



Effect of chemical and biological fertilizers on the morphology and yield of safflower and soybean under monoculture and intercropping

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: Intercropping and biofertilizers application are the most important agricultural methods for moving towards minimizing the risks of agricultural production and increasing production efficiency. Consequently, this experiment was conducted at one year and in 2019. A factorial set of treatments was arranged within randomized complete block design (RCBD) with three replications to investigate the effect of different planting ratios with safflower and soybean (sole cropping, 30:100 soybean to safflower ratio, 60:100 soybean to safflower ratio and 90:100 soybean to safflower ratio) and nutrient levels (100% urea fertilizer, 100% biofertilizer and the combined application of urea and biofertilizer) on growth and yield of these crops. The results of this study indicated that intercropping patterns had the highest plant height, number of grains per plant, biological and grain yields. In addition, the means of the number of heads per plant and the number of grains per head in safflower and the weight of 1000 grains in soybean were increased as intercrops were grown. Maximum of grain number per plant in safflower, leaf number per plant in soybean and biological and grain yields in both crops were attained in urea + biofertilizer. In all of intercropping patterns the values of LER (land equivalent ratio), RVT (relative value total) and RCC (relative crowding coefficient) was more than one, indicating an advantage from intercropping over sole crops.

1. Introduction

Intercropping, as a multiple cropping system, has been used by farmers for many years in various ways and in various countries and has acted as a very significant role in sustainable agriculture (Zhang and Li, 2003) and widely practiced for enhanced production and nutrient acquisition advantages (Ahmed *et al.*, 2020). Many studies have shown the effect of legumes on growth increase, potassium (K), phosphorus (P) and nitrogen (N) uptake, and the yield of intercropped plants compared with sole cropped plants (Tosti *et al.*, 2010; Piri *et al.*, 2011; Raei *et al.*, 2020). The

various benefits of an oilseed legume intercropping system-mainly intercropping legumes (soybeans) with oilseeds (safflowers) take in more skillful use of nutrients, available resources and sunlight, enhance yield and the improve land equivalent ratios (Srinivasarao *et al.*, 2012).

Soybean (*Glycine max* L. Merrill) is one of the most important food legumes in Iran and other parts of the world. It has potential of fixing atmospheric nitrogen besides meeting its own nitrogen requirement and serves as a viable and low cost medium for soil fertility improvement (Muoneke *et al.*, 2007). It is considerable as an important edible oil grain for human alimentation and is worldwide planted on approximately 80 million hectares (FAO, 2020). Safflower (*Carthamus tinctorius* L.) is a prospective oilseed crop because it yields 32-40% seed oil. Safflower oil is widely utilized in industry, mainly for edible and dying purposes. Owing to its considerable drought tolerance compared with other oilseed crops, safflower is usually cultivated in Iran where drought stress is a major restriction in the field. Safflower is a deep-rooted annual crop that can be grown in rotation with other species (La Bella, 2019). Nitrogen is very important for its growth and yield (Leghari *et al.*, 2016), and the nitrogen supply is therefore essential to produce a high yield from this crop. By contrast, the soybean is a legume that can fix nitrogen and release nitrogen compounds into the soil and so intercropping of these species is probably a suitable field management strategy.

A recent trend in sustainable global production is chemical fertilizers being replaced by biofertilizers. Bio-fertilizer, represent a specific complex of microorganisms which enable the movement of nutrients from soil to plants through biological process such as N fixation and solubilization of rock phosphate (Abou-Khadrah *et al.*, 2000). These fertilizers are found to have a positive contribution to soil fertility, resulting in an enhancement in crop yields without causing any environmental, water or soil pollution hazards (Timmusk *et al.*, 1999; Daiss *et al.*, 2008). This suggested that the yield to components were increasing. It was reported the nitrogen and phosphate biofertilizer applications have many important benefits and decrease the inputs of production because of cost deduction compared to chemical fertilizers which increased biological yield. In some studies, it was clearly revealed that biofertilizer application resulted in high productivity for safflower (Mirzakhani *et al.*, 2009; Seyed Sharifi, 2012).

