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The Effect of Different Fertilizer Treatments of Plant Traits on Faba Bean during Flowering Periods

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Abstract: The experiment was conducted at Dicle University Field Crops Department, Southeast Anatolia, Diyarbakir Turkey in the 2018-2019 early spring growth periods. The study aimed to determine the effect of fertilizer treatments (control, phosphorus, nitrogen, organic, and Rhizobium) on plant traits of different faba bean cultivars (Filiz 99, Eresen 87, and Salkim). Plants were harvested in three different periods pre-flowering (43-62 days after sowing), full-flowering (52-75 days), and post-flowering (78-98 days), and shoot and root traits were evaluated. Fresh plant biomass, stem height, fresh and dry root weight, fresh and dry nodule weight, fresh leaf weight, and fresh and dry pod weight were responding to nitrogen fertilizer treatments depending on flowering periods. The number of nodules and fresh and dry nodule weight was affected to a different degree in both nitrogen and phosphorus fertilizer treatments. Nodule weight showed a negative response to rhizobium inoculation. We determined that nitrogen (40 kg ha^{-1}) and phosphorus (80 kg ha^{-1}) fertilizer treatments on faba beans were important. However, we have not belied that fertilizer applications should not be applied for sustainable agriculture because of their high environmental efficiency.

Keywords: faba bean; nitrogen; phosphorus; organic; root; nodule.

1. Introduction

Faba bean is a major crop in many countries including China, Ethiopia, and Egypt, and is widely grown for human food throughout the Mediterranean region and in parts of Latin America. China is a major shareholder in production with 60% (FAO 2019). However, Turkey is not among the major producer countries [5].

Cultivated faba bean is used as human food in developing countries and as animal feed, mainly for pigs, horses, poultry, and pigeons in industrialized countries. Bean can be used as a vegetable, green or dried, fresh or canned. Also, beans, which have an important place in human nutrition, are one of the culture plants with the lowest production costs [11,6,12]. Faba bean is an excellent crop for cropping systems because of its unique ability to fix atmospheric N2 symbiotically which heavily depends on the sufficient populations of effective rhizobia. It can accumulate N both from soil and the atmosphere. Due to their indeterminate growth habit, faba beans continued assimilating N for a longer period, reaching about 315 kg N ha^{-1} after 110 days. The N concentration in the faba bean crop biomass was around 5% a few days before flowering; during the initial stages (30 days) of reproductive growth, the N concentration declined rapidly to c. 2.5–3%, due to the biomass accumulation rate being faster than the N assimilation rate, and the N concentration remained at this level until maturity [9,11]. Faba bean accumulates N from N_2 fixation at an increasing rate until initiation of the maturation process unless other factors such as water availability restrict the N_2 fixation process earlier in growth [1]. It is well known that the amounts of N_2 fixed during seed development were substantial [13], but some researchers reported that major decline in nitrogen fixation during reproductive growth [16], this raised the question of whether the amounts of nitrogen fixed symbiotically are sufficient to meet the needs related to seed setting and formation.

Leguminous crops require an adequate supply of readily available nutrients for optimum growth and yield. Pamiralan and Gok [8] reported application of inhibitor with inducing nitrogen significantly increased the dry matter yield of the plant (root: 29%, shoot: 61%), but nodulation and nodule were not affected. Wojcieska and Kocoñ [14] reported that fertilizer N depressed the nodule formation and nitrogenase activity, but the inhibitory effect of N was smaller when it was supplied to the leaves instead to the soil.

Pulse crops can be bite responsive to P fertilization, particularly where soils test low in available P [7]. Faba bean is generally considered to be sensitive to seed-placed fertilizer and therefore it is not recommended that more than 25 kilograms per hectare (kg/ha), of phosphorus pentoxide, P_2O_5 [5]. Faba beans are responsive to high rates of P fertilizer in low P soils and even respond to rates that are higher than those typically used by most producers. In Turkey, the use of fertilizer for bean plants is applied only in greenhouses for green bean growing. However, in recent years, the producers increasingly want to use fertilizers, organic or inorganic, in legume crops growing. We started this study to get an idea as to whether fertilizer application is necessary for faba bean crops, and we aimed to determine whether the use of fertilizer affects the root and stem parts of the pods in this study.

2. Materials and Methods

An experiment was conducted at Dicle University Field Crops Department, Southeast Anatolia, Diyarbakir Turkey during the early spring growth period in 2018-2019. Meteorological data were given in Table 1. In the 2018 early spring growing season, total precipitation from Feb to April was 146.8 mm, but in May was 157.8 mm, and the mean temperature (Feb to May) has been 11.9 °C. In the 2019 early spring growing season, total precipitation from February to April was 365.2 mm, but May was 45.8 mm, and the mean temperature (Feb to May) was been 11.35 °C. The soil analysis indicate that soils are neutral to slightly alkaline (pH 7.24), un-sufficient in organic matter (0.79%), and phosphorus content (13.2 kg ha^{-1}). The soil texture was 44% clay.

Table 1. Meteorological data in Diyarbakir.

Month	**Mean temperature (°C)**		**Total precipitation (mm)**		**Moisture (%)**	
	2018	**2019**	**2018**	**2019**	**2018**	**2019**
January	5.2	3.8	86.6	67.6	77.3	81.7
February	7.6	5.4	86.4	77.4	74.5	77.0
March	12.3	8.2	11.6	135.2	63.2	74.9
April	15.9	11.8	48.8	152.6	53.0	78.4
May	19.4	20.1	157.8	45.8	67.5	58.5
June	26.5	28.3	14.4	1.0	37.9	32.5
July	31.2	30.3	0.0	0.07	24.2	24.8

The study was conducted to determine the effect of fertilizer treatments (control, phosphorus, nitrogen, organic, and Rhizobium) on plant traits of different faba bean cultivars (Filiz 99, Eresen 87, and Salkım). The experimental design was arranged in a split plot in a randomized complete block design with three replications. Treatments consisted of organic, inorganic, and bio-fertilizers. Inorganic fertilizers were applied at a rate of 40 kg ha^{-1} nitrogen (DAP 18-46%) and 80 kg ha^{-1} phosphorus (TSP 46%). Organic fertilizer consisted of organic materials (45%), organic carbon (20%), organic nitrogen (6%), free amino acids (3.5%), and pH (6-8). Rhizobium leguminosarum as bio-fertilizer was inoculated at 10 g each/kg seed. The dose of organic fertilizer was applied over 40 kg nitrogen per hectare, taking into account the organic nitrogen (6%) contained in the fertilizer content. Before sowing, seeds were inoculated with a specific strain of Rhizobium at 10 g each/kg seed. All fertilizers were applied with sowing. The seeds were sown on 7 February 2018 and 11 February 2019, at 40 cm × 10 cm spacing.

