



Full Length Article

NPK Nutrient Source Influence on Performance and Profitability of Intercropped Safflower (*Carthamus tinctorius*) with Fenugreek (*Trigonella foenum-graecum*)

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Abstract

Intercropping and types of fertilizers may influence the productivity and quality of crops. An experiment was carried out to assess how chemical and biological fertilizers impact the yield and economic outcomes of safflower plants grown in monoculture and intercropped with fenugreek. Six fertilizer treatments, including no fertilizer (control); 100% soil test recommended nitrogen (N) in the form of urea; nitrogen, phosphorus, and potassium (NPK) solution spray; phosphorus biofertilizer (Barvar 2 phosphate) + 100% urea; Barvar 2 phosphate + 50% urea; and Barvar 2 phosphate + NPK spray, were evaluated in a randomized complete block design (RCB) with three replications. The results indicated that safflower monocultures fertilized with urea produced significantly higher grain (332.6 g/m²) and oil yield (85.18 g/m²) than intercropped plots. The Barvar 2 phosphate + NPK treatment in monoculture and the Barvar 2 phosphate + 100% recommended urea treatment in intercrop yielded the most seeds per unit area. The foliar application of NPK fertilizer resulted in the highest biological yield (966.7 g/m²) for sole safflower. Each intercropping pattern had a land equivalent ratio (LER) higher than one. During 2021 and 2022, the most effective treatments involved using Barvar 2 phosphate fertilizer with 100% urea (LER = 2.16), as well as Barvar 2 phosphate with 50% soil test recommended urea in mixed cultivation (LER = 2.7). Furthermore, the relative yield total (RVT) values exceeded one in the mixed cropping treatments that received Barvar 2 phosphate fertilizer with 50% urea. Finally, the results indicated that combining safflower and fenugreek planting with the application of chemical and biological fertilizers effectively increased grain yield, oil yield, and oil content in safflower plants. The findings of this study pave the way for future research to explore the long-term impacts of intercropping systems on soil health and biodiversity.

Keywords: Biofertilizer; Land equivalent ratio; Oil yield; Safflower; Seed yield

Introduction

Improving yield per unit area is an effective solution for boosting agricultural production and food security (Pirzad *et al.* 2003; Mirdoraghi *et al.* 2024). Nitrogen is one of the most important factors affecting agricultural production, with nitrogen content in the dry matter of plants generally between 1% and 2%, and sometimes as high as 4% to 6%. The reaction of plants to nitrogen fertilizers is greater than that of any other nutrient (Baligar *et al.* 2001). Nitrogen affects all components of plant performance, including the number and weight of seeds. A deficiency of this element causes a decrease in the economic yield of the crop, while the presence of high amounts of it increases the susceptibility

of the plant to diseases and lodging (Leghari *et al.* 2016). The Green Revolution has brought about a significant transformation in agriculture and food supply since the 1960s. The development of high-yielding varieties and the use of chemical fertilizers and pesticides have led to a substantial increase in agricultural production worldwide. However, the increased use of chemical inputs and pesticides causes problems in various human environments, such as the reduction of genetic diversity, depletion of water and soil resources, loss of beneficial microorganisms and insects, the emergence of pest and disease activities, and decreased soil health. This leads to the contamination of food chains, reduced quality of agricultural products, and disruption of the natural functioning of the ecosystem (El-Lithy *et al.* 2014).

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One of the fundamental solutions to reduce the harmful effects of excessive use of chemical inputs and pesticides is to move towards sustainable agriculture (Stoltz *et al.* 2013; El-Lithy *et al.* 2014). In this context, important components of sustainable agriculture include mixed farming, crop rotation and the use of biological fertilizers (Sullivan 2003; Raci *et al.* 2022). Mixed cultivation is the practice of growing two or more crops simultaneously on the same land and has many advantages, such as increased efficiency of light, water and nutrient consumption (Akunda 2001; Mndzebele *et al.* 2020), reducing the negative effects of various biotic and abiotic stress factors and enhancing production per unit area (Farhad *et al.* 2014), minimizing the use of chemical fertilizers and improving control of pests, diseases and weeds (Kugbe *et al.* 2018). For example, a study on intercropping barley and vetch reported a significant yield increase in this cropping system compared to monocropping (Aynehband 2008). The authors attributed this to the better utilization of available resources by the intercropped species and improved weed control. A study on intercropping soybeans and mint found that the yield and quality of mint were higher in the intercropping system than in the monoculture system (Maffei and Mucciarelli 2003), while a study on intercropping mint and tomato also reported that intercropping improved economic yield and, consequently, the land equivalent ratio by 25% compared to monocropping (Dikr 2022).

