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Research Article

Effect of phosphate solubilizing bacteria on rain-fed chickpea (*Cicer arietinum* L.) /dragon's head (*Lallemantia iberica*) intercropping

Fahimeh Shokrani, Jalal Jalilian*, Alireza Pirzad, Esmaeil Rezaei- Chiyaneh

Department of Plant Production and Genetics, Faculty of Agriculture, Urmia University, Urmia, Iran

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Abstract

Intercropping and biofertilizers are the effective components of sustainable agriculture that improve the yield quality and quantity of the plants. A two-year factorial field experiment was conducted to find out the effect of phosphate solubilizing bacteria (PSB) on yield, yield components and some physiological aspects of intercropping of rain-fed chickpea and dragon's head. The first factor included five intercropping patterns that were sole cropping chickpea (30 and 40 plant m^{-2}), dragon's head (160 plant m^{-2}) and additive intercropping of both plants. The second factor was the use and non-use of phosphate solubilizing bacteria (*Bacillus lentus* and *Pseudomonas putida*). The results indicated that sole cropping of 30 plants m^{-2} of chickpea and 160 plants m^{-2} of dragon's head had the maximum of 1000-seed weight, seed yield, biological yield and harvest index with PSB inoculation. In both plants, the highest leaf nitrogen (2.97 and 2.76%), total soluble carbohydrates (1.12 and 1.47 mg g^{-1} fresh leaf) and chlorophyll (3.42 and 2.94 mg g^{-1} fresh leaf) obtained from intercropping of dragon's head+30 plant m^{-2} of chickpea inoculated with PSB. The maximum values of LER (>1) for PSB-inoculated (1.72) and non- PSB (1.66) were observed in dragon's head +30 plant m^{-2} of chickpea intercropping of. In general, intercropping of dragon's head-chickpea increased yield and yield components resulted in higher land use efficiency.

Keywords: Chlorophyll, Harvest index, Land equivalent ratio, Plant Nutrients, Proline

Introduction

Water deficit stress is the most important factor limiting plant growth and productivity (Zhao *et al.*, 2019). Therefore, growing drought-resistant crops is a good way to overcome drought stress in dryland condition. Legume–medicinal plants intercropping is a new agricultural pattern in rain-fed conditions. Chickpea (*Cicer arietinum* L.) is drought tolerant and the most important legume in the world as it is rich in protein, carbohydrates, vitamins and minerals (Hussain *et al.*, 2021). The Dragon's head (*Lallemantia iberica*) is a dual-purpose plant whose cultivation for oil production is preferable to its medicinal role, capable of growing well in rain-fed conditions and containing mucilage, fiber, carbohydrates, secondary metabolites, and oil (Heydari and Pirzad, 2021).

Due to the lack of nutrients in the soil of arid and semi-arid regions and the negative effects of intensive agriculture, the use of biological fertilizers can have a beneficial effect on the uptake and supply of plant nutrients. Phosphorus deficiency is evident in most soils of arid and semi-arid regions, phosphorus deficiency reduces root water holding capacity by reducing root hydraulic conductivity (Wittenmayer and Merbach, 2005). Legume-based intercropping systems enhance the beneficial rhizobacterial community such as plant growth promoting rhizobacteria (PGPR), thereby increasing crop yield under stress conditions (Chamkhi *et al.*, 2022). Phosphate solubilizing bacteria (PSB) are PGPR that release some organic acids and dissolve insoluble forms of phosphate (Satyaprakash *et al.*, 2017).

In intercropping, two or more plant species associate during their growing season (Gitari *et al.*, 2018). Well-designed intercropping can help use inputs more imaginatively, increase water use efficiency, optimize soil moisture, reduce soil erosion and reduce weeds and pests than sole cropping (Glaze-Corcoran *et al.*, 2020). Mono-cultivation of chickpea and dragon’s head occurs in arid and semi-arid conditions; however, the relevant information on rain-fed chickpea-dragon's head intercropping is minimal. Therefore, this research was conducted to evaluate the legume-dragon’s head intercropping system and the application of phosphate soluble bacteria to improve the yield and some physiological aspects of chickpea and dragon’s head.

Materials and Methods

Experimental site and meteorological data: This two-year factorial experiment was conducted based on three-replication randomized complete block design at the farm in West Azerbaijan province, Iran (1328 m above sea level, 45°24' E, 36°57'N), during the 2012-2014 growing seasons (Figure 1).

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*Corresponding Author, Email: j.jalilian@urmia.ac.ir

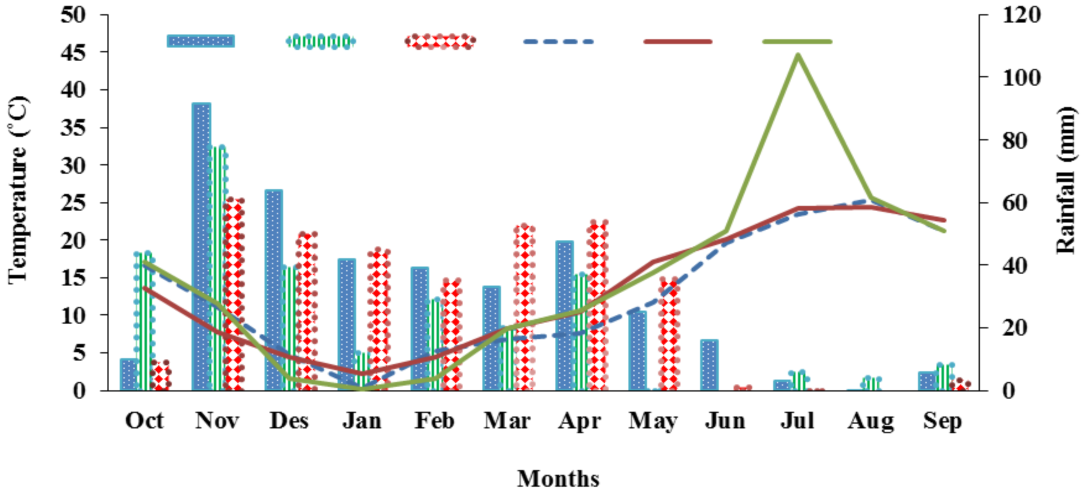

Figure 1. Average rainfall and temperature of West Azerbaijan in the 2012-2014 growing season (based on meteorological data of West Azerbaijan province).