Plants were harvested in three different periods pre-flowering (after 43-62 days after sowing), full-flowering (52-75 days after sowing), and post-flowering (78-98 days after sowing). Before the plant samples were removed from the plots, plots were irrigated with the sprinkler so that plants could be easily removed from the soil with their roots, and the soil was kept until the field capacity. After plants were removed from the plots, were washed off the soil, and dried gently with a soft paper towel to remove any free surface moisture. In dry weight measurements, plants were dried in an oven set to heat (70 °C) overnight [15]. Fresh and dry plant biomass, root, nodule, and leaf weight $plant^{-1}$, plant height, number of nodules $plant^{-1}$, and leaf area $plant^{-1}$ were evaluated in all periods, but fresh and dry pod weight $plant^{-1}$ was evaluated in the post-flowering period. Leaf area was measured by Winfolia2003 software. The crop was irrigated after sown in 2018 due to drought. Weed, disease, and pests were controlled by hand and chemical spray, respectively.

Data of two years were separately and pooled, subjected to analysis of variance, and means were separated using Duncan's Multiple Range Test.

3. Results and Discussion

The effect of fertilizer treatments on fresh plant biomass was significant in all periods. In the flowering period, fresh plant biomass ranged from 26.8 g to 31.2 g, nitrogen treatment gave a high value, followed by organic treatment (29.0 g) and the control group (29.7 g). In the full flowering period, fresh plant biomass ranged from 37.3 g in phosphorus treatment to 48.2 g in nitrogen treatment. In the post-flowering period, the trait was 163.1 g to 237.3 g, the highest fresh plant biomass was obtained from nitrogen treatment. Phosphorus, organic, and rhizobium treatments decreased fresh plant biomass compared to control. Year × variety × treatment interaction was significant, and some cultivars did not respond to fertilizer in some years (Table 2). Nitrogen fertilizer compared to other fertilizers and control were been a treatment that increased the vegetative parts of plants.

Differences among years for plant biomass were significant and varied from 21.7 g to 35.9 g. Differences between the two trial years may be due to climatic conditions. In the 2018 growth period; February, March, and April were quite dry and hot, and the experiment was regularly irrigated by sprinkler irrigation. Irrigation supply water, sunny days and warm weather during the vegetation period, and high rainfall in May had a positive effect on plant development. In the 2019 growth period; since February, March and April were quite rainy and the cloudy weather, the low temperature delayed the plant growth at the beginning of the growing season. Seed germination and emergence were delayed due to low soil temperature, emergence rate and seedling vigor were low and weak. Most seeds that could not emerge due to low soil temperature could not survive under the soil. As a result, the number of plants per parcel decreased. May 2019 was extremely dry and caused negative effects on plants during the generative period. In 2019, the vegetative period started late and progressed heavily. Finally, the drought in the generative period delayed the development of the plant.

Table 2. Fertilizer treatments on fresh plant biomass on faba bean.

	Fresh plant biomass plant^{-1} (g)						
	Filiz 99		**Eresen 87**		**Salkım**		**Mean**
Pre-flowering	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	
Control	40.9 ^{ab}	14.5	39.2 ^{a}	19.4 ^{ab}	41.0 ^{a}	23.2	29.7 ^{ab}
Nitrogen	44.7 ^{a}	19.2	24.5 ^{c}	24.4 ^{a}	45.4^{a}	29.1	31.2 ^{a}
Phosphorus	37.7 ^{abc}	23.6	26.7 ^{c}	13.2 ^{b}	38.3 ^{a}	25.2	27.5 ^{b}
Organic	34.7 ^{bc}	23.3	31.3 ^{b}	22.4 ^{a}	39.6 ^{a}	22.5	29.0 ^{ab}
Rhizobium	30.2 ^{c}	22.8	39.6 ^{a}	17.3 ^{b}	25.3 ^{b}	25.4	26.8 ^{b}
Mean	37.6 ^{a}	20.7 ^{b}	32.3 ^{a}	19.3 ^{b}	37.9 ^{a}	25.1 ^{ab}	
Cultivars	29.2 ^{ab}		25.8 ^{b}		31.5 ^{a}		
Year	35.9 ^{a}	21.7 ^{b}					
Full- flowering							
Control	35.8 ^{b}	33.3 ^{b}	39.7 ^{a}	33.8 ^{b}	47.8 ^{a}	50.95	40.2 ^{bc}
Nitrogen	53.8 ^{a}	62.7 ^{a}	36.5 ^{ab}	51.9 ^{a}	32.5 ^{c}	52.06	48.2 ^{a}
Phosphorus	36.1 ^{b}	32.8 ^{b}	30.7 ^{b}	35.4 ^{b}	33.7 ^{bc}	55.05	37.3 ^{c}
Organic	39.0 ^{b}	33.8 ^{b}	42.7 ^{a}	47.3 ^{ab}	40.3 ^{b}	63.69	44.5 ^{ab}
Rhizobium	39.7 ^{b}	42.1 ^{b}	40.4 ^{a}	33.8 ^{b}	37.6 ^{bc}	48.83	41.2 ^{bc}
Mean	40.9	40.9	38.0	40.4	38.4	54.1	
Cultivars	40.9 ^{ab}		39.7 ^{b}		46.3 ^{a}		
Year	39.1 ^{b}	45.5 ^{a}					
Post -flowering							
Control	229.9	153.5	270.3 ^{b}	117.0 ^{bc}	316.1 ^{b}	141.7 ^{bc}	204.8 ^{b}
Nitrogen	180.7	186.3	325.2 ^{a}	173.5 ^{a}	386.1 ^{a}	172.7 ^{b}	237.4 ^{a}
Phosphorus	190.0	144.4	199.3 ^{c}	129.7 ^{b}	176.2 ^{c}	224.5 ^{a}	177.3 ^{c}
Organic	219.1	176.4	171.0 ^{c}	104.6 ^{c}	295.4 ^{b}	117.9 ^{c}	180.7 ^{c}
Rhizobium	229.9	183.8	245.0 ^{b}	74.75 ^{d}	192.4 ^{c}	105.6 ^{c}	163.1 ^{c}
Mean	209.9 ^{b}	168.9 ^{c}	242.2 ^{a}	131.2 ^{c}	273.2 ^{a}	152.5 ^{c}	
Cultivars	184.1 ^{b}		181.0 ^{b}		212.9 ^{a}		
Year	238.3 ^{a}	147.1 ^{b}					

