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Improving Plant Growth, Seed Yield, and Quality of Faba Bean by Integration of Bio-Fertilizers with Biogas Digestate

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Abstract: Exploring environmentally eco-friendly approaches to enhance crop growth and productivity are essential for sustainable agriculture. Therefore, a field trial was conducted during two growing seasons to study the effects of synthetic (nitrogen, N; phosphorus, P; and potassium, K), biogas digestate (BioD), bio-fertilizer (BioF), and their combinations on the growth, yield, and seed quality of faba bean (*Vicia faba* L.). The number of treatments was seven, as follows: control (zero NPK), NPK (30 kg N ha^{-1}; 45 kg P_2O_5 ha^{-1}: 48 kg K_2O ha^{-1}) as the recommended dose, BioD (2 t ha^{-1}), BioF (plant growth-promoting rhizobacteria; 1 kg ha^{-1}), 50% NPK + 50% BioD, 50% NPK + 50% BioF, and 50% BioD + 50% BioF. The results indicated that all fertilizer sources and their combinations improved the growth, seed yield, and quality of faba bean. However, the highest increase in plant height, leaf area, dry leaf weight, and stem dry weight of faba bean was recorded for the combined application of 50% BioD + 50% BioF. Moreover, the BioD +BioF fertilization enhanced the number of branches, number of seeds, 100 seed weight, and seed yield of faba bean. Similarly, BioD + BioF fertilization enhanced the total chlorophyll and N, P, and K contents of faba bean leaves. BioD fertilization also increased seed quality traits such as N, P, protein, and carbohydrate contents. The outcomes of BioD + BioF fertilization on growth yield and quality parameters of faba bean suggest that the concurrent application of biogas digestate with bio-fertilizer can reduce synthetic fertilizers.

Keywords: *Vicia faba* L.; digestate; bio-fertilizer; mineral fertilizer; productivity; seed chemical composition

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1. Introduction

Faba bean (*Vicia faba* L.) is an important winter-sown legume crop and has the potential to be cultivated as a multi-purpose crop in regions with short growing seasons [1]. Faba bean has high nutritious value because of high carbohydrates (42–47%), protein (up to 35% in dry seeds) content, numerous types of minerals (K, Ca, Mg, Fe, and Zn), and bioactive compounds [2,3]. Growing faba bean in any cropping system can enhance soil fertility and its biological activity due to its symbiotic relationship with Rhizobium bacteria, thus increasing biological nitrogen (N) fixation [1,3]. Faba bean can fix N up to 200 kg ha^{-1} [4], whereas mixing its residues with soil can enhance soil porosity, bulk density, organic matter, and water-holding capacity [5].

Synthetic fertilizers are an important source of nutrients for plant growth, development, yield, and quality parameters [6]. There is abundant evidence that synthetic or inorganic fertilizers are applied to agricultural lands for optimum growth and productivity. However, increasing the rate of synthetic fertilizers in crop production can negatively affect nutrient use efficiency (NUE) [7,8]. For instance, NUE of N, P, and K are 30–35%, 18–20%, and 35–40%, respectively [9], which indicates that a high rate of synthetic fertilizers is lost in fields through volatilization or leaching process [10,11]. Due to the NUE of synthetic fertilizers, farmers use excessive application of fertilizers to enhance crop productivity [12],

which results in severe environmental risks such as soil degradation, groundwater pollution, water eutrophication, air pollution, and human health problems [8,13]. Furthermore, the extensive use of synthetic fertilizers reduces soil organic matter and humus content and increases soil compaction and acidification [14]. Thus, synthetic fertilizers are becoming hazardous for human and animal health, deteriorating the environment and damaging microbial biodiversity [6,14]. Therefore, to reduce the adverse effects of synthetic fertilizers on the environment and human health and to achieve sustainability in agriculture, modern agricultural initiatives have been taken to reduce the use of synthetic fertilizer, substituting it with other organic amendments such as organic manures and bio-fertilizers. Organic and bio-fertilizers not only give essential nutrients to the plants but also maintain the soil health for the subsequent crops [15].