Table 1. Physical and chemical characteristics of the soil

Soil texture	Sand (%)	Silt (%)	Clay (%)	pH	EC × 10^3 (dS m^{-1})	Total N (%)	Organic matter (%)	Available P (mg kg^{-1})	Available K (mg kg^{-1})
loam	22	35	43	7.76	0.55	0.14	0.72	10.7	262

Treatments were additive combinations of chickpea (30 and 40 plants m^{-2}) with dragon's head (160 plants m^{-2}), single cultures of each crop and bio-fertilizer treatments including inoculation with phosphate solubilizing bacteria (PSB) and control (No bio-fertilizer use). Phosphate solubilizing bacteria at 10^9 CFU (colony-forming unit) ml^{-1} are supplied by Green Biotech Ltd. (Zist Fanavar Sabz in Persian, Iran-http://greenbiotech-co.com/page/contact#), and included *Bacillus lentus* and *Pseudomonas putida* that use at a rate of 2 L ha^{-1} in shadow before planting. The ILC482 variety of chickpea and local variety dragon's head seeds were obtained from the Dryland Agricultural Research Institute (DARI) (https://dari.areeo.ac.ir/en-US/dari.areeo.ac/10535/page/Home).

Soil texture was measured using the hydrometer method (Day, 1965), soil pH determined by Carter and Gregorich, 2007 method. EC determined in 1:2.5 soil-water suspension (Okalebo *et al.*, 2002) by EC meter. Soil nitrogen analysed by the Kjeldahl method (Bremner, 1996), organic matter determined by the method of Walkley and Black (1934), P and available K determined by the standard Olsen method (Olsen, 1954) and flame photometer according to Rowell (2014), respectively (Table 1).

Twenty days before planting, tillage was carried out using a tractor. The sowing of chickpea and dragon's head was carried out simultaneously on February 25, 2013 and March 4, 2014. The chickpea and dragon's head were planted in 12 rows with 30 cm spacing between the rows. In additive intercropping, the dragon's head was planted between the rows of chickpea with 15 cm spacing between the rows. Hand control of weeds was carried out throughout the experiment. No irrigation and no chemical fertilizers were used in this study.

Yield and yield components: To determine the yield components for each crop, ten plants were randomly selected on each plot. For chickpea and dragon's head, measurements included plant height (cm), number of branches per plant and number of seeds per plant and 1000-seed weight. At physiological maturity in mid-July 2013 and 2014. To determine the biological and seed yield of the chickpea and dragon's head, 2 m^2 area from each plot were cut at the ground level and separated by hand for. The chickpea and dragon's head plants were hand harvested when yellow in late July, the harvested seeds were dried at room temperature to achieve a moisture content of 14%. The harvest index was calculated as total seed weight divided by total plant dry weight.

Land equivalent ratio (LER): LER was used as a criterion to measure the efficiency of the benefit of intercropping using the resources of the environment compared to monoculture. The land equivalent ratio of dragon's head and chickpea was calculated using Equation 1 (Willey, 1979).

$$LER = \frac{P_1}{M_1} + \frac{P_2}{M_2} \qquad (1)$$

Where P_1, P_2 represent dragon's head and chickpea yield in intercropping, respectively and M_1, M_2 Yield in sole cropping, respectively.

Measurement NPK: Seeds were dried at 75°C for 72 h to constant weight and ground using a mechanical grinder. Samples were stored in airtight plastic containers for chemical analysis. Seed nitrogen content was determined by the Kjeldahl method, seed phosphorus was determined with a spectrophotometer using a red filter at 470 nm (Jackson, 1973). For determination of seed potassium content, seed samples

(5 g) were treated as ash in an oven for 6 h at 500 ºC, the ash was dissolved in chloride acid and seed potassium content was determined by flame emission photometry (Piper, 2019).

Physiological traits: Fresh leaves were secured with aluminium foil at the flowering stage, solidified in liquid nitrogen and placed in plastic sleeves prior to storage at -80 °C. Leaf chlorophyll (a, b and total) was measured according to Lichtenthaler and Buschmann (2001), using 0.25 g fresh leaves. Samples of fresh leaves were extracted with acetone (80% v/v) in sealed tubes kept in the dark at room temperature. The upper zone of the centrifuged extraction (centrifuged at a speed of 3000 rpm for 10 minutes) was taken for reading by a spectrophotometer at wavelengths of 470, 648.8 and 663.2 nm.

For proline and total soluble carbohydrates, 0.5 g freshly harvested leaves were ground in 5 ml 95% (v/v) ethanol. The insoluble fraction of the extract was washed twice with 5 ml of 70% ethanol. All soluble fractions were centrifuged at 3500 rpm for 10 min. the supernatants were collected and stored at 4 °C for the determination of proline and TSS (total soluble carbohydrates). Proline measured with a spectrophotometer at wave length of 515 nm and total soluble carbohydrates at waves length of 625 nm (Paquin and Lechasseur, 1979).

Isolation of the essential oils of dragon's head: Air-dried flowering aerial parts of plant material (100 g) were subjected to hydro distillation using a Clevenger-type apparatus for 3 hours and then chromatographic-mass spectrometry analysis was done using an Agilent 7890/5975C (Santa Clara, CA) GC/MSD. For separation of essential oils components, and HP-5 MS capillary column (5% phenyl methyl polysiloxane, 30 m length, 0.25 mm i.d., 0.25 mm film thickness) was used. Quantification methods were the same as those reported in previous research (Rezaei-Chiyaneh *et al.*, 2021).

Statistical analysis: Combined analysis of variance (ANOVA) of the data was performed using the general linear model (GLM) procedure in the SAS software version 9.1 (SAS Institute, 2000). The mean of experimental treatments was compared using Duncan's test at a 5% level.

Results and discussion

Morpho-physiological traits of chickpea: Combined analysis of variance showed that the significant interaction between the year× intercropping patterns ×biofertilizer were observed for 1000-seed weight, seed yield, biological yield, harvest index, nitrogen content, protein yield and total soluble carbohydrates. Proline, chlorophyll-a, chlorophyll-b and total chlorophyll were significantly affected by the interaction of “intercropping patterns ×biofertilizer”. The significant effect of “year× biofertilizer” on phosphorus, proline, chlorophyll-a, chlorophyll-b and total chlorophyll were observed (Table 2).

