with relatively higher total seed yield in case of the 75% NPK + Vinasse and of cv. Sakha 3. The harvest index (HI) was different only for fertilizer levels ($p = 0.006$). The 75% NPK + Vinasse differed by a very negligible margin from the 100% NPK + Vinasse, but both differed from the other two levels ($p = 0.006$). Along with seed yield, both straw and biological yields were significant for the interaction effects, as well as for the main effects.

3.4 Seed and Straw K^+/Na^+ Ratio

For both ratios, fertilizer x cultivar interactions were different ($p = 0.007$ and 0.008) (Table 5). Within each fertilizer, however, cultivars did not show practical considerable differences. The percentage range for cultivars within each fertilizer x cultivar interaction was 0.57, 0.31, 0.30, and 0.512, making an overall ratio range differential of 0.27% (0.30–0.57%) for straw. For seeds, percentage ranges were 2.06, 1.84, 1.29, and 1.36, making an overall ratio range differential of 0.77% (1.29–2.06%). The main effect of fertilizer combination, for straw, indicated that the presence of Vinasse with either a 25% reduction in NPK or a full amount did make a difference ($p = 0.000$), resulting in means of 2.04 and 2.06%, from its presence with 50% NPK (mean = 1.43%). However, a full percentage NPK resulted in a nonsignificant close mean ratio, of 1.95%. In the case of seed, this K^+/N^+ ratio pattern for these two fertilizer x cultivar interactions was significantly close (6.55% vs. 7.18%) except difference ($p = 0.001$) from both ratios in the case of Vinasse + 50% NPK and of its absence –5.90% vs. 3.79%. In general, overall main effects and interactions, the ratio of these two cations ranged about more than 2–3 folds in the seeds than in straw.

Table 4 Effect of vinasse and mineral fertilization on shoot dry weight and root traits of faba bean Genotypes

Fertilizer Treatment (T)		Root Dry Weight g/Plant	Shoot Dry Weight g/Plant	Nodules No./Pant	Active Nodules/Plant	Nodules Dry Weight g/Plant	Nitrogenase (µmol/g nodule Dry Weight)	Shoot:Root
100% NPK without Vinasse (T1)		18.82^{C} ± 0.57	34.83^{C} ± 0.65	79.66^{C} ± 2.46	73.25^{B} ± 2.53	0.21^{C} ± 0.008	30.62^{C} ± 1.406	1.86^{A} ± 0.031
50% NPK with Vinasse (T2)		15.34^{D} ± 0.38	29.84^{D} ± 0.66	63.00^{D} ± 2.55	57.58^{C} ± 2.58	0.17^{D} ± 0.007	27.92^{C} ± 0.953	1.95^{A} ± 0.038
75% NPK with Vinasse (T3)		20.55^{A} ± 0.48	38.45^{A} ± 0.84	94.33^{A} ± 3.18	87.75^{A} ± 2.39	0.25^{A} ± 0.008	37.40^{A} ± 1.269	1.88^{A} ± 0.017
100% NPK with Vinasse (T4)		19.48^{B} ± 0.57	36.53^{B} ± 0.89	86.66^{B} ± 2.82	81.25^{A} ± 2.74	0.23^{B} ± 0.007	34.62^{B} ± 1.301	1.88^{A} ± 0.026
Genotypes (G)								
Giza-429 (G1)		16.65^{C} ± 0.545	31.58^{D} ± 0.935	71.58^{C} ± 3.763	66.00^{D} ± 3.842	0.19^{C} ± 0.01	28.09^{D} ± 1.24	1.90^{AB} ± 0.036
Giza-843 (G2)		18.41^{B} ± 0.681	36.25^{B} ± 1.153	83.58^{B} ± 4.342	77.91^{B} ± 4.247	0.22^{B} ± 0.011	33.94^{B} ± 1.218	1.97^{A} ± 0.02
Misr-2 (G3)		18.12^{B} ± 0.506	33.91^{C} ± 0.77	77.41^{BC} ± 3.166	71.83^{C} ± 3.24	0.21^{C} ± 0.008	31.31^{C} ± 1.407	1.88^{BC} ± 0.017
Sakha-3 (G4)		21.00^{A} ± 0.708	37.90^{A} ± 1.09	91.08^{A} ± 4.148	84.08^{A} ± 3.545	0.24^{A} ± 0.01	37.22^{A} ± 1.375	1.81^{C} ± 0.024
Interaction of T * G								
100% NPK without Vinasse	Giza-429	16.53^{f} ± 0.176	31.83^{g} ± 0.291	70.33^{hi} ± 2.963	64.00^{fg} ± 3.055		26.10^{gh} ± 2.108	
	Giza-843	18.73^{de} ± 0.441	36.40^{d} ± 0.289	85.0^{bcdefg} ± 2.082	78.66^{bcde} ± 1.453		31.80^{def} ± 1.562	
	Misr-2	18.46^{e} ± 0.406	33.96^{f} ± 0.348	76.33^{fgh} ± 1.202	69.33^{ef} ± 1.667		28.90^{efgh} ± 2.577	
	Sakha-3	21.56^{bc} ± 0.639	37.13^{d} ± 0.384	87.0^{bcdef} ± 5.292	81.00^{bcd} ± 5.568		35.70^{bcd} ± 2.159	
50% NPK with Vinasse	Giza-429	14.00^{h} ± 0.577	26.83^{i} ± 0.935	53.66^{j} ± 3.18	48.00^{h} ± 3.215		24.00^{h} ± 0.577	
	Giza-843	14.83^{gh} ± 0.167	30.13^{h} ± 0.504	61.66^{ij} ± 3.528	56.33^{gh} ± 3.48		29.33^{efg} ± 0.333	
	Misr-2	15.40^{g} ± 0.231	29.90^{h} ± 0.306	62.33^{ij} ± 1.764	56.66^{gh} ± 1.202		26.66^{fgh} ± 1.453	
	Sakha-3	17.13^{f} ± 0.338	32.50^{g} ± 0.346	74.33^{gh} ± 2.906	69.33^{ef} ± 2.404		31.70^{def} ± 0.907	
75% NPK with Vinasse	Giza-429	18.70^{de} ± 0.458	35.13^{e} ± 0.296	84.33^{cdefg} ± 2.906	79.66^{bcde} ± 2.404		32.86^{de} ± 1.795	
	Giza-843	20.93^{c} ± 0.088	40.13^{b} ± 0.328	96.66^{b} ± 4.631	90.00^{ab} ± 4.619		38.73^{abc} ± 0.601	
	Misr-2	19.73^{d} ± 0.203	36.60^{d} ± 0.529	88.33^{bcde} ± 4.256	84.33^{bc} ± 3.756		35.96^{bcd} ± 1.91	
	Sakha-3	22.83^{a} ± 0.176	41.96^{a} ± 0.546	108.00^{a} ± 3.215	97.00^{a} ± 0.577		42.03^{a} ± 2.298	
100% NPK with Vinasse	Giza-429	17.40^{f} ± 0.265	32.53^{g} ± 0.328	78.00^{efgh} ± 4.583	72.33^{def} ± 4.667		29.40^{efg} ± 1.79	
	Giza-843	19.16^{de} ± 0.233	38.36^{c} ± 0.555	91.00^{bcd} ± 4.726	86.66^{abc} ± 4.096		35.90^{bcd} ± 1.852	
	Misr-2	18.90^{de} ± 0.115	35.20^{e} ± 0.208	82.66^{defg} ± 3.18	77.00^{cde} ± 1.732		33.73^{cde} ± 1.919	
	Sakha-3	22.46^{ab} ± 0.285	40.03 b ± 0.41	95.00 bc ± 5.686	89.00^{ab} ± 5.508		39.46^{ab} ± 0.581	
P fertilizer		0.000	0.000	0.000	0.000	0.000	0.000	0.000
P genotypes		0.000	0.000	0.000	0.000	0.000	0.000	0.000
P T * G		0.002	0.000	0.000	0.003	0.7071	0.000	0.0821