Another effective way to reduce the use of chemical fertilizers is to pay attention to the symbiosis of plants with beneficial soil microorganisms and the use of biofertilizers (Mahanty *et al.* 2017). Biofertilizers are living microorganisms that are used as seed dressing and soil application to improve agricultural production, soil fertility, soil properties and plant growth (Ojha *et al.* 2016). Therefore, they have a lot of potential as a complementary, renewable and environmentally friendly source in plant nutrition (Mahanty *et al.* 2017). On the other hand, it has been stated that biofertilizers or chemical fertilizers alone are insufficient for sustainable agricultural production, and combinations of chemical and biofertilizers are necessary (Ajmal *et al.* 2018). A study on the intercropping of fennel and fenugreek (*Trigonella foenum*) found that the use of biofertilizers in intercropping had a positive effect on the seed and essential oil yield of both species (Ghaderimokri *et al.* 2022), but other studies with corn intercropped with either soybeans or safflower found that the combined application of biofertilizers and chemical fertilizers increased the yield of both species while reducing the overall consumption of chemical fertilizers (Naghizade *et al.* 2012; Aydin *et al.* 2021).

Safflower is an annual plant of the daisy family (Asteraceae) that is now cultivated as an important oilseed crop, producing one of the highest quality vegetable oils (Hamdan *et al.* 2009; Maghsoudi *et al.* 2024). Safflower also has valuable agronomic characteristics, such as adaptability to dry and semi-dry climates, cold tolerance,

and relative drought resistance due to its deep roots, salinity resistance, and consistent high seed oil quality (Hussain *et al.* 2016; Kouhi *et al.* 2020).

The seeds of safflower contain 25 to 45 percent oil and 12 to 24% protein, such that safflower meal plays an essential role in the diet of livestock. Additionally, the pigments in its flowers have a relatively high economic value (Menegaes and Nunes 2020). The health benefits of safflower for humans are reported to include anti-thrombosis, anti-hypoxia, anti-liver fibrosis, antioxidant, anti-aging, immune system modulator, anti-coagulant, anti-tumor, anti-pain, anti-inflammatory and anti-diabetic properties (Koyama *et al.* 2008; Mani *et al.* 2020).

There is insufficient understanding of how various NPK sources impact safflowers when intercropped with fenugreek, particularly concerning the application of biofertilizers, as highlighted in the literature review. This research aims to address the challenge of optimizing safflower production in intercropping systems, where competition for resources may impact overall plant performance and nutrient uptake compared to traditional monoculture practices, to fill the gap of findings in these areas. The present study was conducted to investigate the effects of chemical and biofertilizers on the growth, yield and biochemical characteristics of safflower under mixed cultivation with fenugreek, relative to growth, yield, and biochemistry.

Materials and Methods

A field experiment was conducted on the research farm of the Faculty of Agriculture, University of Tabriz, located 23 km east of Tabriz, during the cropping years 2021 and 2022. The geographical location of the area is at 47° 39' E longitude, 38° 07' N latitude and 1360 m altitude above sea level, situated in a cold semi-arid climate with hot summers. According to meteorological data, this area's climate is classified as steppe and semi-arid. While summer does see some rainfall, it predominantly experiences a dry season during this period. Over a span of ten years, the average minimum, mean, and maximum annual temperatures are documented as 3.3 degrees Celsius, 14 degrees Celsius and 16 degrees Celsius, respectively. Additionally, the average yearly precipitation is noted to be 271.2 mL. The average precipitation in the experimental area was 243.33 mm in 2021 and 157.22 mm in 2022. The physical and chemical characteristics of the soil at a depth of 0 to 30 cm are given in Table 1.

The experimental treatments included two crop mixtures (monoculture safflower and intercropped safflower and fenugreek), with six biological and chemical fertilizer treatments, including no fertilizer (controls), 100% urea based on soil analysis recommendations (250 kg/ha), NPK foliar application (40:40:20 kg/ha), simultaneous application of phosphorus biofertilizer (Barvar 2 phosphate, a phosphorus-solubilizing biofertilizer) (seed inoculation) + 100% urea, simultaneous application of Barvar 2 phosphate + 50% urea,

Table 1: Physical and chemical characteristics of the soil of the experimental plot¹

Sample depth	EC dS m ⁻¹	pH of saturated paste soil	Total Neutralizing Value %	Percentage of organic carbon %	Total Nitrogen %	Available phosphorus mg kg ⁻¹	Absorbable potassium mg kg ⁻¹
0-30	2.71 ± 0.11	7.79	7 ± 0.375	0.74 ± 0.11	0.07 ± 0.035	15.8 ± 2.35	410 ± 7.07
30-60	2.49 ± 0.11	7.96	7.75 ± 0.375	0.62 ± 0.11	0.06 ± 0.035	11.1 ± 2.35	400 ± 7.07
Absorbable Zinc mg kg ⁻¹		Absorbable Iron mg kg ⁻¹		Absorbable Manganese mg kg ⁻¹	sand%	silt%	clay%
0.72 ± 0.15		2.6 ± 0.25		3.8 ± 0.55	64	22	14
0.42 ± 0.15		2.1 ± 0.25		2.7 ± 0.55	66	20	14