Using biofertilizer and selection of the best microbial strains have vital role when integrating human society with vulnerable ecosystems. Biological fertilizers, which are called biofertilizers, may be used in a way of to maintain soil fertility and guarantee soil improvement. Biofertilizers are products containing living cells of different types of microorganisms, which have the ability to convert important nutritional elements (N, P ...) from unavailable to available from through biological process such as nitrogen fixation and solubilization of rock phosphate. Biofertilizers differ from chemical and organic fertilizers in that they do not directly supply any nutrients to crops and are cultures of special bacteria and fungi. Some microorganisms have positive effects on plant growth promotion, including the plant growth promoting rhizobacteria (PGPR) such as *Azospirillum* spp., *Azotobacter* spp., *Pseudomonas fluorescens*, and several Gram positive *Bacillus* spp. (Sivasakthi *et al.*, 2014).

Most studies have focused on legume-cereal intercropping as a productive and sustainable system, while intercropping systems such as soybean-safflower have rarely been evaluated. Thus, the present research was carried out to: 1) study the effect of chemical and biological fertilizers on some morphological traits and yields of safflower and soybean mono and intercropping system. 2) to evaluate the influence of cropping system on soybean and safflower performance and finally 3) to investigate the interaction between cropping system and fertilizer.

2. Materials and Methods

Site description

Field experiment was conducted during 2019 growth season at the Heris, East Azerbaijan Province, Iran (Latitude 38°25' N, Longitude 47°12' E, Altitude 1850 m above sea level with the mean annual rainfall of 315.2 mm). Some physical and chemical properties of farm soil (0-30 depth) and means of maximum and minimum temperatures and rainfall during the work in 2019 are shown in Table 1.

Experimental design and treatments

In this experiment a factorial set of treatments within randomized complete block design (RCBD) with three replications was arranged. Factors were cropping patterns (sole cropping's of safflower and soybean, intercropping of safflower/soybean with

Table 1 - Some physical and chemical properties of farm soil (a), and means of maximum and minimum temperatures and rainfall (b) during the work in 2019

Depth (cm)	EC (ds/m)	PH	Organic Carbon (%)	N (%)	P (ppm)	K (ppm)	Sand (%)	Silt (%)	Clay (%)	Soil type
0-35	1.42	8.17	1.29	0.12	51.85	2085	37	50	13	Silty loam

Month	Temperature (°C)	Rainfall (mm)
April	5.9	82.2
May	11.4	28.3
June	18.6	22.5
July	22	0.3
August	22.8	1.7
September	18.6	2.7

the ratios of 30:100, 60:100 and 90:100 soybeans to safflower and nutrient levels (100% recommended urea fertilizer, 100% biofertilizer and 50% biofertilizer + 50% urea. More in details, safflower cultivar Safe and soybean cultivar William were used. The amount of urea given in 100% treatment was 50 kg/ha. The biofertilizer contained Barvar 1 (contains free living nitrogen fixing bacteria) and Barvar 2 (contains phosphate dissolving bacteria) and was used as seed inoculation before the sowing the seeds. The biofertilizers was prepared by Zist Fanavar Sabz company, Iran. Optimum sowing density of soybean and safflower in mono cultures were 50 and 40 seeds per m², respectively.

Measurements

Plant height of two crops. At maturity stage, 5 plants of the middle part of each plot were harvested and plant height (by meter) were determined.

Leaf number per plant of safflower and soybean. Leaf number per plant was measured by hand at maturity stage.

Plant biomass of safflower and soybean. To determine plant biomass, 5 plants were harvested from middle part of each plot with considering marginal effect, then were dried in an oven at 75°C for 48 hours. Finally, plant biomass per unit area were determined.