Values are the mean of 3 replicates ± standard errors. Different letters within columns indicate that there are significant differences at 0.05 level of probability; where uppercase letters indicate the averages of the main effects, while lowercase letters indicate the interaction

Table 5 Effect of vinasse and mineral fertilization on yield and its attributes seed and straw K^{+}/Na^{+} ratio of faba bean Genotypes

Fertilizer treatment (T)		Plant Height	Branchs No./plant	Pods No./plant	Seed No./pod	100 Seed Weight	Seed No./plant	Seed Weight kg/h	Straw Weight kg/h	Biological Yield kg/h	Harvest Index	K:Na in Straw Ratio	K:Na in Seed Ratio
100% NPK without Vinasse (T1)		66.5^{C} ± 1.92	3.33^{BC} ± 0.28	13.83^{B} ± 0.98	2.83^{B} ± 0.11	80.41^{B} ± 0.57	40.08^{B} ± 3.68	3728^{B} ± 330	4007^{BC} ± 343	7735^{BC} ± 673	48.0^{B} ± 0.36	1.95^{A} ± 0.08	5.90^{B} ± 0.32
50% NPK with Vinasse (T2)		60.7^{D} ± 1.59	2.75^{C} ± 0.18	11.33^{C} ± 0.61	2.75^{B} ± 0.13	84.58^{A} ± 1.02	31.33^{B} ± 2.46	3055^{B} ± 212	3344.7^{C} ± 223	6400^{C} ± 434	47.68^{B} ± 0.25	1.43^{B} ± 0.05	3.80^{C} ± 0.24
75% NPK with Vinasse (T3)		81.9^{A} ± 2.28	4.25^{A} ± 0.22	16.75^{A} ± 0.59	3.75^{A} ± 0.13	69.16^{D} ± 1.58	63.16^{A} ± 3.65	5064^{A} ± 195	5258^{A} ± 200	10322^{A} ± 394	49.06^{A} ± 0.193	2.04^{A} ± 0.04	6.55^{AB} ± 0.21
100% NPK with Vinasse (T4)		73.6^{B} ± 1.80	3.67^{AB} ± 0.19	15.75^{AB} ± 0.75	3.33^{A} ± 0.19	75.66^{C} ± 1.63	53.33^{A} ± 4.83	4616^{A} ± 351	4781^{AB} ± 338	9396^{AB} ± 689	48.99^{A} ± 0.228	2.06^{A} ± 0.07	7.18^{A} ± 0.29
Genotypes (G)													
Giza-429 (G1)		63.9^{D} ± 2.34	3.00^{A} ± 0.25	11.83^{C} ± 0.72	2.91^{B} ± 0.15	80.41^{A} ± 1.63	35.33^{C} ± 3.34	3245^{C} ± 258	3478^{B} ± 255	6724^{C} ± 511	48.04^{A} ± 0.42	1.65^{C} ± 0.08	5.09^{C} ± 0.45
Giza-843 (G2)		72.6^{B} ± 2.69	3.75^{A} ± 0.28	15.33^{AB} ± 1.00	3.16^{AB} ± 0.24	76.2^{BC} ± 2.53	50.58^{AB} ± 6.32	4291^{AB} ± 427	4507^{A} ± 417	8799^{AB} ± 844	48.52^{A} ± 0.29	1.95^{A} ± 0.09	6.09^{AB} ± 0.45
Misr-2 (G3)		67.7^{C} ± 2.53	3.33^{A} ± 0.19	14.00^{B} ± 0.88	3.16^{AB} ± 0.17	$78.5A^{B}$ ± 1.77	45.08^{B} ± 4.47	4084^{B} ± 345	4347^{A} ± 341	8431^{B} ± 685	48.29^{A} ± 0.29	1.81^{B} ± 0.09	5.53^{BC} ± 0.42
Sakha-3 (G4)		78.4^{A} ± 2.77	3.92^{A} ± 0.29	16.50^{A} ± 0.65	3.41^{A} ± 0.15	74.75^{C} ± 2.14	56.91^{A} ± 4.06	4843^{A} ± 218	5059^{A} ± 209	9902^{A} ± 426	48.86^{A} ± 0.20	2.08^{A} ± 0.09	6.730A ± 0.39