Biogas digestate is the residual organic matter produced as a by-product of biogas production during the anaerobic decomposition of plants and animal wastes [16]. Digestate can be applied as a bio-fertilizer directly, as a raw material for the production of bio-fertilizers, and as an amendment material to enhance soil physical properties such as porosity, bulk density, and moisture retention capacity of soil [16,17]. Digestates derived from animal and agricultural waste material have good fertilizer qualities, high organic matter content, and different nutrients essential for plant growth and production [18–22]. The incorporation of biogas digestate into the soil can enhance the available P, K, Mg total N, and soil organic carbon content [23]. Odlare et al. [24] and Insam et al. [25] reported an increase in nutrient availability, metabolic activity and soil microbial biomass in soil incorporated with digestate compared to other nutrient sources, including synthetic fertilization and undigested manure. Furthermore, the application of digestate into soil improved the grain quality of maize (*Zea mays* L.) [26], beans (*Phaseolus vulgaris* L.) [27], and soybeans (*Glycine max* L.) as well as increased wheat (*Triticum aestivum* L.) yield. In addition, Andruschkewitsch et al. [28] reported that application of synthetic fertilization and biogas digestate to grasses such as *Festuca rubra* subsp. Rubra, *Trisetum flavescens* and *Lolium perenne* increased the growth; however the highest increase in growth was recorded with biogas digestate fertilization.

On the other hand, bio-fertilizers are comprised of microorganisms that are effective and facilitate plant growth by increasing the uptake of nutrients and secreting phytohormones and metabolites through the interaction within the rhizosphere of plants [29]. Bio-fertilizers are generally comprised of P and K solubilizers, N fixers, cyanobacteria, endo- and ectomycorrhizal fungi, and plant-growth-promoting rhizobacteria (PGPRs) [30]. These microorganisms can enhance the assimilation and availability of nutrients to plants [31]. Plant-growth-promoting rhizobacteria possess the enormous potential to stimulate and increase atmospheric N-fixation, plant nodule formation, solubilization of P and K, and biosynthesis of siderophores and phytohormones in plants. Such PGPRs can enhance the production of hydrolytic enzymes and exo-polysaccharide, induce system resistance, and increase the bioremediation of heavy metals in soil [32]. Applying bio-inoculants comprising N-fixers and P-solubilizers bacteria along with 50% N and P fertilizers has shown an enhancement in plant nutrient uptake, leaf chlorophyll content, and rice yield [33]. The microorganisms can increase the decomposition of organic matter and soil mineralization, enhance plant nutrient availability, and consequently increase the yield by 10–25% [34]. Thus, bio-fertilizers are important for crop growth and development and can play an important role in reducing the use of synthetic fertilizers.

Therefore, the objective of this study was to investigate the effects of biogas digestate, bio-fertilizers, synthetic fertilizers, and their combinations on faba bean growth, yield, and seed quality.

2. Materials and Methods

2.1. Materials, Treatments, and Experimental Design

Two successive field experiments were carried out during two winter growing seasons of 2016/2017 and 2017/2018 at the Experimental Farm, Faculty of Agriculture, Menofiya

University, Egypt (Latitude: 30°33′31″ and Longitude: 31°00′36″) to investigate the effects of synthetic, organic and bio-fertilizers and their combination on growth, productivity, and quality of faba bean (cv. Giza 843). The number of treatments was seven, as follows: control (zero NPK), NPK (30 kg N ha^{-1}; 45 kg P_2O_5 ha^{-1}: 48 kg K_2O ha^{-1}), biogas digestate (2 t ha^{-1}), bio-fertilizer (plant growth-promoting rhizobacteria, 1 kg ha^{-1}), 50% NPK + 50% biogas digestate, 50% NPK + 50% bio-fertilizer, and 50% biogas digestate + 50% biogas digestate.

The analysis of biogas digestate was pH 7.25, EC 0.84 dS m^{-1}, total N 18.5 g kg^{-1}, total P 0.75 g kg^{-1}, and total K 0.56 g kg^{-1}. The biogas digestate was applied to the soil prior to the sowing process at a depth of 3–5 cm. On the other hand, plant-growth-promoting rhizobacteria were obtained from Agricultural Microbiology Department, Soils, Water, and Environment Research Institute, Agricultural Research Centre, Egypt, and consisted of two strains (i.e., *Rhizobium leguminosarum* bv., *viciae,* and *Bacillus circulans*). At sowing, faba bean seeds were coated with PGPR at the rate of 10 g inoculant for each 1 kg of seeds using Arabic gum solution (16%) as the adhesive agent for seed coating. Concerning synthetic fertilization, P (calcium superphosphate; 15.5% P_2O_5) and K fertilizers (potassium sulfate; 48% K_2O) were applied directly prior to sowing at the rate of 45 kg P_2O_5 ha^{-1} and 48 kg K_2O ha^{-1}, respectively, while N fertilizer (ammonium nitrate; 33.5%) was applied with a rate of 30 kg N ha^{-1}.