The effect of treatments on stem height was significant in all periods. In the pre-flowering stem, height ranged from 21.3 cm in nitrogen fertilizer treatment to 24.6 cm in rhizobium inoculation, and all treatments were similar except for bacteria inoculation. Filiz 99 cultivar was not responded to fertilizer treatments in both years, but other cultivars response to it in different years period. In the full flowering period, the highest stem height was obtained from nitrogen treatment (34.1 cm) and control (33.3 cm), but all treatments were similar except for phosphor fertilization. During the post-flowering period, nitrogen fertilizer treatment increased plant height. Year × treatment × cultivar interaction revealed that cultivars were affected differently by fertilizer treatments in both years. Unlike other cultivars, Eresen 87 was affected by bacteria inoculation in both years. Differences among years might result from cloudy, cool, and rainy conditions in 2019 (Table 3).

The effect of treatment on fresh root weight was significant in all periods. In the pre-flowering period, fresh root weight ranged from 7.8 g in rhizobium inoculation to 8.7 g in the control group, similar to the nitrogen (8.2 g) and phosphorus (8.2 g) treatments. In the full flowering period, treatments affected the fresh root weight, and control group (103 g), phosphorus (10.1 g) and nitrogen (9.0 g) treatments were higher than organic (8.1 g) and rhizobium (8.4 g) treatments. Similarly, in the post-flowering period, nitrogen (15.7 g) and phosphorus (14.1 g) treatments were higher than the control group (13.9 g), organic (13.1 g), and rhizobium (11.8 g) treatments (Table 4).

Table 3. Fertilizer treatments on stem height on faba bean.

	Stem height (cm)						
	Filiz 99		**Eresen 87**		**Salkım**		**Mean**
Pre-flowering	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	
Control	27.0	19.0	28.0	18.2 b	32.2 a	17.3	23.6 a
Nitrogen	25.7	21.0	25.0	25.0 a	31.0 a	19.7	24.6 a
Phosphorus	24.7	18.3	25.7	18.3 b	28.0 ab	18.7	22.3 ab
Organic	26.0	20.3	25.3	16.7 b	27.3 ab	20.7	22.7 ab
Rhizobium	22.7	22.2	25.3	18.8 b	24.0 b	15.0	21.3 b
Mean	25.2 a	20.2 b	25.9 a	19.4 b	28.5 a	18.3 b	
Cultivars	22.7		22.6		23.4		
Year	26.5 a	19.3 b					
Full- flowering							
Control	30.0	30.7 bc	29.3 a	32.67	36.7 a	40.3	33.3 a
Nitrogen	28.7	47.7 a	29.7 a	33.33	30.3 b	34.7	34.1 a
Phosphorus	31.7	31.7 bc	28.3 ab	29.00	27.7 b	34.7	30.5 b
Organic	28.0	26.0 c	25.3 b	37.33	30.3 b	42.0	31.5 ab
Rhizobium	29.3	35.3 b	31.7 a	34.0	29.0 b	34.0	32.2 ab
Mean	29.5 b	34.3 ab	28.9 b	33.3 ab	30.8 b	37.1 a	
Cultivars	31.9 b		31.1 b		34.0 a		
Year	29.7 b	34.9 a					
Post -flowering							
Control	65.0 b	85.0	64.7 a	74.0 b	76.3 a	77.7	73.9 b
Nitrogen	87.3 a	80.0	60.3 ab	79.3 ab	55.0 c	81.3	78.0 a
Phosphorus	68.0 b	79.0	49.7 b	81.7 ab	64.3 bc	89.0	71.5 b
Organic	80.3 a	78.3	57.7 ab	77.0 b	55.3 c	80.3	73.8 b
Rhizobium	69.0 b	90.7	66.0 a	90.8 a	67.0 ab	84.3	71.9 b
Mean	73.9 b	82.6 a	59.7 b	80.6 a	63.6 b	82.5 a	
Cultivars	78.3 a		70.1 b		73.1 b		
Year	65.7 b	81.9 a					

Differences among years in pre and full-flowering periods resulted from the high water content of the roots removed from the soil due to high soil moisture.

The effect of treatment on dry root weight was significant in the only pre-flowering period. Dry root weight ranged from 0.94 g to 1.39 g, and the control group (1.39 g) was higher than other treatments. Year × variety × treatment interaction was significant in all periods. Cultivars were affected differently by the treatments, and nitrogen and phosphorus treatments were more effective but not different from the control group (Table 4).

The effect of treatment on several nodules plant^{-1} was significant in all periods. In the pre-flowering period, the number of nodules plant^{-1} ranged from 30.9 to 44.2, and phosphorus (44.2) and bacteria (44.1) treatments were higher than the control group and other treatments. Phosphorus and bacteria may have activated in the early stages of nodule development. However, in full and post-flowering periods, phosphorus and nitrogen fertilizer treatments were important for nodules. In the full flowering period, the highest number of nodules plant-1 was in nitrogen fertilizer treatment (124.4) (Table 5).

Table 4. Fertilizer treatments on fresh and dry root weight on faba bean.