Interaction of T * G													
100% NPK without Vinasse	Giza-429	60.5^{ghi} ± 1.65	2.67^{bc} ± 0.33	10.67^{de} ± 1.86		81.7^{ac} ± 1.20	29.7^{de} ± 7.88	2798^{d} ± 718.4	3030^{e} ± 671	5828^{d} ± 1390		1.7^{def} ± 0.14	4.913^{f} ± 0.67
	Giza-843	69.15^{de} ± 3.47	3.67^{abc} ± 0.33	14.7^{acd} ± 2.40		81.66^{abc} ± 0.9	40.7^{bcde} ± 10	3844^{abcd} ± 97	4162^{abcde} ± 100	8006^{abc} ± 1968		2.03^{abc} ± 0.1	6.13^{de} ± 0.32
	Misr-2	62.89^{efgh} ± 3.71	3.00^{bc} ± 0.001	13.67^{bde} ± 1.76		79^{abcd} ± 1	41^{bcde} ± 5.29	3758^{acd} ± 436	4065^{abcde} ± 556	7822^{acd} ± 992		1.9^{bcd} ± 0.12	5.57^{ef} ± 0.40
	Sakha-3	73.40^{cd} ± 0.87	4.00^{ab} ± 0.001	16.33^{ac} ± 0.67		79.3^{abcd} ± 0.9	49^{bcde} ± 2	4514^{abcd} ± 14	4771^{abcde} ± 197	9285^{abcd} ± 337		2.23^{ab} ± 0.09	6.98^{abc} ± 0.58
50% NPK with Vinasse	Giza-429	54.83^{i} ± 1.65	2.33^{c} ± 0.33	9.67^{e} ± 0.67		86.66^{a} ± 2.19	26.0^{e} ± 4.36	2603^{d} ± 393	2916^{e} ± 481	5519^{d} ± 873		1.28^{g} ± 0.13	2.94^{h} ± 0.08
	Giza-843	61.7^{fghi} ± 1.45	3.00^{bc} ± 0.58	11.33^{de} ± 0.88		84.3^{ab} ± 2.40	30.33^{de} ± 4.91	2949^{cd} ± 404.6	3207^{cde} ± 396	6156^{cd} ± 800		$1.5e^{fg}$ ± 0.07	3.84^{gh} ± 0.28
	Misr-2	58.0^{hi} ± 0.58	2.67^{bc} ± 0.33	10.67^{de} ± 1.33		86.00^{a} ± 1.528	28^{e} ± 4	2796^{d} ± 383.7	3086^{de} ± 380	5882^{d} ± 760		1.36^{fg} ± 0.04	3.622^{h} ± 0.505
	Sakha-3	68.2^{def} ± 0.37	3.00^{bc} ± 0.001	13.7^{bcde} ± 0.88		81.3^{abc} ± 1.20	41^{bcde} ± 2.65	3874^{acd} ± 222	4170^{abcde} ± 292	8043^{abc} ± 513		1.59^{ef} ± 0.06	4.79^{fg} ± 0.284
75% NPK with Vinasse	Giza-429	74.8^{cd} ± 2.19	3.67^{abc} ± 0.33	14.33^{acd} ± 0.33		75^{bcde} ± 3.464	47.7^{bcde} ± 4.3	4125^{acd} ± 185	4324^{abcde} ± 306	8449^{abcd} ± 49		1.9^{bcd} ± 0.05	5.92^{de} ± 0.297
	Giza-843	84.1^{b} ± 2.01	4.67^{a} ± 0.67	18.33^{a} ± 0.33		64.66^{f} ± 1.202	73.33^{a} ± 1.33	5514^{a} ± 79.48	5669^{a} ± 110	11181^{a} ± 174		2.1^{abc} ± 0.08	6.9^{abcd} ± 0.36
	Misr-2	76.7^{c} ± 2.19	4.00^{ab} ± 0.001	16.00^{ac} ± 0.1		70.7^{def} ± 2.19	58.4^{abc} ± 5.04	4980^{ab} ± 276.2	5197^{ab} ± 259	10176^{ab} ± 531		1.98^{ab} ± 0.05	6.19^{cde} ± 0.3
	Sakha-3	91.9^{a} ± 3.25	4.67^{a} ± 0.33	18.33^{a} ± 0.88		66.3^{ef} ± 2.19	73.33^{a} ± 3.528	5640^{a} ± 91.32	5843^{a} ± 134	11483^{a} ± 219		2.21^{ab} ± 0.09	7.21^{abc} ± 0.27