¹soil texture (sandy loam). The recommended fertilizer/amendments were: Urea 250 kg per hectare, Iron sulfate: 100 kg per hectare, Zinc sulfate: 20 kg per hectare Manganese sulfate: 15 kg per hectare. Based on soil analysis and the Soil Lab recommendations

and Barvar 2 phosphate + NPK foliar application). The combination of two crop system and six fertilizer configurations resulted in a total of twelve treatments, which were deployed with three replicates in a randomized complete block design. The spring safflower variety was Zys Chinese, while the fenugreek variety was a local unnamed variety in the region.

A standard germination test was conducted to ensure the germination power of the seeds (Ghassemi-Golezani and Dalil 2013). Before sowing on April 14, 2021 and April 19, 2022, land preparation operations including plowing, disking, and leveling were employed. Safflower and fenugreek were sown by hand in 3 x 3 m square plots. Five planting rows oriented north-south and 50 cm apart were created in each plot, with within-row spacing in monocultures of 5 cm. In intercropping cultivation, the five north-south rows each consisted of one row of safflower and one row of fenugreek, separated by 5 cm with 5 cm within-row spacing. The distance between the plots was one meter, and the distance between the blocks was 1.5 m. The planting depth of fenugreek was 3-4 mm, and that of safflower was 5 cm. At the 3-4 leaf stage, the plants were thinned to obtain a density of ~40 plants per square meter.

Urea chemical fertilizer (46%) was applied as a starter at planting time and simultaneously with the first irrigation, according to the soil test. Barvar 2 phosphate fertilizer was applied as a seed coating, and NPK was applied as a foliar spray at the beginning of safflower flowering. Barvar 2 biofertilizer contains two types of phosphate-solubilizing bacteria from the *Pantoea agglomerans* (strain P25) and *Pseudomonas putida* (strain P13) species, which, respectively, use two mechanisms: organic acid secretion and phosphatase enzyme activity to decompose insoluble phosphorus compounds, making them absorbable by the plant (Tohidinia *et al.* 2013).

Micronutrient fertilizers recommended in the soil test were applied as a foliar spray for all treatments simultaneously with the NPK foliar spray. Irrigation was performed by flooding after planting the seeds. After that, irrigation was carried out after 65 mm evapotranspiration from the class A pan. Weeds were controlled manually when needed. Sampling was performed at the end of the growth period.

At the time of maturity, one square meter was harvested from the center of each experimental unit, eliminating edge effects to determine yield and morphological traits. From the harvested plants, 10 plants

were randomly selected and the traits measured included the number of capitula, the number of branches, the height of the plants, and the number of seeds per capitulum. From the harvested plants (both safflower and the intercropped fenugreek), 10 plants were randomly selected. The traits measured included the number of flower heads, the number of branches, the height of the plants and the number of seeds per flower head.

Biological yield was determined by weighing the dry weight of one square meter of plants after drying at 75 degrees Celsius for 24 h. To determine the weight of 1,000 seeds, three 1,000-seed samples were weighed and their average was recorded. Seed yield was obtained from an area equivalent to one square meter after air-drying the seeds and separating the chaff and straw before weighing. Yield per unit area was also obtained. The harvest index was calculated using Equation 1 (Pirzad *et al.* 2003).

$$\text{Harvest Index} = (100 * \text{Seed Yield}) / \text{Biomass Yield} \quad \text{Eq. 1}$$

Oil percentage

Ten grams of dried safflower seeds were weighed and ground using a mill. They were then packed in filter paper, weighed again, and boiled in a Soxhlet extractor with petroleum ether as the chemical solvent for 5 h. The oil was obtained, the samples were air-dried for two weeks and weighed. The oil percentage was calculated using Equation 2 (Eskandar and Ghanbari 2002). The oil yield was also calculated by multiplying seed yield by oil percentage.

$$\text{Oil percentage} = (W2 - W1) / W2 \quad \text{Eq. 2}$$

Where, W2 is the weight of the sample at the beginning of the experiment and W1 is the weight of the sample after oil extraction.