	Fresh root weight (g)						
	Filiz 99		**Eresen 87**		**Salkım**		**Mean**
Pre-flowering	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	
Control	10.3 ^{a}	6.2 ^{c}	12.9 ^{a}	10.7 ^{a}	6.3 ^{bc}	7.4	8.7 ^{a}
Nitrogen	8.6 ^{ab}	9.7 ^{a}	15.4 ^{a}	7.3 ^{bc}	10.3 ^{a}	9.4	8.2 ^{ab}
Phosphorus	7.5 ^{ab}	8.2 ^{ab}	7.9 ^{b}	8.8 ^{ab}	8.4 ^{ab}	10.8	8.2 ^{ab}
Organic	6.8 ^{b}	7.1 ^{bc}	9.8 ^{b}	8.5 ^{ab}	7.5 ^{b}	7.2	7.3 ^{bc}
Rhizobium	8.9 ^{ab}	7.9 ^{abc}	10.2 ^{b}	4.7 ^{c}	4.8 ^{c}	7.9	7.8 ^{c}
Mean	8.4	7.8	11.2	8.0	7.5	8.5	
Cultivars	8.1		7.5		8.0		
Year	7.7	8.1					
Full- flowering							
Control	7.9 ^{ab}	9.7	8.0 ^{a}	9.7	11.6 ^{a}	14.6	10.3 ^{a}
Nitrogen	7.5 ^{b}	10.4	6.0 ^{b}	11.8	5.8 ^{c}	12.4	9.0 ^{ab}
Phosphorus	9.5 ^{a}	9.1	7.2 ^{ab}	12.9	8.0 ^{b}	14.0	10.1 ^{a}
Organic	4.7 ^{c}	7.7	5.9 ^{b}	10.4	7.4 ^{bc}	12.7	8.1 ^{b}
Rhizobium	8.0 ^{ab}	9.3	7.3 ^{ab}	8.2	6.2 ^{c}	11.6	8.4 ^{b}
Mean	7.5 ^{ab}	9.2 ^{a}	6.9 ^{b}	10.6 ^{a}	7.8 ^{ab}	13.1 ^{a}	
Cultivars	8.4 ^{b}		8.7 ^{b}		10.4 ^{a}		
Year	7.4 ^{b}	10.9 ^{a}					
Post -flowering							
Control	27.3 ^{a}	7.7 ^{b}	12.9 ^{ab}	11.6 ^{b}	11.4 ^{b}	12.3 ^{b}	13.9 ^{b}
Nitrogen	12.7 ^{c}	12.2 ^{ab}	15.4 ^{a}	19.2 ^{a}	15.9 ^{a}	18.6 ^{a}	15.7 ^{a}
Phosphorus	22.0 ^{b}	10.6 ^{ab}	7.9 ^{c}	11.5 ^{b}	14.3 ^{a}	18.3 ^{a}	14.1 ^{ab}
Organic	24.1 ^{ab}	14.8 ^{ab}	9.8 ^{c}	10.4 ^{b}	10.1 ^{b}	14.2 ^{b}	13.9 ^{b}
Rhizobium	16.7 ^{c}	16.7 ^{a}	10.2 ^{bc}	8.6 ^{b}	6.5 ^{c}	12.0 ^{b}	11.8 ^{c}
Mean	20.6 ^{a}	12.4 ^{b}	11.2 ^{b}	12.3 ^{b}	11.6 ^{b}	15.1 ^{b}	
Cultivars	16.5 ^{a}		11.8 ^{b}		13.4 ^{b}		
Year	14.5	13.3					
	Dry root weight (g)						
	Filiz 99		**Eresen 87**		**Salkım**		**Mean**
Pre-flowering	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	
Control	0.76	0.90 ^{a}	1.07	0.73 ^{b}	0.89	1.41	0.96 ^{a}
Nitrogen	0.90	1.07 ^{a}	0.62	1.06 ^{a}	0.70	1.15	0.92 ^{a}
Phosphorus	0.57	0.66 ^{b}	0.67	1.10 ^{a}	0.48	1.12	0.77 ^{b}
Organic	0.83	0.57 ^{b}	0.73	1.31 ^{a}	0.76	1.20	0.90 ^{a}
Rhizobium	0.67	0.75 ^{b}	0.76	1.01 ^{a}	0.67	1.13	0.83 ^{ab}
Mean	0.75 ^{b}	0.79 ^{b}	0.77 ^{b}	1.04 ^{ab}	0.70 ^{b}	1.20 ^{a}	
Cultivars	0.77		0.91		0.95		
Year	0.70 ^{b}	1.20 ^{a}					
Full- flowering							
Control	0.87 ^{ab}	1.65 ^{b}	0.95	2.51	1.24 ^{a}	1.48 ^{a}	1.45
Nitrogen	1.04 ^{a}	1.25 ^{c}	1.1	2.33	0.64 ^{b}	1.80 ^{a}	1.36
Phosphorus	1.17 ^{a}	2.24 ^{a}	0.72	1.50	1.01 ^{ab}	1.70 ^{a}	1.39
Organic	1.08 ^{a}	0.74 ^{d}	0.92	1.88	0.84 ^{b}	0.71 ^{b}	1.03
Rhizobium	0.70 ^{b}	0.79 ^{d}	0.92	1.74	0.86 ^{b}	1.67 ^{a}	1.11
Mean	0.97 ^{b}	1.33 ^{b}	0.92 ^{b}	1.99 ^{a}	0.92 ^{b}	1.47 ^{a}	
Cultivars	1.15 ^{b}		1.46 ^{a}		1.20 ^{b}		
Year	0.92 ^{b}	1.47 ^{a}					
Post -flowering							
Control	4.67 ^{ab}	1.58	2.59 ^{a}	2.14 ^{ab}	2.54	1.96	2.58
Nitrogen	2.76 ^{c}	2.14	2.75 ^{a}	3.03 ^{a}	2.85	2.45	2.66
Phosphorus	3.65 ^{bc}	1.93	1.82 ^{b}	2.06 ^{ab}	2.87	2.66	2.50
Organic	5.20 ^{a}	2.67	1.70 ^{b}	1.40 ^{b}	1.99	1.88	2.47

Rhizobium	3.57 bc	2.86	2.47 a	1.51 b	2.77	1.84	2.50
Mean	3.97 a	2.24 b	2.27 b	2.03 b	2.60 b	2.16 b	
Cultivars	3.10 a		2.15 b		2.38 b		
Year	2.95	2.14					

Table 5. Fertilizer treatments on a number of nodules plant^{-1} on faba bean.