The experimental design was a randomized complete block design, and each treatment was replicated four times. The area of each experimental plot was 12 m^2 (4 m long × 3 m wide). The seeding rate was 85 kg $ha^{-1,}$ as recommended by the Egyptian Agriculture Ministry. Seeds were sown on the 16th and 18th of November in 2016 and 2017, respectively. They were sown on both sides of each ridge. The distance between every two holes was 20 cm, while it was 60 cm between every two ridges. At 15 days after sowing (DAS), seedlings were thinned into two plants per hole. Weeds were controlled using pesticides with the recommended rates.

Before sowing, soil samples were collected for physical and chemical analysis, according to Jackson [35] and Chapman and Pratt [36]. The texture of the soil was clay loam, and the physical properties of the soil were fine sand 23.5%, coarse sand 13.5%, silt 28.1%, and clay 35.0%. While the chemical analysis of soil was pH 7.31, EC 0.69 dS m^{-1}, N 39.25 g kg^{-1}, P 9.02 g kg^{-1}, K 324.2 g kg^{-1}, Fe 3.31 g kg^{-1}, Zn 0.81 g kg^{-1}, and Mn 1.94 g kg^{-1}.

2.2. Measurements

2.2.1. Growth Traits

At the flowering stage, 10 plants were randomly collected from each plot to measure plant height (cm), leaves dry weight (g), stem dry weight (g), leaves area per plant (cm^2), and number of branches per plant.

2.2.2. Total Chlorophyll

Total chlorophyll was measured at 40, 60, 80, and 100 DAS from fully expanded leaves using SPAD (SPAD-502, Sensing Ltd., Amagasaki, Japan).

2.2.3. Yield and Its Components

At the end of the physiological maturation stage, the yield and yield components (i.e., 12% moisture content) such as the number of pods per plant, the number of seeds per pod, 100-seeds weight (g), seed yield per plant, seed and straw yields per ha were measured from the harvested middle plants of each plot using a plot harvester.

2.2.4. NPK Analysis in Leaves and Seeds

Faba bean leaves and seeds were dried at +70 °C for 48 h and ground. Then, the fine powder was passed from a 0.5 mm sieve to be used for the analysis of total N, P, and K. Total N content was analyzed by weighing 250 mg of the DW sample following the described method in Seleiman et al. [37] by the Dumas combustion method (Vario MAX CN

Elementar Analysensysteme GmbH, Hanau, Germany). To analyze P and K, a subsample of 250 mg was weighed and placed in Teflon tubes with HNO_3^- for digestion. The digested samples were filtered and then analyzed via ICP-Optical Emission Spectrometry (iCAP 6200, Thermo Fisher Scientific Inc., Cambridge, UK), as explained by Seleiman et al. [37].

2.2.5. Carbohydrates and Protein in Seeds

Faba bean seeds (~15 g) were well milled, and the fine powder was passed from a 0.5 mm sieve. Total N content was analyzed as described in Section 2.2.5 to get the total crude protein by multiplying total N% × 6.25. The total carbohydrate was analyzed using the protocol described by AOAC [38].

2.3. Statistical Analysis

All data obtained from the effects of synthetic fertilizer, biogas digestate, bio-fertilizer, and their combinations on growth, yield, and quality of faba bean were statistically analyzed through ANOVA using SPSS 21.0 software (IBM Corp, Armonk, NY, USA). The differences among the means of treatments were tested using Tukey and LSD tests at $p \leq 0.05$.

3. Results

Different faba bean plants' growth traits were significantly increased in response to synthetic, organic, and bio-fertilization compared to those grown in controlled-treatment (zero NPK), Table 1. The combined application of BioD + BioF significantly increased plant height, leaves dry weight plant^{-1}, stem dry weight plant^{-1}, leaf area plant$^{-1,}$ and the number of branches plant^{-1} by 29.88, 31.98, 39.71, 22.51, and 43.89% in comparison to those obtained from the negative control, and by 8.16, 7.44, 3.41, 6.16 and 14.71% in comparison to those fertilized with NPK as a positive control (Table 1). Nevertheless, there were no significant differences in leaves' dry weight among plants fertilized with a single application of NPK (7.09 g), BioD (6.99 g), and combined application of NPK+ BioD (7.25 g), and NPK+ BioF (7.19 g).

Table 1. Effect of synthetic, organic, and bio-fertilizers on growth traits of faba bean.