Relative yield total (RYT) and land equality ratio (LER)

The most common index for interpreting the benefits of intercropping is the land equivalent ratio (LER), which indicates the amount of land required to grow a given crop in monoculture to achieve the same yield as intercropping. When the plant density is equal in monoculture and intercropping, the numerical value of the land equivalent ratio is equal to the total relative yield and it allows for a more accurate assessment of the efficiency of the intercropping system compared to monoculture

Table 2: Mean squares of safflower characteristics affected by different treatments in 2021 and 2022

Source of Variation	df	Dry weight of flower	Number of lateral branches	Number of seeds in capitulum	Biological yield	Seed yield	Harvest Index	Oil percentage	Oil yield
Year (Y)	1	4.744 *	0.459 ns	6761.876**	65011.3721**	72331.793*	0.11**	720.544**	481.534 ns
Error 1	4	0.046	1.500	27.871	55537.81	4801.319	0.001	5.743	377.302
Density (D)	1	0.170 ns	24.209 ns	51.562 ns	270641.95**	33141.968 ns	0.0006 ns	53.062*	1513.050 **
Y × D	1	0.059 ns	0.206 ns	4.435 ns	24291.01 ns	532.794 ns	0.005 ns	0.223 ns	0.281 ns
Fertilizers (F)	5	0.134 ns	2.351 ns	36.806 ns	70594.96**	5814.122 ns	0.006*	41.479**	589.425 ns
Y × F	5	0.144 ns	4.027 ns	17.552 ns	61518.31**	3044.504 ns	0.008**	0.876 ns	230.655 ns
D × F	5	0.157 ns	2.611 ns	198.133 ns	84292.48**	10024.137 ns	0.003 ns	24.232**	1379.237 ns
Y × F × D	5	0.096 *	5.519*	174.639 **	58200.50**	2827.849*	0.005*	0.653 ns	313.637 *
Error 2	44	0.030	1.75	47.665	8698.74	862.605	0.009	13.406	116.128
CV (%)	71	19.16	21.76	16.58	14.8	14.24	13.2	12.90	18.72

Ns, *, ** non-significant, significant at 0.05 and 0.01 levels respectively, CV: Coefficient of variation

(Szumigalski and Acker 2006).

Many scientists attribute the superiority of intercropping over monoculture to the total relative yield (RYT) or land equivalent ratio (LER) (Willey 1979). These two indices are similar in terms of calculation and are obtained from Equation 3.

$$LER = RYT = RY_a + RY_b = (Y_{ab}/Y_{aa}) + (Y_{ba}/Y_{bb}) \text{ Eq. 3}$$

Y_{ab}: Yield of species a in intercropping

Y_{aa}: Yield of species a in monoculture

Y_{ba}: Yield of species b in intercropping

Y_{bb}: Yield of species b in monoculture

Values greater than one indicate the superiority of intercropping, while values less than one indicate its inadequacy compared to monoculture. If $RYT = 1$, it indicates that gains in the growth of one crop species are matched by proportional losses in the other crop species due to competition for resources. However, if $RYT > 1$, it indicates some facilitation of growth, partial competition, or a partially complementary mode between the components of the mixture. Finally, if $RYT = 2$, then there is no competition between the components of the intercrop, and there is complete complementarity between the components in the use of environmental resources because both crop species produce similar yields in the intercrop situation as they did in monoculture.

Relative Value Total (RVT)

Alternatively, the value of intercropping can be assessed based on its economic value. Therefore, to justify the economic viability of intercropping, it should be evaluated under the most favorable conditions of the monoculture of two plants. The appropriate index to achieve this goal is the total relative value, which is calculated from Equation 4 (Simmonda and Vandemeer 1989).

$$RVT = (aP_1 + bP_2) / aM_1; \text{ where } aM_1 > bM_2 \quad \text{Eq. 4}$$

In this equation, a is the price of the main product (per kilogram), b is the price of the secondary product (per kilogram), P₁ and P₂ are the yields of the main and secondary plants in intercropping, respectively and M₁ is

the yield of the more valuable crop when planted in monoculture. If RVT is greater than one, intercropping is advantageous; however, if RVT is less than one, intercropping is not advantageous (Javanshir *et al.* 2000).

The normality and homogeneity of the residuals in the data were assessed using the Kolmogorov-Smirnov and Bartlett tests, respectively. Analysis was conducted using SAS software, with means compared through the LSD test at a significance level of $P \leq 0.05$. Figures were created using Excel software.

Results

The combined ANOVA for 2021 and 2022 showed that the dry weight of flowers, the number of seeds per capitulum, seed yield, biological yield, harvest index, and oil percentage of safflower plants were significantly different between the two years of this study. Except for the oil percentage, which was higher in 2022, the other characteristics were higher in 2021 (Table 2).