Yield and yield components of safflower and soybean. At final ripening 5 plants were harvested and head number per plant, grain number per head, grain number per plant and 1000 grains weight were determined in safflower plants. For determine the grain yield an area equal to 1 m² was harvested from middle part of each plot considering marginal effect

grain yields per unit area were determined. Also, in soybean at maturity stage 5 plants from each plot were harvested and pods per plant, grain number per pod, grain number per plant and 1000 grains weight were determined. Grain yield per unit area was determined by threshing all the plants in 1 m² of the plots.

Evaluative indices of intercropping

Land equivalent ratio (LER). Land equivalent ratio (LER), as an agronomic index, indicates the efficiency of intercropping in using the environmental resources compared with mono cultures (Mead and Willey, 1980). The value of unity is the critical value. When the LER is greater than one, the intercropping improves the growth and yield of the cultivars. The LER was calculated as:

$$LER = (Y_{sa_i}/Y_{sa_m}) + (Y_{so_i}/Y_{so_m})$$

Where Y_{sa_m} and Y_{so_m} are the yields of safflower and soybean, respectively, as sole crops and Y_{sa_i} and Y_{so_i} are the yields of safflower and soybean, respectively, as intercrops.

Relative value total (RVT). Relative value total (RVT) as an economic index proposed by Schultz *et al.* (1982). This index is widely used now and has been used by many researchers. The RVT was calculated as:

$$RVT = (aP_1 + bP_2)/aP_1$$

Where a is the key product price, b is a secondary product price, p_1 is the main types yield and p_2 is the secondary species in the mixture. If the RVT is greater than one, it's indicating the intercropping advantage. If this index is smaller than one, it's indicating that monoculture would prefer intercropping. The critical value of RVT is one.

Relative Crowding Coefficient (RCC). RCC is a measure of the relative dominance of one species over the other in a mixture (De Wit, 1960). The RCC was calculated as:

$$RCC = (Y_{sa_i}/Y_{sa_m}) / (Y_{so_i}/Y_{so_m})$$

Where Ysa_m and Yso_m are the yields of safflower and soybean, respectively, as sole crops and Ysa_i and Yso_i are the yields of safflower and soybean, respectively, as intercrops.

If $RCC = 1$, the amount of crop in the mixture will be equal to monocropping. Also, if $RCC < 1$ indicates that the amount of the product in the mixture has decreased relative to sole crop and if $RCC > 1$, the yield of the mixture is higher than that of pure stand of crops and the mixing is beneficial.

3. Results

Analyses of variance is shown in Table 2. In safflower plants showed significant effects of cropping pattern on plant height, head number per plant, grain number per head, grain number per plant, 1000 grains weight and biological and grain yields. Also, the effect of fertilizer factor for grain number per plant, 1000 grains weight and biological and grain yields were significant. The interactions of cropping pattern \times fertilizer was only significant for 1000 grains weight. For soybean plants, analysis of variance showed significant effects of cropping pattern

on plant height, grains number per pod and per plant, 1000 grains weight, biological and grain yields. Also, leaf number per plant, grain number per pod, biological and grain yields were significantly affected by fertilizer factor.

Compared with the sole cropping of safflower and soybean, intercropping patterns had the higher plant height, grain number per plant, biological and grain yields. However, there was not observed significant difference between sole cropping and 90/100 soybean/safflower ratio for soybean traits. Similarly, the means of head number per plant and grain number per head in safflower plants and also 100 grains weight in soybean plants were increased in intercropped patterns compared with pure cultivation (Table 3).

Maximum of grain number per plant in safflower, leaf number per plant in soybean and biological and grain yields in both of these plants were attended in urea + biofertilizer. However, there was no significant differences between 100% biofertilizer and 50% urea + 50% biofertilizer in biological yields of two crops, as same as leaf number per plant in soybean (Table 4).