Yield and yield components: The maximum 1000-seed weight (393.03 g), seed yield (644.07 kg ha^{-1}), biological yield (14519.9 kg ha^{-1}) and harvest index (51.13 %) of chickpea were obtained from the sole cropping of 30 plants m^{-2} under inoculum with PSB. The minimum 1000 seed weight, seed yield, biological yield and harvest index were obtained from the intercropping of dragon’s head and 40 plants m^{-2} chickpea (Table 3).

Seed yield in legumes is mainly affected by the number of pods and the number of seeds in pod. In the present study, the number of pods had an effect on seed yield. Application of PSB increased yield components of chickpea compared to control, the higher yield and yield components in sole cropping could be due to the reduction of interspecific competition in mono cropping, the decrease in yield components and seed yield of both plants in intercropping.

The low number of seeds per pod in the intercrop obtained in the study was explained by low yield compared to the yield in the sole cropping. The higher seed yield under inorganic nutrient sources could be due to the immediate release and availability of nutrients compared to the combined use of inorganic and organic sources, but can achieve yield stability in the long-term (Kumar *et al.,* 2013). The higher yield and yield components in mono-cropping could be due to the reduction in interspecific competition in mono cropping, leading to an increase in seed yield of both plants compared to other different intercropping ratios (Zabih and Saeedipour, 2015). Compared to sole cropping, intercropping decreased yield and yield components in both plant species, mainly as a result of lower plant density and higher interspecific competition for environmental resources when more than one species coexist and compete for available resources in the same environment (Gao *et al.,* 2020).

Physiological traits of chickpea: Mean comparison showed that the highest seed nitrogen content (2.97%), protein yield (94.51 kg ha^{-1}) and total soluble carbohydrates (1.12 mg g^{-1} leaf fresh weight) were obtained from the intercropping of dragon’s head and chickpea (30 plants m^{-2}) under inoculated with PSB in both years. The lowest leaf nitrogen content (2.48%), protein yield (55.91 kg ha^{-1}) and total soluble carbohydrates (0.18 mg g^{-1} leaf fresh weight) were obtained from sole cropping of 30 plants m^{-2} of chickpea and without inoculation with PSB in the first year (Table 3).

Chinthapalli *et al*. (2015) reported that the highest content of chlorophyll-a, -b and total chlorophyll were found in both faba bean and pea plants grown in soils treated with cow manure over the inorganic fertilizer. The lowest chlorophyll-a, -b and total chlorophyll were found in both faba bean and pea plants grown without fertilizer (control treatment). Mean comparisons indicated that, maximum chickpea potassium (1.17%) and minimum chickpea potassium (1.07%) were obtained from the PSB and control treatments respectively (Figure 2 A).

Table 2. Two-year variance analysis for yield, yield components and physiological traits of chickpea intercropped with dragon's head under rain fed condition

Source of variation	df	1000-SW	SY	BY	HI	N	P
Year (Y)	1	5.23	145.9	16557.7	59.78^{*}	0.02	0.0004^{*}
Replication (year)	4	91.08	825.2	1140.5	0.98	0.01	0.00004
Intercropping patterns (I)	3	14010.4^{**}	61061.9^{**}	93169.9^{**}	127.39^{**}	0.1^{**}	0.0007^{**}
Biofertilizer (F)	1	11375.9^{**}	36846.5^{**}	334751.5^{**}	33.55	0.4^{**}	0.12^{**}
Y×I	3	476.9	1142.3	25491.2^{**}	22.19	0.00	0.00017
Y×F	1	5661.2^{*}	1023.9	0.07	20.55	0.08^{**}	0.0003^{*}
F×I	3	358.77	1960.3	5908.2	9.06	0.3^{**}	0.00009
Y×I× F	3	1504.2^{**}	5186.4^{**}	17632.9^{**}	33.04^{*}	0.8^{**}	0.00014
Error	28	201.9	958.6	3185.5	10.55	0.01	0.00008
Coefficient of variation (%)		4.76	6.004	4.88	7.25	3.001	3.43

*** and ** significant at P≤0.05, P≤0.01, respectively. df= degree of freedom. 1000-SW=1000- seed weight, SY= Seed yield, BY= Biological yield, HI= Harvest index, PY= Protein yield, TSC= Total soluble carbohydrates, Chl-a= Chlorophyll-a, Chl-b= Chlorophyll-b, T Chl= Total chlorophyll.**

Continue of table 2.

Source of variation	df	K	PY	TSC	Proline	Chl-a	Chl-b	T Chl
Year (Y)	1	0.02^{*}	31.5	0.23	0.0006	21.8	0.13^{**}	25.4^{**}
Replication (year)	4	0.01^{**}	15.3	0.006	0.00001	0.07	0.005	0.10
Intercropping patterns (I)	3	0.07^{**}	837.0^{**}	0.29^{**}	0.00002^{*}	0.47^{**}	0.016^{*}	0.63^{**}
Biofertilizer (F)	1	0.11^{**}	2198^{**}	0.38^{**}	0.00003	2.41^{**}	0.22^{**}	4.11^{**}
Y×I	3	0.004	22.6	0.10^{**}	0.0001	0.26^{*}	0.05^{**}	0.098
Y×F	1	0.01	11.6	0.26^{**}	0.0012^{**}	0.66^{**}	0.019	0.46^{*}
F×I	3	0.002	77.6^{**}	0.019	0.0001	0.27^{*}	0.026^{**}	0.31^{*}
Y×I× F	3	0.002	52.6^{*}	0.047^{**}	0.00012	0.019	0.12	0.13
Error	28	0.003	12.2	0.006	0.00005	0.07	0.004	0.082
Coefficient of variation (%)		5.50	4.70	18.57	1.30	12.75	11.98	10.49

*** and ** significant at P≤0.05, P≤0.01, respectively. df= degree of freedom. 1000-SW=1000- seed weight, SY= Seed yield, BY= Biological yield, HI= Harvest index, PY= Protein yield, TSC= Total soluble carbohydrates, Chl-a= Chlorophyll-a, Chl-b= Chlorophyll-b, T Chl= Total chlorophyll.**

Table 3. Mean comparison of yield and yield components of chickpea affected by the interaction between year × intercropping patterns × biofertilizer