Dry weight of flower

Comparison of means showed that the highest (1.703 g/flower head) and the lowest values (0.43 g/flower head) of dry flower weight were, respectively, observed in the treatment combinations of NPK application under pure safflower cultivation in 2021 and the simultaneous application of Barvar 2 phosphate fertilizer and NPK foliar application under the intercropping system in 2022 (Fig. 1).

Number of lateral branches

Maximum (8.66) and minimum (4.18) values for the number of lateral branches were related to treatment combinations of Barvar 2 phosphate fertilizer with 50% urea under pure safflower cultivation and the combined application of Barvar 2 phosphate and NPK foliar application under intercropping (Fig. 2). Additionally, it was shown that the control treatments (without fertilizer) in pure cultivation had more lateral branches than those in mixed

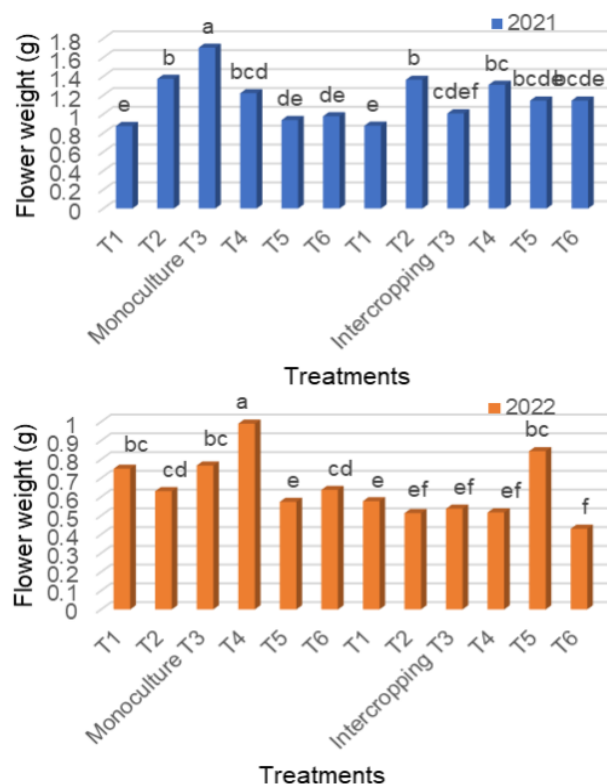


Fig. 1: Changes in dry safflower flower weight were affected by cropping pattern levels [pure safflower cultivation and mixed safflower and fenugreek cultivation under fertilizer treatments in 2021 and 2022]. Means with at least one letter in common are not statistically significantly different at the 5% probability level using the least significant difference (LSD) method. Results on the left side show safflower flower weights with different fertilizer treatments when grown in monoculture. Results on the right side show safflower flower weights with different fertilizer treatments when intercropped with fenugreek. Treatments include T1 (no fertilizer), T2 (application of 100% urea), T3 (NPK foliar application), T4 (application of Barvar 2 phosphate + 100% urea), T5 (application of Barvar 2 phosphate + 50% urea) and T6 (Barvar 2 phosphate + NPK foliar application)

cultivation.

Number of seeds in the capitulum

Results suggest that the treatment combination of Barvar 2 phosphate + NPK in pure cultivation in 2021 had the highest number of seeds at 61.80, while the treatment of Barvar 2 phosphate + 100% urea in mixed cultivation in 2022 had the highest number of seeds at 64.47 (Fig. 3). In contrast, the lowest number of seeds per plant was observed in the mixed cultivation treatment combination with NPK foliar spray at 24.94 (Fig. 3).

Seed yield

The results of the mean comparison showed that the

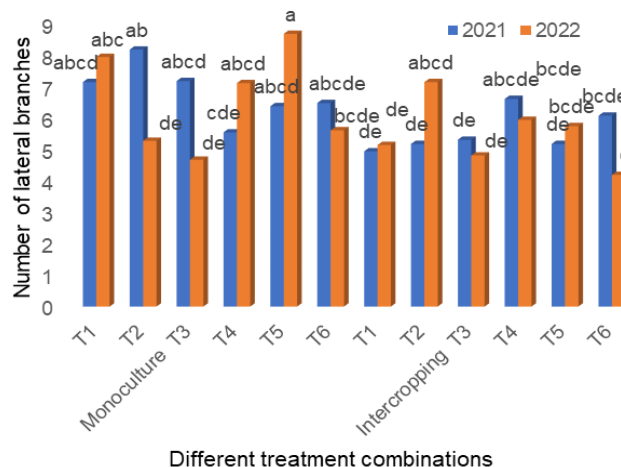


Fig. 2: Changes in the number of lateral branches affected by cropping pattern levels [pure safflower cultivation and mixed safflower and fenugreek cultivation under fertilizer treatments in 2021 and 2022]. Means with at least one letter in common are not statistically significantly different at the 5% probability level using the least significant difference (LSD) method. Results on the left side show safflower flower weights with different fertilizer treatments when grown in monoculture. Results on the right side show safflower flower weights with different fertilizer treatments when intercropped with fenugreek. Treatments include T1 (no fertilizer), T2 (application of 100% urea), T3 (NPK foliar application), T4 (application of Barvar 2 phosphate + 100% urea), T5 (application of Barvar 2 phosphate + 50% urea) and T6 (Barvar 2 phosphate + NPK foliar application)