Traits / Treatments	Plant Height (cm)	Leaves Dry Weight Plant^{-1} (g)	Stem Dry Weight Plant^{-1} (g)	Leaf Area Plant^{-1} (cm^2)	Number of Branches Plant^{-1}
Control (zero NPK)	70.68 ^{e}	5.21 ^{d}	7.24 ^{c}	1952.33 ^{d}	2.25 ^{e}
NPK	92.57 ^{c}	7.09 ^{b}	11.60 ^{b}	2364.33 ^{b}	3.42 ^{c}
Biogas digestate (BioD)	85.91 ^{d}	6.99 ^{b}	11.53 ^{b}	2358.66 ^{b}	3.43 ^{c}
Bio-fertilizer (BioF)	84.79 ^{d}	6.91 ^{bc}	11.35 ^{b}	2263.00 ^{c}	3.28 ^{d}
NPK+ BioD	94.38 ^{bc}	7.25 ^{b}	11.95 ^{a}	2403.33 ^{b}	3.63 ^{b}
NPK+ BioF	96.85 ^{b}	7.19 ^{b}	11.91 ^{a}	2388.33 ^{b}	3.52 ^{bc}
BioD + BioF	100.80 ^{a}	7.66 ^{a}	12.01 ^{a}	2519.67 ^{a}	4.01 ^{a}
$LSD_{0.05}$	1.63 **	0.21 **	0.30 **	43.98 **	0.13 **

$LSD_{0.05}$ = Least significant differences at 5% level; columns with different letters are significantly different from each other, as indicated by the Tukey test ($p \leq 0.05$); ** = $p \leq 0.01$.

The number of branches plant^{-1} was significantly increased in plants fertilized with BioD + BioF (4.01) followed by NPK + BioD (3.63) and NPK + BioF (3.52) in comparison to those obtained from the control (Table 2). Synthetic and organic fertilizers significantly enhanced the number of pods plant^{-1}, seeds pod^{-1}, 100-seed weight, seed yield, and straw yield of faba bean in comparison to the control treatment or those fertilized with bio-fertilizers (Table 2). For example, the combined application of BioD + BioF significantly increased the number of seed pods^{-1}, 100 seeds weight, seed yield plant^{-1}, seed yield ha$^{-1,}$ and straw yield ha^{-1} by 17.43, 18.39, 47.17, 36.75, and 36.83% in comparison to those obtained from the control, and by 8.87, 6.64, 18.00, 8.80, and 28.32% in comparison to those

obtained from faba fertilized with bio-fertilizer as a single application. Nevertheless, there were no significant differences between the effects of NPK+ BioD and NPK+ BioF on the above-mentioned traits, and they have ranked the second highest treatments in terms of improving growth and yield traits of faba bean in the current study.

Table 2. Effect of synthetic, organic, and bio-fertilizers on yield and yield components of faba bean.

Traits / Treatments	Number of Pods Plant^{-1}	Number of Seeds Pod^{-1}	100-Seeds Weight (g)	Seed Yield Plant^{-1} (g)	Seed Yield ha^{-1} (kg)	Straw Yield ha^{-1} (kg)
Control (zero NPK)	13.74 ^{d}	3.07 ^{d}	61.91 ^{d}	24.91 ^{e}	2968 ^{c}	3731 ^{d}
NPK	18.28 ^{b}	3.48 ^{b}	70.69 ^{b}	41.22 ^{c}	4355 ^{b}	5336 ^{b}
Biogas digestate (BioD)	18.29 ^{b}	3.44 ^{bc}	70.43 ^{b}	41.24 ^{c}	4340 ^{b}	5157 ^{b}
Bio-fertilizer (BioF)	16.24 ^{c}	3.39 ^{c}	69.68 ^{c}	38.67 ^{d}	4280 ^{b}	4234 ^{c}
NPK+ BioD	19.40 ^{ab}	3.54 ^{b}	72.64 ^{ab}	44.04 ^{b}	4404 ^{b}	5340 ^{b}
NPK+ BioF	18.27 ^{b}	3.52 ^{b}	71.50 ^{b}	42.99 ^{b}	4322 ^{b}	5276 ^{b}
BioD + BioF	19.73 ^{a}	3.72 ^{a}	74.64 ^{a}	47.16 ^{a}	4693 ^{a}	5907 ^{a}
LSD $_{0.05}$	0.80 **	0.06 **	2.04 **	1.65 **	125 **	202 **

$LSD_{0.05}$ = Least significant differences at 5% level; columns with different letters are significantly different from each other as indicated by the Tukey test ($p \leq 0.05$); ** = $p \leq 0.01$.