Year	Intercropping patterns	Biofertilizer	1000–SW (g)	SY (kg ha^{-1})	BY (kg ha^{-1})	HI (%)	N (%)	PY (kg ha^{-1})	TSC (mg g^{-1} of fresh leaf)
2013	A	Control	302.48^{c}	536.30^{bcd}	1052.6^{ef}	44.37^{bcd}	2.44^{f}	72.01^{defg}	0.24^{fg}
		PSB	321.95^{bc}	561.90^{bcd}	1104.40^{de}	45.71^{abc}	2.69^{cd}	83.01^{bc}	0.35^{def}
	B	Control	328.08^{bc}	574.17^{bc}	1143.17^{de}	50.227^{ab}	2.48^{ef}	55.91^{h}	0.18^{g}
		PSB	393.03^{a}	644.07^{a}	1451.9^{a}	51.13^{a}	2.67^{cd}	71.03^{efg}	0.31^{efg}
	C	Control	253.25^{d}	424.5^{f}	$963.10f^{g}$	44.26^{bcd}	2.39^{f}	78.33^{cd}	0.56^{bc}
		PSB	255.07^{d}	442.37^{f}	$1123.8d^{e}$	38.68^{de}	2.77^{bc}	81.78^{bc}	0.52^{bc}
	D	Control	262.18^{d}	442.37^{f}	1051.73^{ef}	44.15^{bcd}	2.76^{bcd}	66.06^{g}	0.46^{bcd}
		PSB	301.63^{c}	521.27^{cd}	1198.83^{cd}	43.56^{cde}	2.97^{a}	94.51^{a}	1.12^{a}
2014	A	Control	308.22^{c}	543.77^{bcd}	1147.60^{de}	47.22^{abc}	2.44^{f}	72.99^{def}	0.24^{fg}
		PSB	322.53^{bc}	563.07^{bcd}	1192.07^{cd}	42.18^{cde}	2.52^{ef}	78.12^{cd}	0.43^{cde}
	B	Control	315.68^{bc}	562.70^{bcd}	1293.63^{bc}	47.19^{abc}	2.48^{ef}	76.89^{cde}	0.24^{fg}
		PSB	336.68^{b}	$574.17^{b}c$	1338.40^{b}	50.99^{a}	2.62^{ef}	85.25^{b}	0.55^{bc}
	C	Control	218.93^{e}	355.8^{g}	948.5^{g}	38.69^{de}	2.61^{ed}	51.15^{h}	0.40^{cde}
		PSB	301.42^{c}	507.5^{de}	1197.67^{cd}	42.48^{cde}	2.75^{bcd}	76.7^{cde}	0.62^{b}
	D	Control	273.05^{d}	464.10^{ef}	1071.37^{e}	$43.34^{cd}e$	2.69^{cd}	$68.7f^{g}$	0.52^{bc}
		PSB	305.88^{c}	523.10^{cd}	1197.60^{cd}	43.68^{cde}	2.85^{ab}	79.9^{bc}	0.41^{cde}

The same letters at each column show the non-significant differences. A: Sole cropping of chickpea (40 plants m^{-2}), B: Sole cropping of chickpea (30 plants m^{-2}), C: Intercropping of chickpea (40 plants m^{-2}) and dragon's head (160 plants m^{-2}), D: Intercropping of chickpea (30 plants m^{-2}) and dragon's head (160 plants m^{-2}). PSB: Phosphate-solubilizing bacteria. 1000-SW=1000- seed weight, SY= Seed yield, BY= Biological yield, HI= Harvest index, PY=Protein yield, TSC= Total soluble carbohydrates

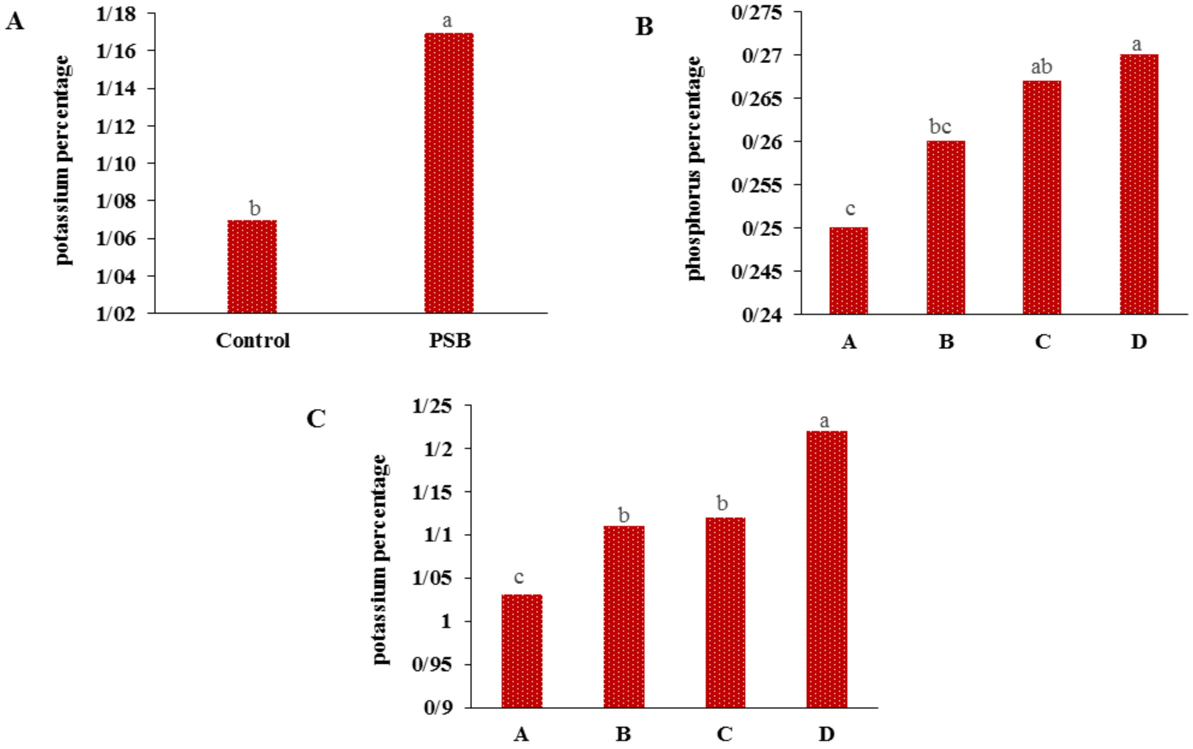

Figure 2. Means comparison for potassium (A and C) and phosphorus (B) content of chickpea seed affected by fertilizer and intercropping patterns. The same letters in each column show the non-significant differences. PSB: Phosphate-solubilizing bacteria, A: Sole cropping of chickpea (40 plants m^{-2}), B: Sole cropping of chickpea (30 plants m^{-2}), C: Intercropping of chickpea (40 plants m^{-2}) and dragon's head (160 plants m^{-2}), D: Intercropping of chickpea (30 plants m^{-2}) and dragon's head (160 plants m^{-2}).