maximum safflower seed yield (332.6 g per square meter) was achieved from pure cultivation with 100% urea treatment in 2021 and the lowest safflower seed yield (91.27 g per square meter) was obtained from the intercropping treatment with Barvar 2 phosphate fertilizer in 2022 and NPK (Fig. 4). Additionally, the yield in the pure control treatment is higher than that of the intercropping treatments. In pure cultivation, due to the lack of interspecific competition, all available resources are accessible to safflower. Therefore, under these conditions, each plant maximizes the available resources, which can be one of the factors increasing the yield of this plant per unit area. In intercropping, the conditions of intercropping safflower with fenugreek lead to increased plant density in the desired area, which heightens interspecific competition for available resources and, as a result, reduces crop yield in intercropping compared to pure cultivation.

Biological yield

Based on the results obtained, it was determined that pure safflower cultivation had the highest biological yield (total biomass) under the influence of a combination of NPK application in 2021 (966.7 g per square meter) and the lowest biological yield in the simultaneous application of Barvar 2 phosphate fertilizer and NPK foliar

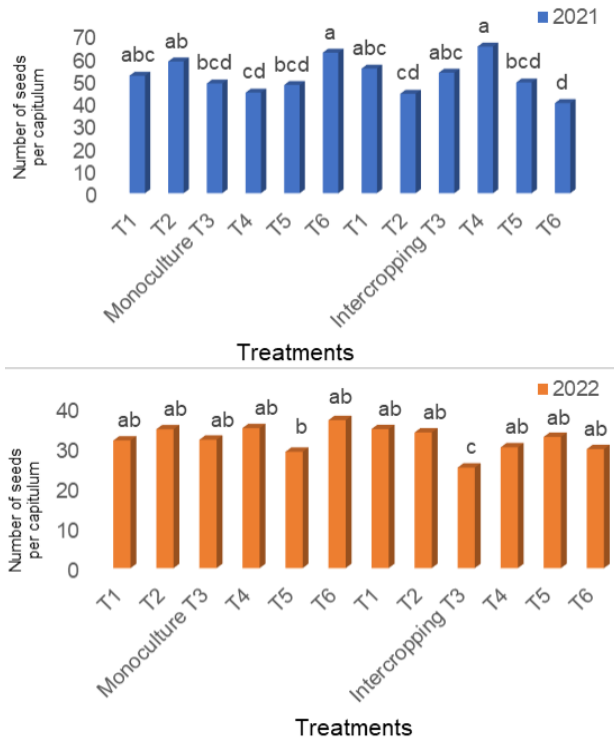


Fig. 3: Changes in the number of seeds per capitulum in the safflower plant were affected by cropping pattern levels [pure safflower cultivation and mixed safflower and fenugreek cultivation under fertilizer treatments in 2021 and 2022]. Means with at least one letter in common are not statistically significantly different at the 5% probability level, using the least significant difference (LSD) method. Results on the left side show safflower flower weights with different fertilizer treatments when grown in monoculture. Results on the right side show safflower flower weights with different fertilizer treatments when intercropped with fenugreek. Treatments include T1 (No fertilizer), T2 (application of 100% urea), T3 (NPK foliar application), T4 (application of Barvar 2 phosphate + 100% urea), T5 (application of Barvar 2 phosphate + 50% urea) and T6 (Barvar 2 phosphate + NPK foliar application)

application under intercropping conditions in 2022 (283.8 g per square meter) (Fig. 5).

Harvest index

Based on the obtained results, it was determined that the harvest index (seed mass/total plant mass) was highest in the no-fertilizer treatment under mixed cultivation conditions in 2021 (0.4595), while the lowest harvest index occurred in pure cultivation with the combination of Barvar 2 phosphate fertilizer and 50% urea under pure safflower cultivation conditions in 2022 (0.2415) (Fig. 6).