The chlorophyll content of a plant generally shows the nutrient status and nutrient response of the plants to different agricultural practices. In the current study, the total chlorophyll content of faba bean was significantly increased with the application of fertilizers compared to those obtained from the control treatment (Figure 1). Compared to all synthetic, organic, and bio-fertilizer treatments, the combined application of BioD + BioF significantly enhanced the total chlorophyll content at 40, 60, 80, and 100 DAS. However, the combined application of NPK+ BioD and NPK+ BioF, as well as the single application of NPK at 40 DAS, resulted in similar total chlorophyll contents without significant differences. Similarly, the single application of NPK or the combined application of NPK + BioF at 60 DAS had a non-significant difference in total chlorophyll content. Nevertheless, faba bean plants fertilized with the single application of BioF resulted in lower total chlorophyll content at 40, 60, 80, and 100 DAS than all other treatments except the control treatment.

Analysis of faba bean leaves for macro elements such as N, P, and K showed that synthetic, organic, and bio-fertilizers significantly affected their concentration (Figure 2). The highest N concentration (36.62 g kg^{-1} DW) in faba bean leaves was obtained from those fertilized with the combined treatment of BioD + BioF without a significant difference with those grown in plots treated by NPK + BioF (36.44 g kg^{-1} DW). Plants in controlled-treatment (zero NPK) showed the lowest N concentration (27.19 g kg^{-1} DW). Similarly, the highest P content was obtained from faba bean leaves fertilized with BioD + BioF (4.56 g kg^{-1} DM), NPK+ BioD (4.52 g kg^{-1} DM), and NPK+ BioF (4.12 g kg^{-1} DM), while the lowest P content (2.91 g kg^{-1} DM) was recorded from those grown in the negative control treatment. Potassium concentration was significantly increased when NPK + BioF (27.32 g kg^{-1} DM), BioD + BioF (26.95 g kg^{-1} DM), and NPK+ BioF (26.60 g kg^{-1} DM) were applied in comparison to those grown in the control treatment. However, the single application of NPK or BioD resulted in higher K content than those obtained from plants fertilized with the combined application of NPK + BioF.

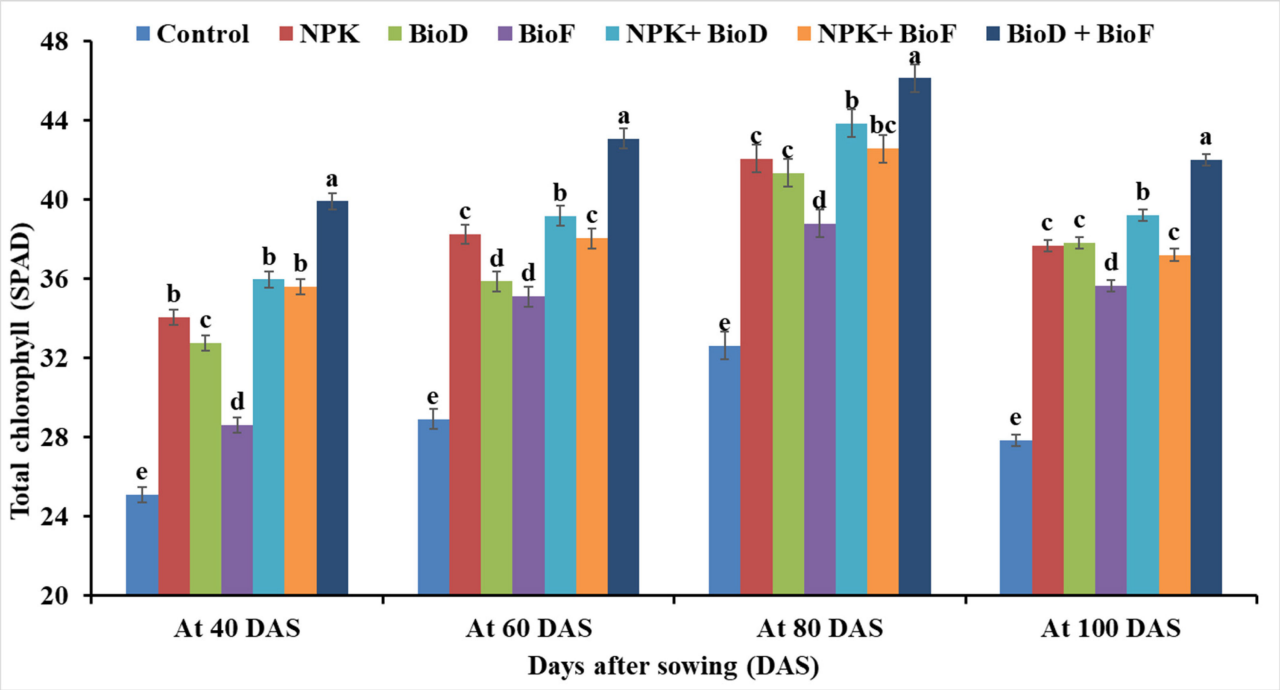


Figure 1. Effect of synthetic, organic, and bio-fertilizers on total chlorophyll content (SPAD) of faba bean. Columns with different letters significantly differed from each other as indicated by the Tukey test ($p \leq 0.05$).