The results showed that the content of phosphorus and potassium in intercropping are higher than sole crops. The highest phosphorus (0.27%) and potassium (1.22%) content was obtained from intercropping of dragon's head and 30 plants m^{-2} chickpea, the lowest phosphorus (0.25%) and potassium percentage (1.03%) was obtained from the sole cropping of 40 plants m^{-2} chickpea (Figure 2 B and C).

Plant growth is influenced by several factors, including soil nutrient availability, temperature, and water and light availability. Accessibility to macronutrients (e.g. N, P, and K) and micronutrients is important for physiological and biochemical activities in plants. Khan *et al*. (2014) reported that intercropping of peanut and maize improves soil nutrition and enzymatic activity to prevent soil deterioration. These results also reduce reliance on fertilizers for growth and yield. Mean comparison indicated that the maximum chlorophyll-a (2.70 mg g^{-1} fresh leaf weight), chlorophyll-b (0.66 mg g^{-1} fresh leaf weight) and total chlorophyll (3.42 mg g^{-1} fresh leaf weight) were observed in intercropping of dragon's head and 30 plants m^{-2} chickpea under inoculation with PSB, same as in intercropping of dragon's head and 40 plants m^{-2} of chickpea. The minimum levels of chlorophyll-a (1.82 mg g^{-1} fresh leaf weight), chlorophyll-b (0.45 mg g^{-1} fresh leaf weight) and total chlorophyll (2.28 mg g^{-1} fresh leaf weight) were observed in the sole cropping of chickpea (40 Plants m^{-2}) without inoculation PSB (Table 4). The biofertilizer increased the level of chlorophyll-a, -b and total chlorophyll, the increase in chlorophyll levels after the application of biofertilizer can be attributed to an increased uptake of nutrients. The amount of chlorophyll in intercropping was higher than in the sole crop.

The maximum and minimum phosphorus content (0.28% and 0.24%), proline (0.565 and 0.54 mmol g^{-1} fresh leaf weight), chlorophyll-a (3.17 and 1.59 mg g^{-1} fresh leaf weight) and total chlorophyll (3.80 and 2.21 mg g^{-1} fresh leaf weight) obtained in the first and second year and using and non-using PSB, respectively (Table 5).

Morpho-Physiological traits of dragon's head: The mean comparison showed that in both years the seed yield, chlorophyll-a and chlorophyll- b were improved by the application of bio-fertilizer in all intercropping patterns. 1000-seed weight, biological yield, harvest index, nitrogen content, and phosphorus content were affected by the interaction between intercropping patterns × bio-fertilizers. There was a significant effect between year× bio-fertilizer on biological yield and harvest index (Table 6). Mean comparison showed that the largest 1000-seed weight (5.42 g) and harvest index (20.95%) of dragon's head belonged to the sole cropping of the dragon's head in the first year. The lowest amounts of 1000-seed weight (4.26 g) and harvest index (16.44 %) of the dragon's head were obtained from intercropping of the dragon's

Table 4. Mean comparison for the chlorophyll content chickpea of chickpea affected by intercropping patterns × biofertilizer

Intercropping patterns	Fertilizer	Chl-a	Chl-b	T Chl
		(Mg g^{-1} of fresh leaf)		
A	Control	1.82^{c}	0.45^{f}	2.28^{d}
	PSB	2.24^{b}	0.64^{abc}	2.91^{bc}
B	Control	2.14^{bc}	0.49^{ef}	2.64^{cd}
	PSB	2.08^{bc}	0.57^{cde}	2.58^{cd}
C	Control	1.96^{bc}	0.50^{def}	2.47^{d}
	PSB	2.58^{a}	0.66^{ab}	3.22^{ab}
D	Control	1.81^{c}	0.59^{bcd}	2.41^{d}
	PSB	2.7^{a}	0.72^{a}	3.42^{a}

The same letters in each column show the non-significant differences. A: Sole cropping of chickpea (40 plants m-2), B: Sole cropping of chickpea (30 plants m^{-2}), C: Intercropping of chickpea (40 plants m^{-2}) and dragon's head (160 plants m^{-2}), D: Intercropping of chickpea (30 plants m^{-2}) and dragon's head (160 plants m^{-2}). PSB: Phosphate-solubilizing bacteria. Chl-a= Chlorophyll-a, Chl-b= Chlorophyll-b, T Chl=Total chlorophyll.

Table 5. Mean comparison of physiological traits of chickpea influenced by year × biofertilizer

Year	biofertilizer	Phosphorus (%)	Proline (mmol g^{-1} fresh leaf)	Chlorophyll -b (mg g^{-1} fresh leaf)	Total chlorophyll (mg g^{-1} fresh leaf)
2013	Control	0.27^{b}	0.558^{b}	2.49^{b}	3.86^{b}
	PSB	0.28^{a}	0.565^{a}	3.17^{a}	3.08^{a}
2014	Control	0.24^{c}	0.54^{c}	1.59^{c}	2.21^{c}
	PSB	0.25^{c}	0.555^{b}	1.38^{c}	1.82^{d}

The same letters in each column show the non-significant differences. PSB: Phosphate-solubilizing bacteria

Table 6. Two-year variance analysis for yield, yield components and physiological traits of dragon's head in different intercropping of chickpea under rain-fed condition

Source of variation	df	1000-SW	SY	BY	HI	N	P
Year (Y)	1	0.00	341.63	6944.4	0.49	0.28	0.032
Replication (year)	4	0.057	90.30	13111.1	1.78	0.01	0.002
Intercropping patterns (I)	2	3.59^{**}	26462.2^{**}	461519.4^{**}	30.44^{**}	0.06^{*}	0.029^{**}
Biofertilizer (F)	1	6.34^{**}	14532.3^{**}	8526400^{**}	471.32^{**}	0.31^{*}	0.080^{**}
Y×I	2	0.17^{**}	397.4	14519.4	5.57^{**}	0.064^{*}	0.02
Y×F	1	0.023	73.10	236844.4^{**}	13.76^{**}	0.030	0.005
F×I	2	1.38^{**}	369.8	54008.3^{**}	11.51^{**}	0.06^{*}	0.014^{**}
Y×I× F	2	0.003	1066.5^{*}	4019.4	3.95	0.030	0.0002
Error	20	0.034	304.2	14277.7	2.16	0.015	0.0016
Coefficient of variation (%)		3.88	5.78	6.76	7.95	4.92	10.81

*** and ** significant at P≤0.05, P≤0.01, respectively. df= degree of freedom. 1000-SW=1000- seed weight, SY= Seed yield, BY= Biological yield, HI= Harvest index, TSC= Total soluble carbohydrates, Chl-a= Chlorophyll-a, Chl-b= Chlorophyll-b, T Chl= Total chlorophyll**

Continued of table 6.