Table 5 (continued)

Fertilizer treatment (T)		Plant Height	Branchs No./plant	Pods No./plant	Seed No./pod	100 Seed Weight	Seed No./plant	Seed Weight kg/h	Straw Weight kg/h	Biological Yield kg/h	Harvest Index	K:Na in Straw Ratio	K:Na in Seed Ratio
100% NPK with Vinasse	Giza-429	65.4^{ef} ±1.55	3.33^{abc} ±0.67	12.67^{ce} ±0.88		78.3^{abcd} ±1.8	38^{cde} ±2.646	3456^{bcd} ±212	3641^{bcde} ±164	7098^{bcd} ±371		1.77^{cd} ±0.05	6.58^{bcd} ±0.24
	Giza-843	75.6^{cd} ±1.98	3.67^{abc} ±0.33	17.00^{ab} ±1.16		74^{cde} ±4.04	$58a^{bc}$ ±14.19	4861^{ab} ±1008	4989^{abcd} ±978	9850^{abc} ±198		2.18^{ab} ±0.12	7.47^{ab} ±0.599
	Misr-2	73.3^{cd} ±2.58	3.67^{abc} ±0.33	15.67^{ac} ±1.45		78.3^{abcd} ±1.2	$53ab^{cd}$ ±9.85	4804^{ac} ±819.6	5041^{abc} ±77	9844^{abc} ±159		1.99^{ab} ±0.1	6.75^{bcd} ±0.80
	Sakha-3	80.3^{bc} ±0.78	4.00^{ab} ±0.001	17.67^{ab} ±0.88		72^{def} ±4.73	64.3^{ab} ±4.33	5344^{ab} ±83.48	5452^{ab} ±27	10796^{ab} ±104		2.29^{a} ±0.10	7.940^{a} ±0.36
P fertilizer	**0.000**		**0.000**	**0.003**	**0.011**	**0.000**	**0.000**	**0.000**	**0.008**	**0.000**	**0.006**	**0.000**	**0.001**
P genotypes	**0.000**		**0.151**	**0.007**	**0.016**	**0.002**	**0.001**	**0.003**	**0.007**	**0.000**	**0.243**	**0.001**	**0.004**
P T * G	**0.000**		**0.012**	**0.012**	**0.981**	**0.014**	**0.003**	**0.005**	**0.003**	**0.000**	**0.914**	**0.007**	**0.008**

Values are the mean of 3 replicates ± standard errors. Different letters within columns indicate that there are significant differences at 0.05 level of probability; where uppercase letters indicate the averages of the main effects, while lowercase letters indicate the interaction

3.5 Protein Content and Uptake, Recovery, and Use Efficiency of NPK

Al 10 measured parameters statistically varied for fertilizer x cultivar interaction, in addition to main effects ($p < 0.01$) (Fig. 1 and Table 6). The 50% NPK + Vinasse x cultivar interaction resulted in the least protein contents for all cultivars relative to those of the Control and both the 75% and 100% NPK (+ Vinasse), reaching relatively more seed protein for the 4 cultivars within the 75% NPK + Vinasse. The performance of cultivar simple effects, within each fertilizer level, was consistently similar, where both Giza 843's and Sakha 3's protein contents were relatively higher. Protein content for the latter two cultivar main effects supported this result since theirs over yielded the other two cultivars.

The N, P, and K uptake, each varied ($p \leq 0.007$) for the fertilizer (Fig. 1A) x cultivar (Fig. 1B) interaction (Fig. 1C). All three uptakes (kg h^{-1}) among cultivars within each fertilizer level mimicked the same response for protein content. Cultivar means might be split into two subgroups within each fertilizer level: i) Giza 843 and Sakha 3, and ii) Giza 429 and Misr 2, resulting in a positive linear response towards Saka 3 for every fertilizer level. Among the 4 linear responses, the 75% NPK + Vinasse caused the highest relative response, regardless of the cultivar, for each individual element uptake; the uptake (kg h^{-1}) ranges were 206.8–312.8 for N, 22.5–35.7 for P, and 126.6–188.3 for K Fig. 1).

Neither interaction effects for recovery efficiency (RE) nor use efficiency (UE) for each of N, P, and K ($p \leq 0.01$), did vary from that of their mean uptakes regarding each of the: i) positive linear response, (ii) relative outyield of 75% NPK x cultivar combination and of iii) both cultivars Giza 429 and Sakha 3 within each interaction effect (Table 6). Moreover, both main effects did differ –where p values were generally ≤ 0.009– as for all these of the three mean element uptakes.

3.6 Seed Yield Response Index (SYRI)

The SYRI of the four cultivars is shown in (Fig. 2). At low NPK rate, the mean seed yield was 3055.5 kg h^{-1}. At a rate of 54.2 kg N h^{-1}, 74.2 kg P h^{-1}, and 124.3 kg K h^{-1}, SYRIs were 65.6, 42.3, and 27.6 kg seeds NPK kg h^{-1}. For each particular cultivar at low NPK: Sakha3 was categorized as both efficient and nonresponsive (ENR) since both its yield and SYRI were greater than the mean seed yield; Giza 429 was neither efficient nor responsive (NENR) for total mean seed yield was greater than both its yield and SYRI; Misr 2 and Giza 843 were categorized as nonefficient but responsive (NER) where both their seed yield were lower than the mean seed yield, yet their SYRIs were greater.