	Number of nodules plant^{-1}						
	Filiz 99		**Eresen 87**		**Salkım**		**Mean**
Pre-flowering	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	
Control	68.0 a	62.6 a	28.0 b	25.8 b	21.1 d	19.0 d	37.4 b
Nitrogen	28.7 d	26.4 d	14.0 c	12.9 c	61.2 ab	55.8 ab	33.2 c
Phosphorus	55.7 b	51.2 b	18.3 c	16.8 c	64.5 a	58.9 a	44.2 a
Organic	32.7 d	30.0 d	28.0 b	25.7 b	36.4 c	32.8 c	30.9 c
Rhizobium	46.3 c	42.6 c	37.7 a	34.6 a	54.0 b	49.4 b	44.1 a
Mean	46.3 a	42.6 a	25.2 b	23.2 b	47.4 a	43.2 a	
Cultivars	44.4 a		24.2 b		45.3 a		
Year	39.6	36.3					
Full- flowering							
Control	92.0 a	131.3 b	38.0 b	56.0 c	24.7 c	116.3 ab	76.4 b
Nitrogen	30.0 cd	275.0 a	35.3 b	216.7 a	67.0 a	122.3 ab	124.4 a
Phosphorus	25.0 d	185.7 b	49.7 a	94.0 bc	27.0 c	187.7 a	94.8 b
Organic	37.0 c	186.7 b	34.7 bc	140.7 b	34.7 b	120.3 ab	92.3 b
Rhizobium	67.0 b	155.3 b	28.3 c	91.3 bc	61.7 a	102.0 b	84.3 b
Mean	50.2 d	186.8 a	37.2 d	119.7 c	43.0 d	129.7 b	
Cultivars	118.5 a		78.5 b		86.4 b		
Year	43.5 b	145.4 a					
Post -flowering							
Control	113.3 a	174.3 b	111.0 b	102.7 b	47.3 b	117.0 c	110.9 b
Nitrogen	77.3 c	226.0 a	161.0 a	140.7 a	92.4 a	183.3 b	146.8 a
Phosphorus	99.3 b	119.2 bc	105.3 b	150.0 a	55.0 b	296.3 a	137.5 a
Organic	45.7 d	97.7 c	34.0 c	96.1 b	47.5 b	219.3 b	90.0 c
Rhizobium	26.3 e	175.7 ab	62.7 c	110.7 b	89.8 a	132.3 c	99.6 bc
Mean	72.4 c	158.6 a	94.8 bc	120.0 b	66.4 c	189.6 a	
Cultivars	115.5 ab		107.4 b		128.0 a		
Year	77.9 b	156.1 a					

In the post-flowering period, nitrogen (146.8) and phosphorus (135.7) treatments for these traits were high. Differences among years were significant, rainy from Feb to April in 2019 increased soil moisture content, and root systems in 2019 year were more growth than those in 2018. In addition, soil moisture had generally a positive effect on the nodulation system. The detection of root nodules in the faba bean was quite difficult. Because after the roots were removed from the soil, darkening starts on the root and root nodule and in a short time all the nodules become black. The nodules cannot be exactly and easily counted although there were many nodules in the main and secondary roots. Wojcieska and Kocoñ [14] reported that fertilizer N depressed the nodule formation and nitrogenize activity, but the inhibitory effect of N was smaller when it was supplied to the leaves instead of to the soil.

The effect of treatment on fresh nodule weight plant-1 was significant in all periods. In the pre-flowering period, fresh nodule weight plant-1 ranged from 0.31 g to 0.77 g, and nitrogen fertilizer treatment was higher than the control group and other treatments. Cultivar × treatment interaction was significant, but Filiz 99 cultivar was no response to treatments. In the full flowering period, nitrogen (1.18 g) and phosphorus (1.06 g) fertilizer treatments were higher than other ones. In the post-flowering period, control (1.64 g), nitrogen (1.58 g) and phosphorus (1.64 g) treatments were higher than organic (1.09 g) and rhizobium (1.03 g) treatments (Table 6).

Table 6. Fertilizer treatments on fresh nodule weight plant^{-1} on faba bean.