Source of variation	df	K	TSC	Proline	Chl-a	Chl-b	T Chl
Year (Y)	1	0.018	0.93	0.00001	0.34	0.44	1.08
Replication (year)	4	0.012	0.023	0.00005	0.12^{**}	0.011	0.03
Intercropping patterns (I)	2	0.049^{**}	1.83^{**}	0.00027^{**}	2.10^{**}	0.008	2.73^{**}
Biofertilizer (F)	1	0.05^{*}	0.091	0.0001	0.60^{**}	0.13^{**}	1.54^{**}
Y×I	2	0.023^{*}	0.79^{**}	0.00014	0.27	0.015	0.22^{*}
Y×F	1	0.019	0.066	0.00004	0.040	0.023	0.01
F×I	2	0.011	0.031	0.00003	0.17^{**}	0.10^{**}	0.14
Y×I× F	2	0.013	0.042	0.00014	0.12^{*}	0.057^{*}	0.10
Error	20	0.063	0.021	0.00004	0.026	0.011	0.065
Coefficient of variation (%)		5.96	20.60	1.20	10.26	18.23	11.70

*** and ** significant at P≤0.05, P≤0.01, respectively. df= degree of freedom. 1000-SW=1000- seed weight, SY= Seed yield, BY= Biological yield, HI= Harvest index, TSC= Total soluble carbohydrates, Chl-a= Chlorophyll-a, Chl-b= Chlorophyll-b, T Chl= Total chlorophyll**

head+ chickpea (40 plants m^{-2}) in the first year (Table 7). The maximum 1000 seed weight (6.10 g), biological yield (2438.3 kg ha^{-1}) and harvest index (24.83 %) were obtained from sole cropping of dragon's

Table 7. Mean comparison for yield components of dragon's head affected by the interaction between year × intercropping patterns

Year	Intercropping patterns	1000-SW (g)	HI	N	K	TSC	Chl-a	Chl-b	T Chl
			(%)			(Mg g^{-1} of fresh leaf)			
2013	A	5.22^{a}	19.21^{ab}	2.27^{b}	1.36^{b}	0.18^{e}	0.95^{d}	0.37^{e}	1.63^{c}
	B	4.26^{d}	16.44^{c}	2.60^{a}	1.28^{b}	0.39^{d}	1.30^{c}	0.46^{de}	1.72^{c}
	C	4.72^{c}	17.35^{bc}	2.57^{a}	1.40^{a}	0.65^{c}	1.54^{bc}	0.53^{cde}	2.09^{b}
2014	A	5.42^{a}	20.95^{a}	2.39^{b}	1.25^{b}	0.95^{b}	1.69^{b}	0.59^{cd}	2.41^{b}
	B	4.20^{d}	18.48^{b}	2.57^{b}	1.38^{a}	0.60^{c}	1.66^{b}	0.43^{de}	2.30^{b}
	C	4.98^{b}	18.60^{b}	2.61^{a}	1.41^{a}	1.47^{a}	2.03^{a}	0.83^{ab}	2.94^{a}

The same letters in each column, show the non-significant differences. A: Sole cropping of dragon's head, B: Intercropping of dragon's head (160 plants m^{-2}) and chickpea (40 plants m^{-2}), C: Intercropping of dragon's head (160 plants m^{-2}) and chickpea (30 plants m^{-2}). 1000-SW=1000- seed weight, HI= Harvest index, TSC= Total soluble carbohydrates, Chl-a= Chlorophyll-a, Chl-b= Chlorophyll-b, T Chl=Total chlorophyll.

head under inoculated with PSB, and the minimum of 1000 seed weight (4.13 g), biological yield (1121.6 kg ha^{-1}) and harvest index (21.61%) were obtained from intercropping of dragon's head and chickpea (40 plants m^{-2}) without PSB inoculated (Table 8). The highest amount of seed yield and biological yield of dragon's head are observed in monoculture compared with intercropping.

Results showed that the sole dragon's head produced the highest grain and biological yield. Plant performance and productivity under these conditions could be attributed to N- fixing and P- and K-solubilizing bacteria that increase nutrient availability to plants, increasing growth parameters, such as plant height and rate of photosynthesis (Nasar *et al.*, 2021).

The maximum seed yield (373.67 kg ha^{-1}) was obtained from the sole cropping of the dragon's head under inoculated with PSB. The minimum seed yield (240 kg ha^{-1}) was obtained from intercropping of dragon's head and chickpea (40 plants m^{-2}) without inoculation with PSB (Figure 3).

In intercropping with high plant density of chickpea (40 plants m^{-2}), the fertilizer had no positive effect on yield compared to the control, but in lower plant density of chickpea (30 plants m^{-2}), biofertilizer showed a positive effect on seed yield. Ghamari *et al.* (2016) reported that the highest seed yield of dragon's head and purslane was observed in monoculture with fertilizer application. In intercropping systems, competition for environmental resources (e.g. light, water, and nutrient) can reduce the yield of each individual species. The intercropping of fennel with fenugreek in a 1:2 row ratio significantly reduced fennel yield, where it recorded the lowest seed and biological yield compared to the 1:1 row ratio and sole fennel, respectively. The 1:2 row ratio intercropping produced less seed and biological yield of fennel, the maximum seed and biological yield of fennel belonged to sole cropping of fennel (Boori *et al.*, 2017).