Maximum of 1000 grains weight of safflower in different cropping patterns was observed in cropping

Table 2 - Analysis of variance of the agronomic traits in safflower and soybean plants under different cropping patterns and fertilizer treatments

Source	df	Mean square							
		Plant height	Leaf number per plant	Head number per plant	Grain number per head	Grain number per plant	1000 grains weight	Biological yield	Grain yield
Safflower (Carthamus tinctorius L.)									
Replication	2	35426	3326	0.756	0.562	2418347	5477	944302.4	928992
Cropping pattern	3	705.209 *	11.273 NS	3.645 *	18.465 **	12574.412 **	74.811 **	8603907.64 *	5528674.70 **
Fertilizer (F)	2	55.243 NS	22.166 NS	1.441 NS	5.384 NS	7400.101 *	28.189 **	8379561.03 *	3426726.11 **
C × F	6	134.942 NS	7.165 NS	1.109 NS	3.5 NS	1464.802 NS	16.372 **	2104488 NS	722194.6 NS
Error	22	207923	11704	0.946	1.92	1495934	4058	2377343	510591.7
Cv %	-	19.34	18.72	12.27	6.88	14.43	5.98	16.26	19.49
Soybean (Glycine max L.)									
		Plant height	Leaf number per plant	Pods per plant	Grain number per pod	Grain number per plant	100 grains weight	Biological yield	Grain yield
Replication	2	17606	1245	2462	0.022	6611	0.801	147657.5	34858.45
Cropping pattern	3	76.843 **	8.261 NS	6.223 NS	0.037 *	49.205 *	6.429 **	28566822.68	4661478.68 **
Fertilizer (F)	2	28.492 NS	15.208 *	0.984 NS	0.212 **	44.766 NS	1.672 NS	1547677.03 *	511204.56 **
C × F	6	31.105 NS	0.656 NS	4.983 NS	0.039 **	32.575 NS	1.326 NS	631704 NS	14713.6 NS
Error	22	13331	3369	2584	0.01	13146	1174	414173.6	57485.11
Cv %	-	5.88	15.35	12.52	4.17	12.07	5.84	14.4	12.66

NS, * and **: non-significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Table 3 - Means of the agronomic traits in safflower and soybean plants under different cropping patterns

Traits	Plant height (cm)	Head number per plant	Grain number per head	Grain number per plant	Biological yield (kg/ha)	Grain yield (kg/ha)
<i>Safflower</i>						
Pure cultivation	63.54 b	7.044 b	18.26 c	217.6 b	8107 b	2697 b
30:100	83.11 a	7.933 ab	21.69 a	295.9 a	10330 a	4423 a
60:100	80.10 a	8.533 a	20.59 ab	296.8 a	9999 a	4166 a
90:100	71.41 ab	8.189 a	20.08 b	261.6 a	9503 ab	3382 b
<i>Soybean</i>						
Traits	Plant height (cm)		Grain number per plant	100 grains weight (g)	Biological yield (kg/ha)	Grain yield (kg/ha)
Pure cultivation	58.82 b		26.87 b	17.49 b	5654 a	2345 a
30:100	65.87 a		32.46 a	18.71 a	1983 c	912.7 c
60:100	62.51 ab		30.74 a	19.54 a	4379 b	1807 b
90:100	61.38 b		30.07 ab	18.5 ab	5858 a	2510 a

Different letters in each column indicate significant difference at $p \leq 0.05$ (Duncan test).

Table 4 - Means of the agronomic traits in safflower and soybean plants under different fertilizer treatments

Traits	Grain number per plant	Biological yield (kg/ha)	Grain yield (kg/ha)
<i>Safflower</i>			
100% urea fertilizer	252 b	8831 b	3358 b
100% biofertilizer	255.4 b	9194 ab	3359 b
50% Urea + 50% biofertilizer	296.6 a	10430 a	4284 a
<i>Soybean</i>			
Traits	Leaf number per plant	Biological yield (kg/ha)	Grain yield (kg/ha)
100% urea fertilizer	10.73 b	4122 b	1706 b
Urea + biofertilizer	12.95 a	4839 a	2115 a

Different letters in each column indicate significant difference at $p \leq 0.05$ (Duncan test).

system of 30/100 (soybean/safflower) with integration application of 50% urea + 50% biofertilizers. Minimum of this trait was related to monoculture and biofertilizer (Fig. 1a). Additionally, it was shown that the entrance of soybean plant to intercropping patterns led to an increase for 1000 grains weight in the ratio. Grain number per pod in soybean plants affected by cropping patterns and fertilizer treatments and significantly, maximum grain number per pod in different cropping patterns was observed in intercropping with 60 to 100 soybean to safflower ratio and urea + biofertilizer treatment (Fig. 1b).