	Fresh Nodule weight plant $^{-1}$ (g)						
	Filiz 99		**Eresen 87**		**Salkım**		**Mean**
Pre-flowering	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	
Control	0.71	0.59	0.61 ^{bc}	0.51 ^{bc}	0.49 ^{cd}	0.40 ^{cd}	0.55 ^{bc}
Nitrogen	0.50	0.42	0.87 ^{a}	0.72 ^{a}	1.16 ^{a}	0.96 ^{a}	0.77 ^{a}
Phosphorus	0.57	0.47	0.73 ^{ab}	0.61 ^{ab}	0.86 ^{ab}	0.71 ^{ab}	0.66 ^{b}
Organic	0.58	0.48	0.47 ^{c}	0.39 ^{c}	0.65 ^{bc}	0.53 ^{bc}	0.52 ^{c}
Rhizobium	0.24	0.20	0.48 ^{c}	0.39 ^{c}	0.29 ^{d}	0.24 ^{d}	0.31 ^{d}
Mean	0.52	0.43	0.63	0.52	0.69	0.57	
Cultivars	0.48 ^{b}		0.59 ^{a}		0.63 ^{a}		
Year	0.61	0.51					
Full- flowering							
Control	0.92 ^{a}	0.66 ^{b}	1.43 ^{ab}	0.56 ^{b}	0.75 ^{b}	0.98	0.88 ^{bc}
Nitrogen	0.63 ^{b}	1.44 ^{a}	1.57 ^{a}	1.69 ^{a}	0.65 ^{bc}	1.09	1.18 ^{a}
Phosphorus	1.11 ^{a}	1.01 ^{a}	0.22 ^{d}	1.07 ^{ab}	1.74 ^{a}	1.21	1.06 ^{ab}
Organic	0.29 ^{c}	0.97 ^{ab}	0.73 ^{c}	0.60 ^{b}	0.64 ^{bc}	1.33	0.76 ^{c}
Rhizobium	0.46 ^{bc}	0.86 ^{b}	1.05 ^{bc}	0.91 ^{b}	0.29 ^{c}	0.93	0.75 ^{c}
Mean	0.68 ^{c}	0.99 ^{a}	1.00 ^{a}	0.97 ^{a}	0.81 ^{b}	1.11 ^{a}	
Cultivars	0.83 ^{b}		0.98 ^{a}		0.96 ^{a}		
Year	0.83	1.02					
Post -flowering							
Control	2.61 ^{a}	1.09	1.16 ^{b}	1.25 ^{bc}	1.06	2.69 ^{a}	1.64 ^{a}
Nitrogen	1.08 ^{b}	1.09	1.82 ^{a}	2.39 ^{a}	1.30	1.77 ^{b}	1.58 ^{a}
Phosphorus	2.31 ^{a}	1.35	0.92 ^{b}	1.83 ^{ab}	0.63	2.78 ^{a}	1.64 ^{a}
Organic	0.55 ^{bc}	1.15	0.37 ^{c}	1.26 ^{bc}	0.56	2.64 ^{a}	1.09 ^{b}
Rhizobium	0.35 ^{c}	1.89	0.89 ^{b}	0.73 ^{c}	1.21	1.28 ^{b}	1.06 ^{b}
Mean	1.38 ^{b}	1.31 ^{b}	1.03 ^{b}	1.49 ^{b}	0.95 ^{c}	2.23 ^{a}	
Cultivars	1.35		1.26		1.59		
Year	1.12 ^{b}	1.68 ^{a}					
	Dry Nodule weight plant $^{-1}$ (g)						
	Filiz 99		**Eresen 87**		**Salkım**		**Mean**
Pre-flowering	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	
Control	0.041	0.034	0.011 ^{b}	0.009 ^{b}	0.076 ^{a}	0.058 ^{a}	0.038 ^{a}
Nitrogen	0.014	0.012	0.068 ^{a}	0.056 ^{a}	0.028 ^{b}	0.023 ^{b}	0.034 ^{a}
Phosphorus	0.030	0.025	0.069 ^{a}	0.057 ^{a}	0.046 ^{b}	0.037 ^{b}	0.044 ^{a}
Organic	0.045	0.037	0.014 ^{b}	0.012 ^{a}	0.017 ^{b}	0.014 ^{b}	0.023 ^{b}
Rhizobium	0.039	0.032	0.028 ^{b}	0.022 ^{a}	0.014 ^{b}	0.012 ^{b}	0.025 ^{b}
Mean	0.034	0.028	0.038	0.031	0.036	0.029	
Cultivars	0.031		0.032		0.035		
Year	0.036	0.030					
Full- flowering							
Control	0.063 ^{a}	0.103 ^{b}	0.082 ^{a}	0.079 ^{b}	0.079 ^{a}	0.185	0.099 ^{b}
Nitrogen	0.017 ^{b}	0.276 ^{a}	0.026 ^{b}	0.298 ^{a}	0.046 ^{ab}	0.158	0.137 ^{a}
Phosphorus	0.061 ^{a}	0.105 ^{b}	0.046 ^{ab}	0.181 ^{ab}	0.072 ^{ab}	0.218	0.114 ^{ab}
Organic	0.025 ^{b}	0.174 ^{ab}	0.032 ^{b}	0.111 ^{b}	0.019 ^{ab}	0.191	0.090 ^{b}
Rhizobium	0.047 ^{ab}	0.134 ^{b}	0.048 ^{ab}	0.123 ^{b}	0.013 ^{b}	0.142	0.084 ^{b}
Mean	0.043 ^{b}	0.158 ^{a}	0.047 ^{b}	0.158 ^{a}	0.046 ^{b}	0.179 ^{a}	
Cultivars	0.101		0.103		0.113		
Year	0.045 ^{b}	0.165 ^{a}					
Post -flowering							
Control	0.48 ^{b}	0.24	0.28 ^{bc}	0.38 ^{ab}	0.84 ^{a}	0.34 ^{b}	0.42 ^{b}
Nitrogen	0.26 ^{bc}	0.24	0.49 ^{b}	0.53 ^{a}	0.15 ^{c}	0.43 ^{ab}	0.35 ^{bc}

Phosphorus	0.75 a	0.30	1.51 a	0.35 bc	0.40 b	0.48 ab	0.63 a
Organic	0.16 c	0.21	0.23 c	0.33 bc	0.13 c	0.69 a	0.29 cd
Rhizobium	0.10 c	0.42	0.15 c	0.20 c	0.28 bc	0.37 b	0.25 d
Mean	0.35 b	0.28 b	0.53 a	0.36 b	0.36 b	0.462 a	
Cultivars	0.32 b		0.44 a		0.41 a		
Year	0.41	0.37					

Bacteria inoculation depressed nodulation, and the decreased nodule may be due to incompatibility of existing bacteria in soil with the inoculated Rhizobium race. Nodulation or nodule weight was increased from the pre-flowering to pod setting period, and the highest nodule weight was measured in N fertilizer and a post-flowering period where growth is maximum.

The effect of treatment on dry nodule weight plant^{-1} was significant in all periods. In pre-flowering period, dry nodule weight plant-1 ranged from 0.044 g to 0.023 g, and control (0.038 g), nitrogen (0.034 g) and phosphorus (0.044 g) fertilizer treatment were higher than organic (0.023 g) and rhizobium (0.025 g) treatments. In the full flowering period, dry nodule weight plant^{-1} ranged from 0.084 g in phosphorus to 0.137 g in nitrogen fertilizer treatment. In the post-flowering period, the highest dry nodule weight was in phosphorus fertilizer treatment. Although differences between the treatments and varieties became, phosphorus treatment was more effective on nodule quality (Table 6).