Physiological traits of dragon's head: Mean comparison showed that the highest leaf nitrogen (2.61%), potassium (1.41%), total soluble carbohydrates (1.47 mg g^{-1} fresh leaf weight) and total chlorophyll (2.94 mg g^{-1} fresh leaf weight) were obtained from intercropping of dragon's head and chickpea 30 plants/m^2 in the second year, as in the first year.

The lowest leaf nitrogen (2.27%), potassium (1.36%), total soluble carbohydrates (0.18 mg g^{-1} fresh leaf weight) and total chlorophyll (1.63 mg g^{-1} fresh leaf weight) were obtained from the sole cropping of dragon's head in the first year (Table 7). The observed increase in available soil N, P, and K could be attributed to higher soil enzyme activities in the green garlic and cucumber intercropping system (Xiao *et al.*, 2012).

Mean comparison revealed that the maximum proline (0.545 mmol g^{-1} fresh leaf weight) was obtained in intercropping of dragon's head and 40 plants m^{-2} of chickpea, that no different with intercropping of dragon's head and 30 plants m^{-2} of chickpea. The minimal proline (0.536 mmol g^{-1} fresh leaf weight) was obtained in the sole cropping of dragon's head (Figure 4). Intercropping patterns had a significant impact on proline, which improved with increasing chickpea plant density.

Mean comparison indicated that the maximum nitrogen (2.76%) and phosphorus content (0.5%) was observed in intercropping of dragon's head and chickpea (30 plants m^{-2}) under inoculation with PSB, the minimum nitrogen (2.36%) and phosphorus content (0.30%) were observed from sole cropping of dragon's head without inoculation with PSB (Table 8). The results indicated that the content of N and P in different intercropping patterns after application of bio fertilizer in dragon's head seed were higher than in mono-cropping without bio fertilization. The accumulation of biomass and most nutrient elements (N, P, K, Ca and Mn) in intercropped cucumber was significantly greater than that found in mono cropped cucumber and the effect was even sustained to the second growing season (Xiao *et al.*, 2013). The highest chlorophyll-a (2.28 mg g^{-1} fresh leaf weight) and chlorophyll-b (0.94 mg g^{-1} fresh leaf weight) levels belonged to the intercropping of dragon's head and chickpea (30 plants m^{-2}) in the second year and inoculated with PSB. The lowest chlorophyll-a (0.95 mg g^{-1} fresh leaf weight) and chlorophyll-b (0.37 mg g^{-1} fresh leaf weight) belonged to sole cropping of dragon's head without inoculation with PSB in the first year, as well as in the second year (Table 7).

It has been reported that the chlorophyll content of

Table 8. Two-year mean comparison for yield and yield components of dragon's head affected by interaction between intercropping patterns × biofertilizer.

Intercropping patterns	Biofertilizer	1000 –SW (g)	BY (Kg ha^{-1})	HI	N	K
					(%)	
A	Control	4.54^{c}	1331.6^{c}	15.33^{c}	2.36^{b}	0.30^{c}
	PSB	6.10^{a}	2438.3^{a}	24.83^{a}	2.52^{b}	0.33^{c}
B	Control	4.13^{d}	1121.6^{d}	15.47^{c}	2.45^{b}	0.34^{c}
	PSB	4.47^{c}	1960.0^{b}	13.86^{c}	2.51^{b}	0.43^{b}
C	Control	4.33^{cd}	1388.3^{c}	19.93^{b}	2.41^{b}	0.32^{c}
	PSB	5.23^{b}	2363.3^{a}	21.61^{b}	2.76^{a}	0.50^{a}

The same letters in each column, show the non-significant differences. A: Sole cropping of dragon's head, B: Intercropping of dragon's head (160 plants m^{-2}) and chickpea (40 plants m^{-2}), C: Intercropping of dragon's head (160 plants m^{-2}) and chickpea (30 plants m^{-2}). PSB: Phosphate-solubilizing bacteria. 1000-SW=1000- seed weight, SY= Seed yield, BY= Biological yield, HI= Harvest index

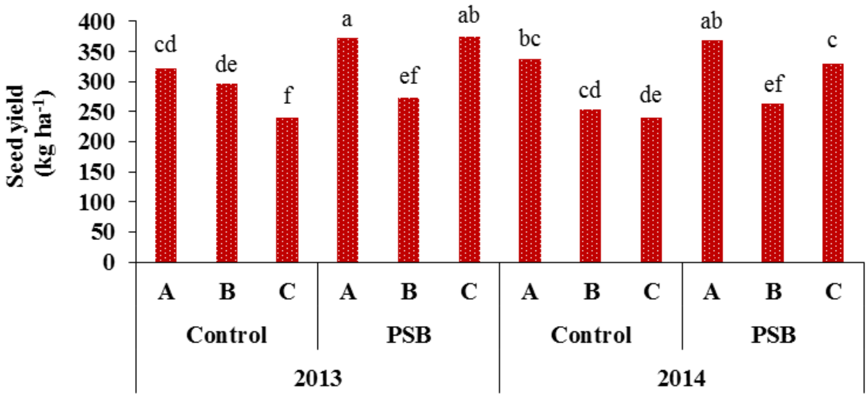

Figure 3. Mean comparison for year × intercropping patterns × biofertilizer on dragon's head seed yield. The same letters in each column, show the non-significant differences. A: Sole cropping of dragon's head, B: Intercropping of dragon's head (160 plants m^{-2}) and chickpea (40 plants m^{-2}), C: Intercropping of dragon's head (160) and chickpea (30 plants m^{-2}). PSB: Phosphate-solubilizing bacteria

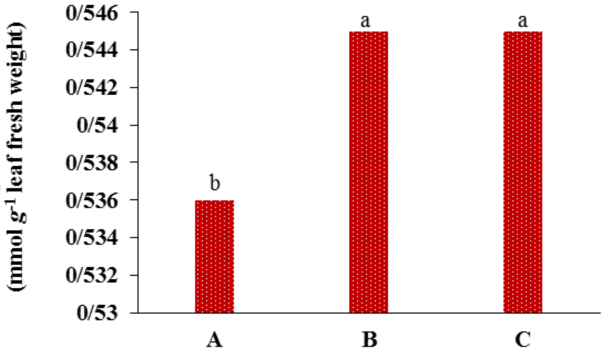

Figure 4. Mean comparison for dragon's head proline affected by intercropping patterns. The same letters in each column show the non-significant differences. A: Sole cropping of dragon's head. B: Intercropping of dragon's head (160 plants m^{-2}) and chickpea (40 plants m^{-2}). C: Intercropping of dragon's head (160 plants m^{-2}) and chickpea (30 plants m^{-2}).

pepper plants was significantly improved by garlic intercropping, which could be due to better plant nutrient availability and higher light use efficiency in intercrop treatment (Ahmad *et al.*, 2013). Machiani *et al.* (2018) reported that the accumulation of chlorophyll in the leaves of crop species grown in intercropping system was higher due to better growth and light absorption.