Oil percentage

It was clear that the maximum oil percentage of safflower seeds (32.58%) was achieved through mixed cultivation

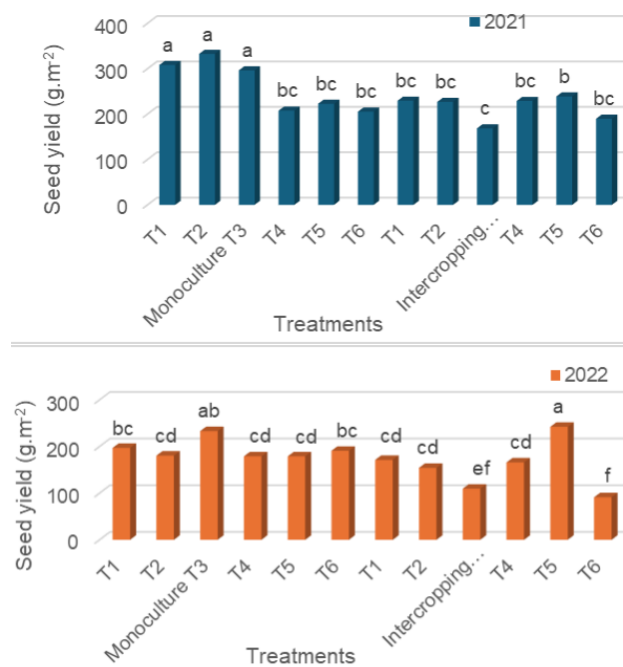


Fig. 4: Changes in seed yield per unit area of safflower were affected by cropping pattern levels [pure safflower cultivation and mixed safflower and fenugreek cultivation under fertilizer treatments in 2021 and 2022]. Means with at least one letter in common are not statistically significantly different at the 5% probability level using the least significant difference (LSD) method. Results on the left side show safflower flower weights with different fertilizer treatments when grown in monoculture. Results on the right side show safflower flower weights when intercropped with fenugreek. Treatments include T1 (No fertilizer), T2 (Application of 100% urea), T3 (NPK foliar application), T4 (Application of Barvar 2 phosphate + 100% urea), T5 (Application of Barvar 2 phosphate + 50% urea) and T6 (Barvar 2 phosphate + NPK foliar application)

with the application of Barvar 2 phosphate and NPK in 2022, while the lowest percentage (23.68%) was obtained from the pure cultivation of safflower with the application of Barvar 2 phosphate and 100% of the recommended fertilizer in 2021 (Fig. 7).

Oil yield

The results of the comparison of means showed that the maximum safflower oil yield (85.18 g.m⁻²) was obtained from the pure cultivation of this plant along with the application of 100% urea treatment, while the lowest oil yield (32.80 g.m⁻²) was obtained from mixed cultivation under the fertilizer treatment of Barvar 2 phosphate and NPK (Fig. 8). This probably indicates a high percentage of cellulose and a lower amount of oil in the embryo of the plant.