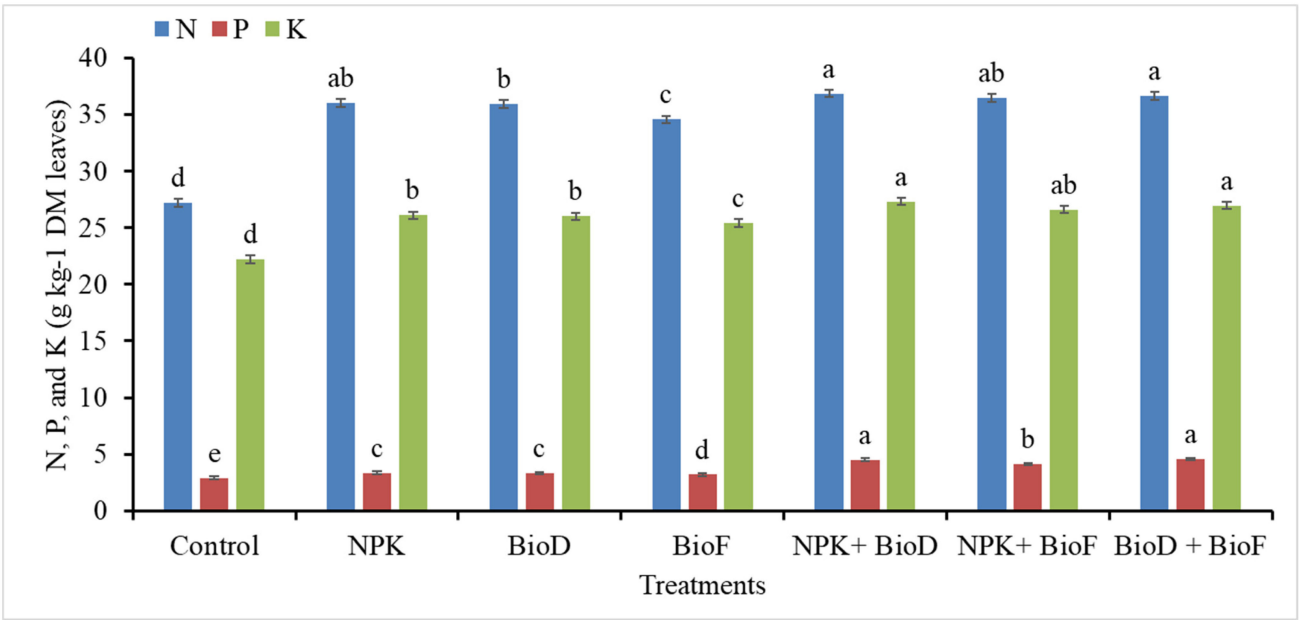


Figure 2. Effect of synthetic, organic, and bio-fertilizers on macro elements analysis of faba bean leaves. Columns with different letters significantly differed from each other as indicated by the Tukey test ($p \leq 0.05$).

Faba bean seed analysis showed that synthetic, organic, and bio-fertilizers enhanced the N, P, protein, and carbohydrate contents compared to controlled-treatment (zero NPK) (Table 3). The highest seed N content (4.35–4.42%) was recorded from those fertilized with the combined treatments of BioD + BioF, NPK + BioD, and NPK + BioF, followed by the single application of BioD in comparison to other treatments. Seed P content was significantly increased (0.482 to 0.544%) with the combined treatments of BioD + BioF, NPK

+ BioF, and NPK + BioD, followed by the single application of NPK and BioD, while the lowest P content (0.346%) was obtained from those grown in the control treatment.

Table 3. Effect of synthetic, organic, and bio-fertilizers on the percentage of N, P, protein, and carbohydrates of faba bean seeds.