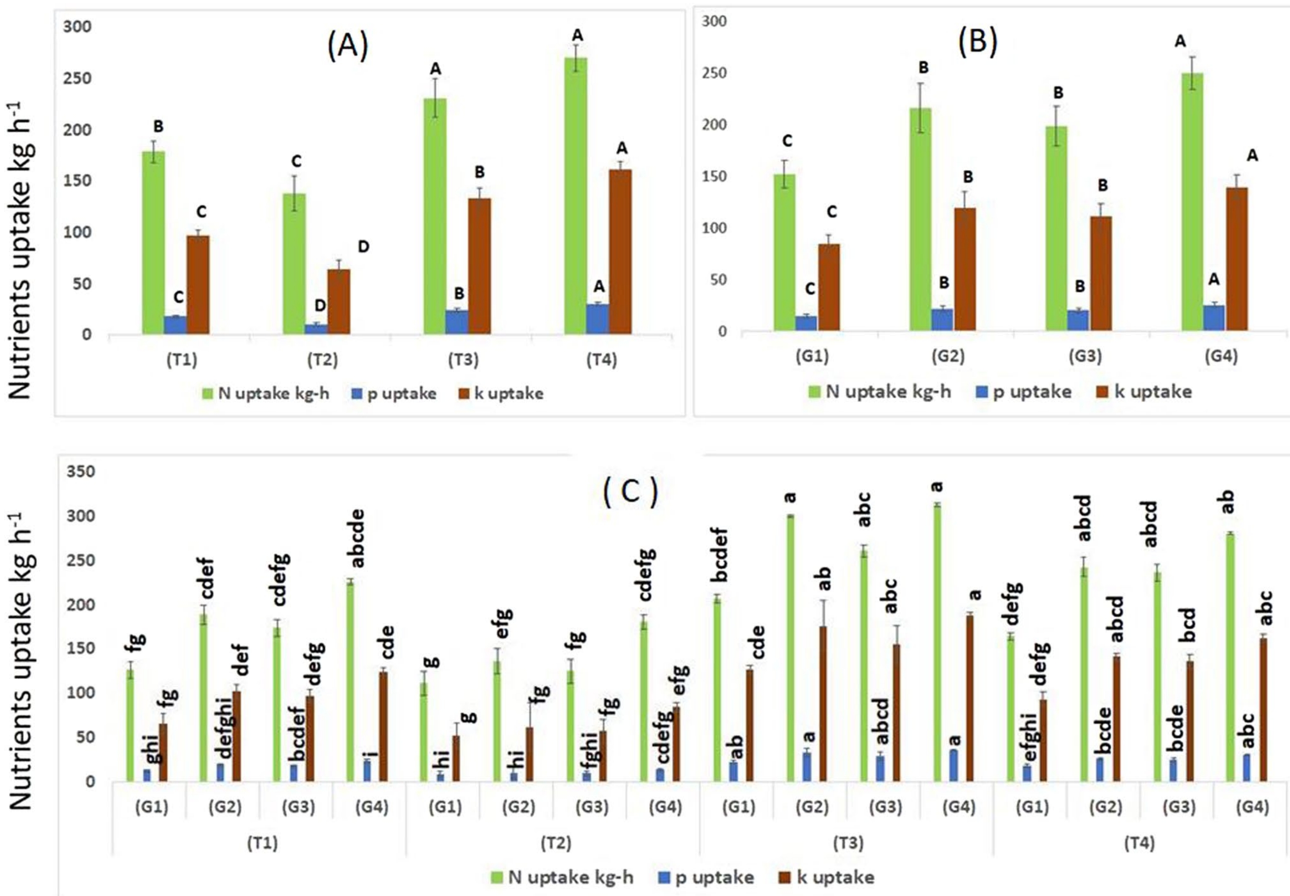

Fig. 1 Effect of vinasse and mineral fertilization (**a**), faba bean genotypes (**b**) the interaction between the fertilization and genotypes (**c**) on NPK uptake. Values are the mean of 3 replicates ± standard errors. Different letters within columns indicates that there are significant differences at 0.05 level of probability; where uppercase letters indicate the averages of the main effects, while lowercase letters indicate the interaction. Whereas T1 = 100% NPK without vinasse, T2 = 50% NPK with vinasse, T3 = 75% NPK with vinasse, T4 = 100% NPK with vinasse, G1 = Giza-429, G2 = Giza-843, G3 = Misr-2, G4 = Sakha-3

4 Discussion

Addition of vinasse to each of 100% and of 75% NPK significantly affected each of chlorophyll a &b content and their ratio, carotenoids, and total photosynthetic pigments (Table 2). And proline, phenolic content, membrane stability index (MSI%), and osmotic potential (Table 3) Organic fertilizers, e.g. vinasse, cause reduction in soil acidification, and improves soil nutrient availability (Abou Hussien et al. 2017). In addition, vinasse contains sugar which is essential for building up energy required by soil microbes. In a newly-reclaimed soil in Egypt, which was treated by sugar cane by products, its pH dropped and essential nutrient content improved (Otieno et al. 2009). Vinasse also contains cytokinins and gibberellic acid (Clementson et al. 2016). These growth regulators, and the like, act as stimulants for transporting and supplying photosynthetic assimilates, by which source-sink translocation improves (Desoky et al. 2021; Rafique et al. 2021). Vinasse increases absorbed N and Mg which are structural components of chlorophyll, and this, therefore, is likely to enhance chlorophyll accumulation, thereby increases photosynthetic rate. Both humic acid and P improve N, Fe, and Mg ion contents, and P induces Chlorophyll a &b contents to affect their ratio, in addition to the carotenoids, total photosynthetic pigments, proline, phenolic content, MSI, and enhance osmotic potential (Frydenvang et al. 2015; Carstensen et al. 2018). The combined effect of vinasse with recommended full NPK rate, only 50% and 75% was found effective on maintaining each of faba bean leaf proline, phenolic content, and osmotic potential. Proline and phenols act as indicators of abiotic stress when a plant experiences an osmotic stress (Raza et al. 2023). Supplementary vinasse with NPK fertilizer may have an indirect effect on proline and phenols to adjust plant osmotic potential. Moreover, vinasse contains zinc and an amino acid this amino acid has a role in osmotic protection when a plant suffers from osmotic stress Zn plays a role in plant metabolic pathways