Although it is known that nitrogen generally adversely affects nodule formation, we determined that nitrogen dose and application time contributed to nodule formation in this study. Also, although some researchers reported that major decline in nitrogen fixation during reproductive growth [16], we obtained the highest fresh nodule weight in the post-flowering pod setting period. However, dry nodule weight was decreased in the post-flowering period.

The effect of treatment on fresh leaf weight plant^{-1} was significant in the full period. In the full-flowering period, fresh leaf weight plant^{-1} ranged from 16.82 g to 21.05 g, and nitrogen fertilizer treatments were higher than treatments. Cultivar × treatment interaction was significant in all periods. The effect of treatment on dry leaf weight plant^{-1} was not significant in all periods. In pre and full-flowering periods, cultivar x treatment interaction was significant in only Filiz 99, all treatments were higher than the control group in both periods and years (Table 7).

Table 7. Fertilizer treatments on fresh and dry leaf weight plant^{-1} on faba bean.

	Fresh leaf weight plant^{-1} (g)						
	Filiz 99		**Eresen 87**		**Salkım**		**Mean**
Pre-flowering	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	
Control	10.5	8.0 b	13.8 ab	10.5 ab	11.8 ab	9.0 ab	10.6
Nitrogen	12.3	9.4 ab	9.6 ab	7.3 ab	18.6 a	14.2 a	11.9
Phosphorus	10.1	7.7 b	14.6 a	11.1 a	11.5 ab	8.7 ab	10.6
Organic	10.6	8.1 b	11.8 ab	9.0 ab	10.4 b	7.9 b	9.6
Rhizobium	15.6	11.9 a	7.7 b	5.8 b	11.9 ab	9.1 ab	10.3
Mean	11.82	9.02	11.5	8.74	12.84	9.78	
Cultivars	10.4		10.1		11.3		
Year	12.0	9.2					
Full- flowering							
Control	17.5 b	11.8 b	23.3 ab	11.6	19.9 ab	16.8	16.8 b
Nitrogen	20.7 ab	22.4 a	16.2 ab	16.7	31.4 a	18.9	21.0 a
Phosphorus	17.1 b	13.4 ab	24.7 a	11.9	19.3 ab	18.6	17.5 b
Organic	18.0 b	15.0 ab	19.9 ab	16.4	17.5 b	19.9	17.8 ab
Rhizobium	26.3 a	14.9 ab	12.9 b	16.1	20.1 ab	17.2	17.9 ab
Mean	19.92	15.5	19.4	14.54	21.64	18.28	
Cultivars	17.7 b		16.9 b		20.0 a		
Year	20.3	16.11					
Post -flowering							

Control	84.0 a	43.1 ab	73.2 a	40.1 bc	76.5 b	51.7 b	61.4
Nitrogen	58.6 b	55.4 ab	80.1 a	60.3 a	97.1 a	57.1 b	68.1
Phosphorus	94.8 a	40.7 b	82.7 a	50.5 ab	57.7 c	79.4 a	67.6
Organic	100.4 a	89.5 ab	44.1 b	35.2 bc	50.9 c	30.5 c	58.4
Rhizobium	87.7 a	120.3 a	64.8 ab	24.0 c	58.8 c	35.7 c	65.2
Mean	85.1	69.8	68.98	42.02	68.2	50.88	
Cultivars	77.5 a		55.5 b		59.6 b		
Year	74.1	54.2					
	Dry leaf weight plant-1 (g)						
	Filiz 99		**Eresen 87**		**Salkım**		
Pre-flowering	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	**Mean**
Control	0.86 b	0.65 b	2.83	2.16	1.73	1.32	1.66
Nitrogen	1.70 ab	1.30 ab	1.84	1.40	2.13	1.62	1.80
Phosphorus	2.22 a	1.69 a	2.41	1.84	1.53	1.16	1.33
Organic	1.27 ab	0.97 ab	1.81	1.39	1.45	1.10	1.61
Rhizobium	2.19 a	1.17 a	1.84	1.40	1.47	1.11	
Mean	1.65	1.16	2.15	1.64	1.66	1.26	
Cultivars	1.45		1.89		1.46		
Year	1.82	1.39					
Full- flowering							
Control	1.44 b	1.57 b	4.78	1.42	2.92	2.22	2.40
Nitrogen	2.87 ab	3.21 a	3.10	2.17	3.58	2.36	2.89
Phosphorus	3.74 a	1.63 b	4.07	2.04	2.57	2.19	2.71
Organic	2.14 ab	1.98 ab	3.06	2.11	2.44	3.54	2.55
Rhizobium	3.69 a	2.37 ab	3.10	1.89	2.47	1.48	2.50
Mean	2.78	2.15	3.62	1.93	2.80	2.36	
Cultivars	2.46		2.78		2.58		
Year	3.07	2.15					
Post -flowering							
Control	14.73 ab	6.56	13.95 a	5.82	14.10 b	5.34 b	10.10
Nitrogen	11.81 c	8.68	14.23 a	5.41	17.41a	8.34 b	10.91
Phosphorus	16.80 a	6.63	7.99 b	7.21	10.97 c	12.8 a	10.44
Organic	13.69 bc	9.42	7.64 b	5.12	10.09 c	5.54 b	8.65
Rhizobium	13.97 bc	9.11	12.47 a	4.10	10.46 c	5.10 b	9.20
Mean	14.20	8.10	11.33	5.6	12.6	7.4	
Cultivars	11.14 a		8.39 c		10.02 b		
Year	12.69	7.02					

Treatments affected cultivars to varying levels; some cultivars did not respond to treatments for some years. Fekadu et al. [4] reported that the application of organic and inorganic amendments significantly increased the number of leaves, pod weight, and plant height in Ethiopia in acidic soils.

Leaf area was evaluated in only the 2019 growing season. The effect of treatments on leaf area plant-1 was significant in full and post-flowering periods. Nitrogen fertilizer treatment (582.4 cm^2 and 2015.0 cm^2, respectively) was affected highly in both periods. In the full period, control (369.6 cm^2) and rhizobium inoculation (400.2 cm^2) were low for the leaf area. In the post-flowering period, nitrogen and other treatments highly affected the leaf area compared to the control group (Table 8).

Leaf area is an important factor affecting radiation interception, transpiration and photosynthesis are important in agronomy studies for crop growth and development [10].