Traits / Treatments	N (%)	P (%)	Protein (%)	Carbohydrate (%)
Control (zero NPK)	3.36 ^{e}	0.346 ^{d}	21.00 ^{d}	52.95 ^{d}
NPK	3.70 ^{d}	0.461 ^{b}	27.16 ^{a}	57.24 ^{b}
Biogas digestate (BioD)	4.13 ^{b}	0.454 ^{b}	25.85 ^{b}	57.03 ^{b}
Bio-fertilizer (BioF)	3.87 ^{c}	0.415 ^{c}	24.20 ^{c}	54.28 ^{c}
NPK+ BioD	4.35 ^{a}	0.512 ^{a}	27.24 ^{a}	57.79 ^{b}
NPK+ BioF	4.33 ^{a}	0.482 ^{ab}	27.09 ^{a}	57.46 ^{b}
BioD + BioF	4.42 ^{a}	0.544 ^{a}	27.67 ^{a}	59.68 ^{a}
LSD $_{0.05}$	0.16 *	0.039 **	1.01 *	1.25 **

$LSD_{0.05}$ = Least significant differences at 5% level; Columns with different letters significantly differed from each other as indicated by the Tukey test ($p \leq 0.05$); ** = $p \leq 0.01$; * = $p \leq 0.05$.

In the current investigation, all fertilizer sources enhanced the protein content of faba bean seeds; however, the highest values were recorded from those fertilized with BioD + BioF (27.67%), NPK + BioD (27.24%), NPK (27.26%) and NPK + BioF (27.09%) without significant differences with each other, while the lowest value was obtained from those grown in the control treatment (21.00%). The application of different sources of fertilizers increased the carbohydrate content of faba; however, the combined application of BioD + BioF resulted in the highest carbohydrate content (59.68%) in seeds, while the lowest value of carbohydrate content (52.95%) was obtained from those grown in the control treatment (Table 3). However, the single application of NPK, BioD, and BioF, as well as the combined application of NPK + BioD or NPK + BioF, resulted in non-significant differences among each other for seed carbohydrate content.

4. Discussion

In the current study, the focus was to investigate the comparative effects of synthetic fertilizer (NPK), biogas digestate (BioD), and bio-fertilizer (BioF) and their combinations on the growth and yield of faba bean. The results obtained from the current study indicated that plant height, leaves, stem dry weight, leaf area, number of branches and pods, seeds within pods, 100-seed weight, seed yield, and quality of grains in faba bean were positively and significantly affected by the application of BioD, BioF, NPK, and their combinations. However, the application of BioD+ BioF as a combination enhanced growth and improved yield and quality of faba bean in comparison to all other treatments. Thus, such a combination treatment of BioD+ BioF can decrease the dependence on synthetic fertilizers in agricultural systems. The enhancement in plant height, leaf area, number of branches, and yield traits of faba bean in response to combined treatment of BioD + BioF might be due to the positive effects of BioD + BioF on plant growth, cells development, enzymes regulation, and improvement in the photosynthetic process. In addition, it can be related to the ability of microorganisms present in BioF to produce phytohormones such as indole acetic acid [39]. Such microorganisms in BioF can solubilize and mobilize P and K, fix N, and increase the availability of these nutrients to the plants; consequently, they can enhance the growth and productivity of crops [40,41]. In addition, BioD is considered a good source of macro- and micronutrients essential to plant growth, yield, and quality. The BioD can improve soil properties due to the high organic matter content. For example, soil bulk density was reduced, and total porosity was increased in soil treated with biogas slurry compared to synthetic fertilizers [42]. In addition, biogas slurry application enhanced

water-holding capacity by 13.83% and available water content by 25.87% in comparison to synthetic fertilizer, respectively [42].

In the current study, N, P, and K contents were increased in leaves of faba bean fertilized with different sources of fertilizers, particularly the combined treatments of BioD + BioF, in comparison to the control treatment. The digestates, particularly those made from agricultural or animal wastes, are a valuable source of organic matter, microbial origin, phytohormones, and macro- and micronutrients that can improve soil characteristics as well as plant growth and productivity [18–22,43,44]. Bio-organic fertilization can enhance microbial activities by increasing enzyme activities such as dehydrogenase, acid phosphatase, mycorrhizal, and bacterial levels in the rhizosphere of plants compared to synthetic fertilizers [44]. Thus, BioD and BioF concurrently stimulate each other through their physical and biosynthetic activities and, subsequently, increase the physiosynthetic functions of plants.

Some researchers have found positive effects of bio-fertilizers and organic fertilizers on the growth and productivity of other crops. For example, Gao et al. [45] have shown that the combined application of bio-fertilizer with organic fertilizers increased the nutrient uptake, growth, and yield of maize. Indeed, the beneficial effects of the organic and bio-fertilizers are probably associated with the recycling of nutrients, solubilization of nutrients, particularly P, K, and Fe, decomposition of soil organic matter, production of phytohormones and antibiotics, and improvement in soil aggregation and structure [45–47].