Table 6 Effect of vinasse and mineral fertilization on protein yield and NPK physiological parameters of faba bean Genotypes

Fertilizer Treatment (T)		Protein Yield kg h^{-1}	NRE	NUE	PRE	PUE	KRE	KUE
100% NPK without Vinasse (T1)		903.75^{B} ±8.466	368.544^{C} ±3.502	78.327^{C} ±6.936	24.729^{C} ±3.046	57.101^{C} ±4.482	85.206^{C} ±0.969	32.636^{B} ±2.895
50% NPK with Vinasse (T2)		696.74^{C} ±5.194	517.22^{B} ±3.589	106.126^{AB} ±9.075	28.021B^{C} ±4.752	73.935^{B} ±5.696	94.232^{BC} ±1.367	45.078^{A} ±3.131
75% NPK with Vinasse (T3)		1315.52^{A} ±5.837	472.615^{B} ±3.215	124.463^{A} ±6.913	54.119^{A} ±3.878	70.031^{B} ±3.501	168.085^{A} ±1.091	52.612^{A} ±2.026
100% NPK with Vinasse (T4)		1153.89^{A} ±9.298	601.21^{A} ±3.56	87.775^{BC} ±6.13	33.313^{B} ±3.935	84.606^{A} ±4.746	107.136^{B} ±1.022	37.143^{B} ±2.832
Genotypes (G)								
Giza-429 (G1)		768.55^{C} ±6.897	375.614^{C} ±3.357	79.066^{C} ±6.949	25.939^{C} ±2.621	50.52^{B} ±5.596	85.282^{C} ±0.833	33.387^{C} ±3.432
Giza-843 (G2)		1080.69^{B} ±11.708	480.505^{B} ±5.12	102.777^{AB} ±7.371	36.751^{B} ±2.426	82.001^{A} ±6.996	119.501^{B} ±0.756	43.386^{AB} ±3.533
Misr-2 (G3)		989.06^{B} ±9.217	664.162^{A} ±3.851	97.447^{B} ±4.794	34.137^{B} ±2.993	90.906^{A} ±5.576	110.07^{B} ±0.789	41.133^{B} ±3.182
Sakha-3 (G4)		1231.59^{A} ±7.083	439.308^{BC} ±3.481	117.401^{A} ±6.692	43.356^{A} ±2.707	62.245^{B} ±5.729	139.807^{A} ±0.859	49.563^{A} ±4.908
Interaction of T * G								
100% NPK without Vinasse	Giza-429	640.97^{ef} ±15.951	265.199^{h} ±6.382	58.78^{e} ±5.092	16.899^{f} ±4.601	37.912^{h} ±9.734	57.875^{g} ±1.228	24.492^{e} ±0.629
	Giza-843	953.77^{bcdef} ±23.837	396.7de^{fgh} ±9.808	80.754c^{de} ±2.284	26.047^{def} ±7.544	52.085^{efgh} ±13.083	89.423d^{efg} ±2.353	33.647^{cde} ±0.845
	Misr-2	880.53^{cdef} ±9.664	365.644^{fgh} ±4.065	78.943^{cde} ±9.162	24.472^{def} ±3.868	50.917^{fgh} ±5.91	84.744^{efg} ±1.173	32.893^{cde} ±0.382
	Sakha-3	1139.71^{abcd} ±4.693	474.877^{def} ±1.833	94.832^{bcd} ±2.952	31.498^{cde} ±1.911	61.166^{defg} ±1.904	108.783^{cde}f ±0.488	39.513^{bcd} ±0.123
50% NPK with Vinasse	Giza-429	562.24^{f} ±7.458	387.656^{efgh} ±5.342	90.408^{bcde} ±3.642	22.54^{ef} ±3.703	69.856^{cdef} ±10.541	76.482^{efg} ±1.68	38.401^{bcd} ±0.579
	Giza-843	687.61^{def} ±10.655	473.45^{def} ±6.874	102.429^{bc} ±4.054	26.756d^{ef} ±4.313	79.144^{bcd} ±10.859	91.771^{defg} ±1.166	43.507^{b} c±0.597
	Misr-2	631.23^{ef} ±8.386	433.646d^{efg} ±5.647	97.119^{bcd} ±3.329	25.705^{def} ±3.854	75.042^{cd} ±10.299	84.426^{efg} ±1.148	41.252^{bcd} ±0.566
	Sakha-3	905.86^{cdef} ±4.807	627.268^{bc} ±3.69	134.549^{a} ±7.695	37.082^{cd} ±4.771	103.963^{a} ±5.946	124.251^{cd} ±0.694	57.151^{a} ±0.327
75% NPK with Vinasse	Giza-429	1037.79^{abcde} ±4.497	508.45^{cde} ±2.267	101.358^{bc} ±4.534	40.408^{bc} ±3.555	74.031^{cde} ±3.312	131.585^{bc} ±0.895	42.845^{bc} ±0.192
	Giza-843	1453.89^{a} ±2.803	737.525^{ab} ±1.293	135.499^{a} ±1.953	59.483^{a} ±2.658	98.967^{ab} ±1.427	183.251^{a} ±0.412	57.277^{a} ±0.083
	Misr-2	1273.95^{abc} ±7.758	641.771^{abc} ±3.691	122.383^{ab} ±6.789	52.411^{ab} ±3.702	89.387^{abc} ±4.958	161.877^{ab} ±0.859	51.732^{ab} ±0.287
	Sakha-3	1496.45^{a} ±2.77	768.901^{a} ±1.215	138.611^{a} ±2.244	64.174^{a} ±1.814	101.24^{ab} ±1.639	195.628^{a} ±0.428	58.592^{a} ±0.095