Table 8. Fertilizer treatments on leaf area plant-1 on faba bean.

	Leaf area plant^{-1} (cm^2)			
Pre-flowering	**Filiz 99**	**Eresen 87**	**Salkım**	**Mean**
Control	210.5	242.1 ^{ab}	208.9	220.5
Nitrogen	239.3	189.0 ^{ab}	234.2	220.8
Phosphorus	217.3	270.5 ^{a}	197.4	228.4
Organic	227.2	209.4 ^{ab}	198.7	211.8
Rhizobium	287.6	147.2 ^{b}	201.9	212.2
Mean	236.4	211.6	208.2	
Full- flowering				
Control	313.2 ^{b}	304.3 ^{c}	491.3 ^{b}	369.6 ^{c}
Nitrogen	749.5 ^{a}	460.9 ^{b}	536.9 ^{b}	582.4 ^{a}
Phosphorus	408.5 ^{b}	385.9 ^{bc}	514.4 ^{b}	436.3 ^{b}
Organic	373.7 ^{b}	466.4 ^{a}	629.2 ^{a}	489.8 ^{b}
Rhizobium	500.4 ^{ab}	300.0 ^{c}	400.3 ^{c}	400.2 ^{c}
Mean	469.1 ^{ab}	383.5 ^{b}	514.4 ^{a}	
Post –flowering				
Control	1973.2	906.3 ^{b}	1125.1 ^{d}	1334.9 ^{b}
Nitrogen	2102.0	1928.6 ^{a}	2014.4 ^{b}	2015.0 ^{a}
Phosphorus	1474.4	1648.8 ^{ab}	2481.3 ^{a}	1868.2 ^{ab}
Organic	2220.1	1284.2 ^{ab}	1256.3 ^{d}	1586.9 a^{b}
Rhizobium	1493.8	1389.9 ^{ab}	1651.9 ^{c}	1511.9 ^{ab}
Mean	1431.6	1705.8	1663.4	

The effect of treatments on fresh and dry pod weight plant-1 was significant. The highest fresh pod weight was obtained from phosphorus (82.02 g) and nitrogen (81.02 g) treatment, and the lowest value was in rhizobium inoculation (45.84 g). Dry pod weight ranged from 8.48 g to 12.66 g. phosphorus and nitrogen fertilizer increased pod weight compared to the control group, but bacteria inoculation significantly decreased fresh and dry pod weight (Table 9).

Table 9. Fertilizer treatments on fresh and dry pod weight plant-1 on faba bean.

	Fresh pod weight plant^{-1} (g)						
	Filiz 99		**Eresen 87**		**Salkım**		**Mean**
Post -flowering	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	
Control	61.26 ^{b}	53.19	124.0 ^{b}	15.98 ^{b}	64.82 ^{d}	41.71 ^{a}	60.17 ^{c}
Nitrogen	67.92 ^{b}	51.57	124.7 ^{b}	34.29 ^{a}	172.7 ^{a}	34.91 ^{ab}	81.02 ^{a}
Phosphorus	73.36 ^{b}	43.81	145.1 ^{a}	21.09 ^{b}	183.8 ^{a}	24.92 ^{bc}	82.02 ^{a}
Organic	105.1 ^{a}	47.93	90.88 ^{c}	19.90 ^{b}	131.8 ^{b}	31.75 ^{abc}	71.22 ^{b}
Rhizobium	34.58 ^{c}	49.40	67.40 ^{d}	17.93 ^{b}	84.14 ^{c}	21.56 ^{c}	45.84 ^{d}
Mean	68.44	49.18	110.42	21.84	127.45	30.97	
Cultivars	58.81 ^{b}		66.14 ^{b}		79.21 ^{a}		
Year	102.11 ^{a}	33.99 ^{b}					
	Dry pod weight plant^{-1} (g)						
Control	8.10 ^{a}	6.31	24.23 ^{ab}	2.19 ^{b}	9.09 ^{d}	4.81 ^{a}	9.12 ^{bc}
Nitrogen	9.71 ^{a}	6.56	30.97 ^{a}	4.78 ^{a}	19.55 ^{b}	4.37 ^{ab}	12.66 ^{a}
Phosphorus	9.98 ^{a}	5.32	17.35 ^{ab}	3.11 ^{b}	35.72 ^{a}	3.05 ^{ab}	12.42 ^{a}
Organic	10.0 ^{a}	6.02	15.48 ^{b}	3.13 ^{b}	21.50 ^{b}	3.94 ^{ab}	10.01 ^{b}
Rhizobium	5.15 ^{b}	6.05	21.04 ^{ab}	2.28 ^{b}	13.96 ^{c}	2.43 ^{b}	8.48 ^{c}
Mean	8.59	6.05	21.81	3.10	19.96	3.72	
Cultivars	7.32 ^{b}		12.46 ^{a}		11.84 ^{a}		
Year	16.79 ^{a}	4.29 ^{b}					

4. Conclusions

This study examined the effects of nitrogen, phosphorus, and organic and bacterial applications on root and stem characteristics of faba beans during the flowering period. Fresh and dry plant biomass, root, nodule, and leaf weight plant^{-1}, plant height, number of nodules plant$^{-1,}$ and leaf area plant^{-1} were evaluated in all periods, but fresh and dry pod weight plant^{-1} was evaluated in the post-flowering period. Fresh plant biomass, stem height, fresh and dry root weight, fresh and dry nodule weight, fresh leaf weight, and fresh and dry pod weight were responded to nitrogen fertilizer treatments depending on flowering periods. The number of nodules and fresh and dry nodule weight was affected to a different degree by both nitrogen and phosphorus fertilizer treatments. Nodule weight showed a negative response to rhizobium inoculation. The effect of organic fertilizers is not remarkable, and there is no noticeable difference in almost all traits. We determined that nitrogen (40 kg ha-^{1}) and phosphorus (80 kg ha^{-1}) fertilizer treatments on faba beans were important. However, we have not belied that fertilizer applications should not be applied for sustainable agriculture because of their high environmental efficiency.

Conflicts of Interest: The authors declare no conflict of interest.

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