Evaluation of intercropping efficiency of treatments indicated high land equivalent ratio value (LER >1) in all intercropping patterns which indicate those treatments produced biomass more efficiently than

monocropping. Maximum of LER, relative value total (RVT), and relative crowding coefficient (RCC) were achieved in 60/100 soybean/safflower ratio (Table 5).

4. Discussion and Conclusions

Some important benefits of intercropping in this research are increasing in plant performance such as plant height, grain number, 1000 grains weight, biological yield and production of grain yield per unit area compared to sole cropping (Table 3), due to the effective use of resources, including water, nutrients and solar energy (Nasri et al., 2014). This is probably due to the fact that in intercropping system plants can achieve a better absorption. Intercropping is pre-

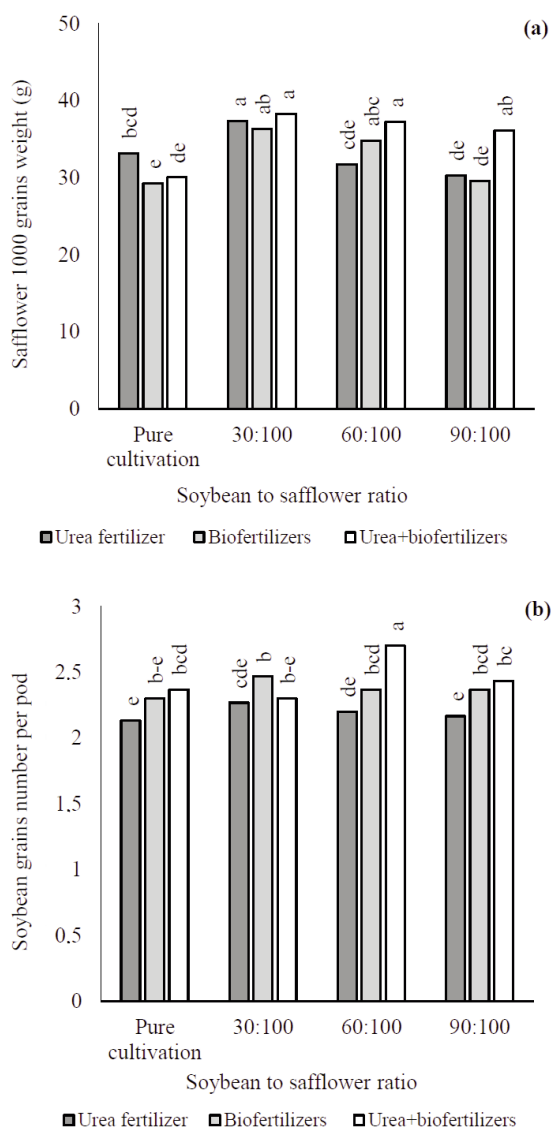


Fig. 1 - Means of safflower 1000 grains weight (a) and soybean grains number per pod (b) for interaction of cropping pattern × fertilizer treatments.

ferred to sole cropping as a result of superior yield due to better absorption of resources, and this is especially realized when legumes are used (Sachan and Uttam, 1992), because they improve soil fertility due to increased nitrogen fixation (Manna *et al.*, 2003). Intercropping of legumes (soybean) and Asteraceae (safflower) families results in increased crop yield (Table 3), maximized resource consumption and enhanced productivity of cultivation system (Singh Rajesh *et al.*, 2010). Interspecific interaction between species in the rhizosphere can also affect the nutrient availability and uptake in intercropping (Li *et al.*, 2010). Light, water and nutrients may be more completely absorbed and converted to crop

biomass by intercropping. This is a result of differences in the competitive ability for growth factors between intercrop components (Amini *et al.*, 2013).