Essential oil constituents: According to GC-MS analyses, a total of 42 components were identified in the essential oil of dragon's head, the main constituent of dragon's head was Alpha-thujone, α-Pinene, 1-octen-3-ol, 3-octanone, α-phellandrene, Gama- Terpinene, Myrtenal, cis-Carveol and Spathulenol. Mean comparison indicated that the maximum of α-phellandren, α-Pinene and Alpha-thujone was obtained under inoculum with PSB, the minimum of α-phellandren, α-Pinene and Alpha-thujone was obtained without PSB (Table 9).

The composition of essential oils in medicinal and aromatic plants is influenced by several agricultural and environmental factors such as nutrient availability,

Table 9. Mean comparison for some constituent essential oil of dragon's head affected by biofertilizer

Biofertilizer	α-phellandren (%)	α-Pinene (%)	Alpha-thujone (%)
Control	1.06^{b}	1.08^{b}	1.05^{b}
PSB	1.40^{a}	1.48^{a}	1.34^{a}

The same letters in each column, show the non-significant differences. PSB: Phosphate-solubilizing bacteria

Table 10. Mean comparison for some constituent essential oil of dragon's head affected by intercropping patterns

Intercropping patterns	1-octen-3-ol (%)	3-octanone (%)	cis-Carveol (%)
A	1.06^{b}	0.88^{b}	1.10^{b}
B	1.38^{a}	1.50^{a}	1.86^{a}
C	1.15^{ab}	1.21^{ab}	1.45^{ab}

The same letters in each column, show the non-significant differences. A: Sole cropping of dragon's head, B: Intercropping of dragon's head (160 plants m^{-2}) and chickpea (40 plants m^{-2}), C: Intercropping of dragon's head (160 plants m^{-2}) and chickpea (30 plants m^{-2}).

Table 11. Mean comparison for some constituent essential oil of dragon's head affected by treatments

Intercropping patterns	Biofertilizer	Gama- Terpinene (%)	Myrtenal (%)	Spathulenol (%)
A	Control	0.93^{c}	0.56^{b}	4.36^{b}
	PSB	1.20^{bc}	1.10^{a}	6.0^{ab}
B	Control	1.50^{abc}	0.93^{ab}	6.66^{a}
	PSB	2.03^{a}	1.30^{a}	6.80^{a}
C	Control	1.36^{bc}	1.30^{a}	5.46^{ab}
	PSB	1.60^{ab}	1.23^{a}	4.86^{ab}

A: Sole cropping of dragon's head, B: Intercropping of dragon's head (160 plants m^{-2}) and chickpea (40 plants m^{-2}), C: Intercropping of dragon's head (160 plants m^{-2}) and chickpea (30 plants m^{-2}). PSB: Phosphate-solubilizing bacteria

Table 12. Partial land equivalent ratio (PLER) and total land equivalent ratio (LER) for seed yields of dragon's head and chickpea at intercropping patterns

Intercropping patterns	PLER of chickpea		PLER of dragon's head		Total LER	
	PSB	Control	Control	PSB	PSB	Control
A	0.80	0.72	0.72	0.73	1.53	1.44
B	0.84	0.83	0.83	0.88	1.72	1.66

A: Intercropping of dragon's head (160 plants m^{-2}) and chickpea (40 plants m^{-2}), B: Intercropping of dragon's head (160 plants m^{-2}) and chickpea (30 plants m^{-2}). PSB: Phosphate-solubilizing bacteria

metrological factors and the presence of specific pathogens (Verma *et al.*, 2016). Comparison of mean values indicated that the maximum levels of 1-octen-3-ol (1.38%), 3-octanone (1.50%) and Cis-Carveol (1.86%) were obtained in intercropping of dragon's head and chickpea (40 plants m^{-2}) (Table 10). Minimum 1-octen-3-ol (1.06%), 3-octanone (0.88%) and Cis-Carveol (1.10%) were obtained in sole cropping of dragon's head.

The maximum content of Gama- Terpinene (2.03%), Myrtenal (1.30%) and Spathulenol (6.80%) was in intercropping of dragon's head and chickpea (40 plants m^{-2}), which was similar to intercropping of dragon's head and chickpea (30 plants m^{-2}) under inoculum with PSB. The minimum levels of Gama- Terpinene (0.93%), Myrtenal (0.56%) and Spathulenol (4.36%) belonged to the sole cultivation of dragon's head without PSB (Table 11). It was also noted that the amounts of essential oil component of the dragon's head were higher in the intercropping system compared to monocultures(Verma *et al.*, 2016).

Land equivalent ratio (LER): The partial land equivalent ratio (PLER) of chickpea and dragon's head intercropping showed that most PLER of chickpea and dragon's head belonged to intercropping of dragon's head and chickpea (30 plants m^{-2}) with PSB inoculation (Table 12).

The LER was greater than one for all intercropping treatments. The maximum value of the total LER inoculated with PSB was 1.72 and the total LER without inoculated with PSB was 1.66 achieved in intercropping of dragon's head and chickpea (30 plants m^{-2}) (Table 12). From this it can be concluded that the intercropping system performed better than mono-cropping. The higher LER of the intercropping system may be related to the proper arrangement and complementary use of nutrients, water, and radiation by the components of the intercropping system.

Conclusion

However, PSB-inoculated sole cropping of both plants showed the highest yield and yield components, but the maximum LER values (1.72) were observed in the intercropping of dragon's head+30 plant m^{-2} of chickpea. Intercropping PSB-inoculated of dragon's head/chickpea by improving yield, leaf nitrogen, total soluble carbohydrates and chlorophyll in both plants, increased land use efficiency.

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Conflicts of interest

The authors declare no competing interests.

Abbreviations and symbols

PSB	phosphate solubilizing bacteria
I	Intercropping patterns
LER	Land equivalent ratio

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