Table 6 (continued)

Fertilizer Treatment (T)		Protein Yield kg h^{-1}	NRE	NUE	PRE	PUE	KRE	KUE
100% NPK with Vinasse	Giza-429	833.19^{cdef} ±6.213	312.873^{gh} ±2.224	65.72^{de} ±4.037	23.906^{def} ±1.809	46.605^{gh} ±2.863	75.187^{fg} ±0.371	27.81^{de} ±0.171
	Giza-843	1227.49^{abc} ±25.861	461.167^{de} ^{f}±9.545	92.426^{bcd} ±9.169	34.717^{cde} ±6.821	65.544d^{efg} ±13.594	113.56c^{de} ±2.274	39.111b^{cd} ±0.811
	Misr-2	1170.53^{abc} ±19.379	449.39^{defg} ±7.329	91.345^{bcd} ±5.584	33.959^{cde} ±6.177	64.776d^{efg} ±11.052	109.234^{cde}f ±1.679	38.653b^{cd} ±0.659
	Sakha-3	1384.35^{ab} ±3.281	533.793^{cd} ±1.24	101.61^{bc} ±1.587	40.67^{bc} ±0.859	72.056^{cdef} ±1.126	130.564^{bc} ±0.208	42.997^{bc} ±0.067
P fertilizer		0.001	0.000	0.004	0.000	0.009	0.008	0.009
P genotypes		0.000	0.000	0.008	0.000	0.002	0.001	0.007
P T * G		0.000	0.002	0.015	0.001	0.005	0.004	0.012

Values are the mean of 3 replicates ± standard errors. Different letters within columns indicates that there are significant differences at 0.05 level of probability; where uppercase letters indicate the averages of the main effects, while lowercase letters indicate the interaction

to reduce some salt stress adverse effects. Another indicator of stress tolerance in plants is the chlorophyll stability index (CSI); the higher its value, the lower the stress effect on plants' chlorophyll content. This leads to a more photosynthetic rate and dry matter production CSI is also used to screen genotypes for abiotic stress (Mohan et al. 2000; Kakar et al. 2019). Some other salinity tolerance lab screening indicators are total chlorophyll content, phenols, proline, percentage MSI, and osmotic potential (Deivanai et al. 2010; Ebaid et al. 2019).

All root traits (Table 4) positively responded to the application of both vinasse + 75% or + 100% NPK, in addition to full 100% NPK. Vinasse contains N, P, K, Fe, Mn, Cu, Zn, Mo, and organic C (Clementson et al. 2016), these nutrients improve root traits (Singh Gahoonia and Nielsen 2004; Gahoonia et al. 2006; Khalil 2019), and plant growth promoters (Indole-3-butyric acid, Gibberellic Acid and naphthalene acetic acid) which promote root growth, expansion of root hairs, and cotyledon cells (Chhun et al. 2004; Elmongy et al. 2018). Vinasse also has a positive impact upon per plant total nodule number, active ones, nodule dry weight, and nitrogenase activity, this in turn enhances atmospheric N fixation efficiency and nutrient uptake (Rafique et al. 2021), and by improving root traits, total plant growth is enhanced (Elmongy et al. 2018; Mao et al. 2018). Vinasse also contributes P which affects main root length and its dry weight, total surface area, total root tips and forks (Ramtekey et al. 2021). Phosphorus also stimulates N fixation efficiency, nitrogenase activity, and N uptake via improving active nodule number and dry weight (Li et al. 2022). Nodule number formed on faba bean roots are much initiated by applied mineral P fertilizer relative to the control (Otieno et al. 2009, Mohamed et al. 2021).

Faba bean cultivar plant height and seed yield components and yield were also varied by the contribution of vinasse to the applied NPK fertilizer, especially by the reduction of 25% of the recommended NPK or when added with the full amount (Table 5). The treatment of vinasse + 75% NPK showed higher growth, physiological and biochemical performance. This may be attributed to the high dose of nitrogen for the treatment (vinasse + 100% NPK) negatively affecting the nitrogen fixation process in the root nodules compared to vinasse + 75% NPK as shown in Table 4. The reduction in soil pH and the availability of soil nutrients may partially explain the role added organic fertilizer played in maintaining relatively higher seed yield and yield components. Vinasse also contains plant growth promotors (Togay et al. 2008; Rafique et al. 2021). Similarly, the role of P, N and K elements in the photosynthesis process, and sink-source relationship may lead to increasing seed yield and its components (Togay et al. 2008; Nget et al. 2022; Yang et al. 2022). The legume crops have more requirement for P for optimal N-fixation compared to cereals for it has a fundamental role in nodule energetic transformations (Husssien