The results of our study indicated that the combined application of BioD + BioF increased the total chlorophyll contents in faba bean leaves. The increase in total chlorophyll contents of faba bean could be due to the increase in N uptake from both organic and bio-fertilizers, which may induce phytohormones production that stimulates photosynthesis and chlorophyll contents. In this respect, Awan and Baloch [48] reported a positive correlation between chlorophyll and N content in plants. Consequently, plant growth can be enhanced. The chlorophyll content shows a direct response to the N and P in plant leaves, and studies showed that organic and bio-fertilizers are rich sources of N, P, K, and other micronutrients [49,50], which can enhance growth and chlorophyll content of faba bean.

The current study indicated that a combination of BioD + BioF resulted in higher, without significant differences with other combinations treatments, N, P, and K contents in faba bean leaves than those obtained from a single application of NPK or BioD and BioF. The increased N, P, and K contents in faba bean leaves could be related to relatively high nutrient contents of BioD and BioF in the available forms in slow release manner that were sufficient to meet the requirements of growing plants. For instance, bio-fertilizers can be effective and support plants by increasing the uptake of nutrients and secreting phytohormones and metabolites through their interactions within the rhizosphere once applied via seed or soil [29]. The enhancement in nutrient contents in faba bean plants under bio-organic treatments might be due to an improvement in biological N_2 fixation and/or biosynthesis of organic acids, which could cause nutrient solubilization for P, K, and Fe. Imagine, the soil fertility category was improved from "high" (200–300 mg kg^{-1}) to "very high" (>300 mg kg^{-1}) according to P_2O_5 by using 170 kg ha^{-1} of N in solid digestate rate [17]. In addition, the biosynthesis of specific growth-promoting substances has been reported after the application of bio-fertilizers that can effectively influence root development and, consequently, their function in water and nutrient uptake [45,51]. Wu et al. [51] documented that bio-fertilization enhanced the assimilation of nutrients (N, P, and K) in maize plants. Similarly, bio-fertilizer containing arbuscular mycorrhizal fungi and humic acid increased nutrient availability in the plant's rhizosphere and improved soil health [41,52]. Thus, enhancing the nutrient uptake of faba bean plants in the current study through the combined treatment of BioD + BioF not only can increase seed yield but also can improve nutrient contents in seeds.

In the current study, the improvement in seed quality traits might be due to the fact that bio-fertilizer and biogas digestate collectively provided sufficient quantities of nutrients

to plants by increasing N_2 fixation and/or provision of organic acids and phytohormones. For instance, biogas digestate can play an important role in the balance of carbon and N metabolisms and, thus, can increase grain amino acid content [53]. Increasing nutrient contents such as N, P, and K in leaves and grains of crops fertilized with organic or bio-fertilizers can enhance phytohormones production and the photosynthesis process [45]. Consequently, this can enhance protein [54], carbohydrate, and soluble sugars in seeds of crops fertilized with organic and/or bio-fertilizers [45]. Similarly, Różyło et al. [55] reported that the application of biogas digestate resulted in higher wet gluten, phenols, and protein content in wheat grains than those obtained from wheat fertilized with NPK fertilization. Thus, applying biogas digestate and/or bio-fertilizers can potentially improve seed quality by enhancing nutrient uptake and improving photosynthetic assimilates, which ultimately translocate to the seeds for increasing grain nutrient status, protein, and carbohydrates.

5. Conclusions

Based on the obtained results, it could be concluded that the application of synthetic fertilizers, biogas digestate (BioD), bio-fertilizer (BioF), and their combination increased the growth, seed yield, and related traits, nutrient content, and seed quality traits in comparison to those obtained from the control. However, the highest increase in these parameters was obtained when the combined application of BioD with BioF was used. The combination of BioD with BioF resulted in an increase in faba bean growth and physiology due to increased uptake of N, P, and K contents, probably due to better root growth. An improvement in the uptake of plant nutrients increases the quality of seeds, thus increasing the N, P, protein, and carbohydrate contents. This study suggests that biogas digestate and bio-fertilizers, in combination, could improve soil microbial activity, which in turn increases faba bean growth and development. Thus, the present study showed that the concurrent application of biogas digestate and bio-fertilizer could serve as an effective and alternative fertilizer to reduce the consumption of synthetic fertilizers for sustainable agriculture.

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