Combined application of urea + biofertilizer significantly improved the grain number per plant in safflower and leaf number per plant in soybean plants. Also, biological and grain yields of two crops were significantly increased by this treatment (Table 4). Nitrogen is a chemical fertilizer that has an important role in enhancing the growth and yield of plants (Kulekci *et al.*, 2009). However, intensive utilization of chemical fertilizers entails several ecological issues and increases the production costs and food insecurity. Integrated plant nutrient management and irrigation are practically two elements of crop production. The application of biofertilizers is critical in the agricultural sector for sustainability of soil fertility, plant growth and development, and final yield performance (Bhardwaj *et al.*, 2014). Biofertilizers contain living cells or efficient strains of symbiotic and non-symbiotic microorganisms. These beneficial bacterial or fungal inoculants accelerate the uptake of nutrients in the rhizosphere once applied over seed and soil. Various studies have documented that plant growth-promoting rhizobacteria can promote plant growth by various mechanisms such as fixation of atmospheric nitrogen, production of siderophores that chelate metal elements and make them accessible to plant roots, solubilization of minerals such as phosphorus, and synthesis of phytohormones (Gusain *et al.*, 2015).

Results indicated that the interaction effect of cropping pattern × fertilizer treatments was significant. The introduction of soybean plants to cropping patterns resulted in the significant increase for safflower 1000 grains weight and soybean grains number per pod as integrated nutrition was applied (Fig. 1a). Intercropping with soybean can improve available nitrogen through supplementary in nutrient resources achieved from N_2 fixation (Agegnehu *et al.*, 2006). This nitrogen resource is anticipated to: (i) alleviate interference between safflower and soybean for nitrogen absorption and; (ii) increase the available nitrogen for the next crops by improving the nitrogen content of the soil after the decomposition of the leguminous debris (Hauggaard-Nielsen *et al.*, 2008).

In this study, the values of LER, as the most common agronomic index was more than one (Table 5) and used for suitability intercropping evaluate. It can be attributed to differences in traits such as rooting

depth, maximum absorption for nutrient elements and especially no significant competition for resource such as nitrogen on the basis of complementary resources due to dispute in space, time and form. Another indicator used in assessment of intercropping is RVT, which evaluate intercropping in terms of economic value. By placing the numbers associated with each parameter in the formula of this index, the economic value of each treatments of intercropping can be calculated and interpreted. In calculations of this research, the daily price of two crops was used. The value of RVT in all of intercropping patterns is more than one indicating economic superiority of intercropping over monocropping. The highest value was obtained in 60 to 100 soybean to safflower ratio (Table 5). Relative crowding coefficient (RCC) is the ability of a species to use limited resources in intercropping relative to its ability to gain the same resource in monocropping system by using yield comparing. It shows the competitive advantage of intercropping components (Snaydon, 1991) and RCC of 60 to 100 soybean to safflower ratio was higher than those of other intercropping patterns (Table 5).

Table 5 - Evaluation of intercropping efficiency of treatments

Soybean to safflower ratio	Land equivalent ratio (LER)	Relative value total (RVT)	Relative crowding coefficient (RCC)
30:100	2.02	1.95	2.9
60:100	2.31	2.17	17.4
90:100	2.18	2.13	10.16

Agronomic traits in safflower and soybean plants showed intercropping patterns and urea + biofertilizers were superiority treatments compared to other treatments. Evaluation of different treatments of intercropping by LER and RVT showed that in all the treatments the value of LER and RVT was more than one. This is due to high density of vegetation and better use of environmental resource. These results are referred to a one-time trial, therefore would need confirmations and more in deep investigation.

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