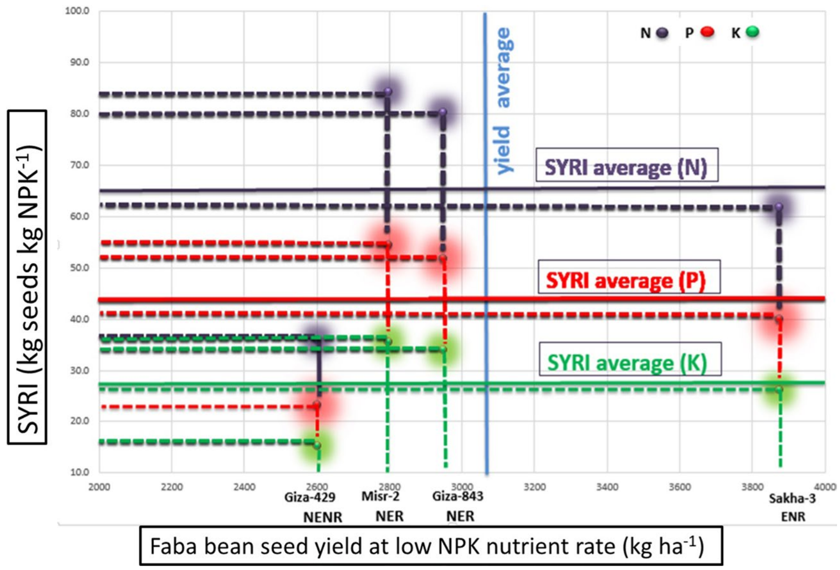

Fig. 2 Seed yield response index (SYRI) of the tested faba bean genotypes fertilized by nitrogen (N), phosphors (P), and potassium (K) with vinasse bundle at a rate of 52.6 N; 74.2 P and 124.3 K (kg h^{-1}). The purple lines represent N fertilizer while red lines P fertilizer likewise green lines K fertilizer. Efficient and responsive (ER); efficient and non-responsive (ENR); neither efficient but responsive (NER) and neither efficient nor responsive (NENR)

et al. 2020). Phosphorus leads to maximum yield and yield attributes (Gidago et al. 2011; Ali et al. 2020). Phosphorus supplement in legumes has great potential for promoting growth and yield and its attributes (Ndakidemi et al. 2011; El-Hady et al. 2022). According to (Hamer et al. 2009), soil microorganisms may be triggered by the supplied organic substrates (vinasses and molasses), thereby, enhancing the mineralization of nutrients. The ratios K^+ /Na^+ and Ca^{2+}/Na^+ in faba bean straw and seed decreased by increasing salinity levels, and reached their lowest values at the severe salinity (Afzal et al. 2022). The K^+/Na^+ ratio is a fundamental trait that signals salinity stress resistance in plants, and it may be used as a screening tool for plant breeders (Oyiga et al. 2018). Under salt stress, Na^+ and K^+ transporters are critical for maintaining Na^+ and K^+ homeostasis in cells and plants (Azhar et al. 2017; Ebaid et al. 2019).

N accumulated in faba bean seed has been reported in different studies, most studies report that at least 50% of total plant N is found in the seed by maturity, and typically proportions in the seed are higher. For example, (Dinesh et al. 2010) reported that faba bean seed held over 50% and 78% of plant N by maturity, respectively, (Tarek and Kh A Mohamed 2020), reported that faba bean seed contained 2/3 of the total plant N. Hossain et al. (Hossain et al. 2016) attributed the higher seed N uptake by faba bean to greater seed yield and greater ability to fix N_2 compared to the other pulses in the study. Nitrogen and phosphorus are considered the first and second most influential nutrients in crop growth, respectively (Anglade et al. 2015; Li et al. 2010; Papakaloudis and Dordas 2023). Therefore, measurements to quantify N and P removal by a crop are essential to fully assess the influence of any crop, including the faba bean on soil fertility and nutrient requirements in a sustainable cropping system. It is likely that raising the potassium rate of the soil fertilizer boosts nutrient uptake, allowing for greater nutrient assimilation, resulting in larger potassium content in faba bean seed (Shaban et al. 2013; Taha et al. 2016; Shawer 2019).

The four faba bean cultivars showed varied potentials for absorbing and utilizing available N, P, and K. The cv. Saka 3 was the only cultivar that had a higher seed yield than the total mean seed yield in response to applied NPK levels. Both Misr 2 and Giza 843 have higher SYRI values than the average (Fig. 2) might be explained based on their genetic potential response to NPK. By applying 52.6 kg N, 74.2 kg P, and 124.3 kg K, cv. Misr 2 had SYRI values of 84.4, 54.4, and 35.5 kg seeds for every kg of N, P, and K h^{-1}, and cv. Giza 843 had SYRI values of 80.3, 51.8, and 33.8 kg seeds for every kg of N, P, and K h^{-1}. These are relative to the mean SYRI of 65.6; 42.3 and 27.6 kg seeds. SYRI varied among some faba bean genotypes (Liu et al. 2022) and among some faba bean ones that refer to their better genetic potency to exploit and utilize NPK in soil and the exogenous supply.

5 Conclusion

In this study, vinasse in combination with mineral NPK were utilized as fertilizers to improve faba bean plant growth. The rate of 75% NPK/vinasse exhibited the highest performance compared to the other's 50% or 100% NPK rates particularly to cultivars Giza 843 and Saka 3. All three vinasse-NPK fertilizer rates x cultivar interactions enhanced the trait means in comparison with full NPK rate. These significant variations cannot be related back only to the action vinasse, yet to the combination of the organic/mineral fertilizer. A futuristic cost–benefit study needs be conducted to evaluate performance when deducting a specific percentage of or applying full NPK when mixed with vinasse.

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Data availability All data of this study are available in the article. Source data are available from the corresponding author upon reasonable request.

Declarations

Competing interest The authors declare no competing interests.

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