Mixed cropping evaluation

Land Equivalent Ratio (LER) and Relative Yield Total

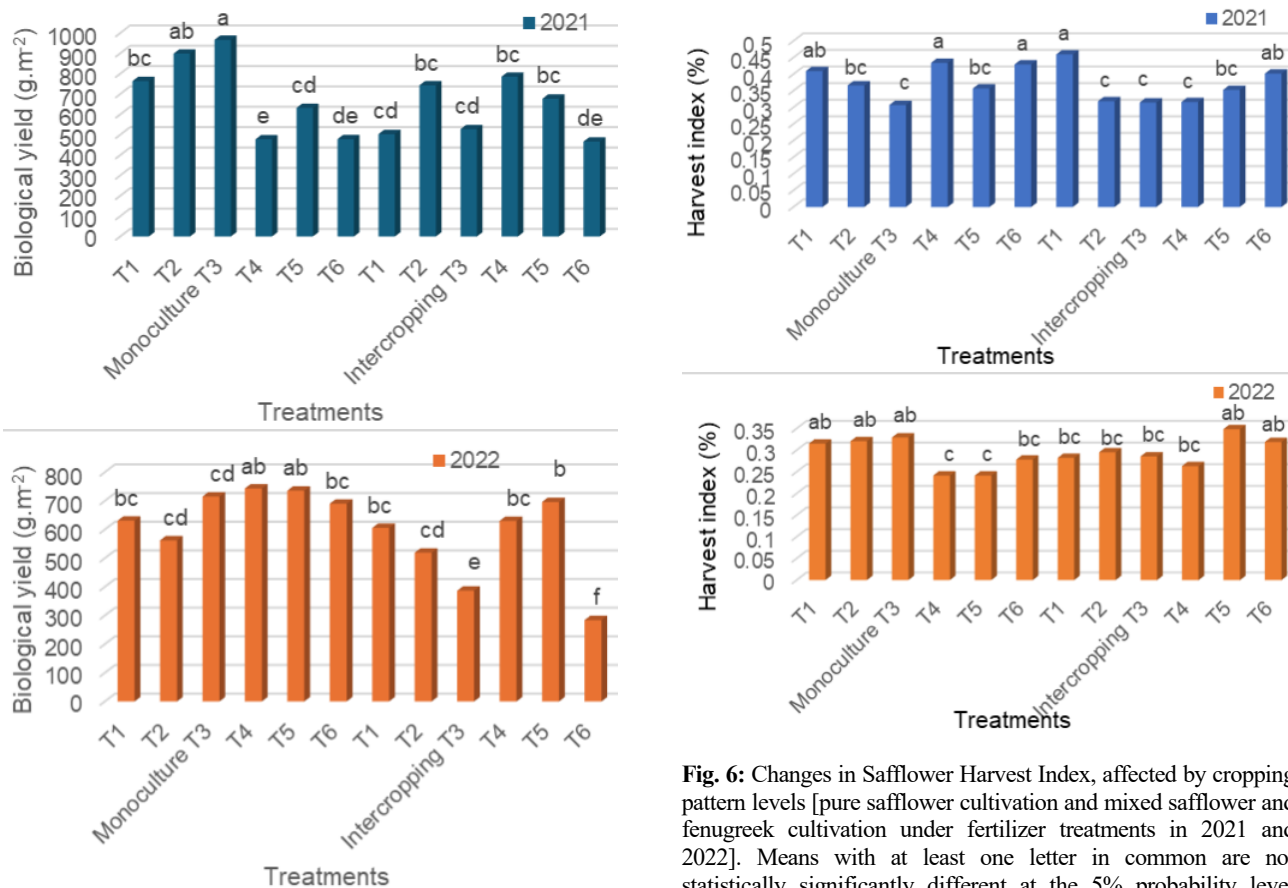


Fig. 5: Changes in the biological yield of safflower, affected by cropping pattern levels [pure safflower cultivation and mixed safflower and fenugreek cultivation under fertilizer treatments in 2021 and 2022]. Means with at least one letter in common are not statistically significantly different at the 5% probability level using the least significant difference (LSD) method. Results on the left side show safflower flower weights with different fertilizer treatments when grown in monoculture. Results on the right side show safflower flower weights with different fertilizer treatments when intercropped with fenugreek. Treatments include T1 (No fertilizer), T2 (Application of 100% urea), T3 (NPK foliar application), T4 (Application of Barvar 2 phosphate + 100% urea), T5 (Application of Barvar 2 phosphate + 50% urea) and T6 (Barvar 2 phosphate + NPK foliar application)

(RYT): As can be seen in Table 3, the land equivalent ratio of safflower for the treatments of phosphate fertilizer 2 + 100% soil-recommended urea and phosphate fertilizer 2 + 50% soil-recommended urea in 2021 was higher than one. The land equivalent ratio of fenugreek in mixed cropping combinations with fertilizer treatments of 100% urea, Barvar 2 phosphate fertilizer + 100% urea, and Barvar 2 phosphate fertilizer + 50% urea was also higher than one. In 2022, the land equivalent ratio in mixed cultivation with the fertilizer treatment of Barvar 2 phosphate fertilizer + 50% urea was greater than one. Additionally, according to Table 2, the land equivalent ratio in both years for mixed cultivation with the fertilizer treatment of Barvar 2

Fig. 6: Changes in Safflower Harvest Index, affected by cropping pattern levels [pure safflower cultivation and mixed safflower and fenugreek cultivation under fertilizer treatments in 2021 and 2022]. Means with at least one letter in common are not statistically significantly different at the 5% probability level using the least significant difference (LSD) method. Results on the left side show safflower flower weights with different fertilizer treatments when grown in monoculture. Results on the right side show safflower flower weights with different fertilizer treatments when intercropped with fenugreek. Treatments include T1 (No fertilizer), T2 (Application of 100% urea), T3 (NPK foliar application), T4 (Application of Barvar 2 phosphate + 100% urea), T5 (Application of Barvar 2 phosphate + 50% urea) and T6 (Barvar 2 phosphate + NPK foliar application)

phosphate fertilizer + 50% soil-test-recommended urea was greater than one. LER ranged from 1.34 to 2.20 in 2021 and from 1.53 to 2.09 in 2022.

In mixed cropping conditions, the application of Barvar 2 phosphate fertilizer + 50% urea increased the land equivalent ratio in both years (Table 3). Based on the average data from the two cropping years, 2021 and 2022, the highest LER belonged to the treatments of Barvar 2 phosphate fertilizer + 100% soil test-recommended urea and mixed cultivation of Barvar 2 phosphate fertilizer + 50% soil test-recommended urea, respectively. The lowest LER also belonged to mixed cultivation with the NPK fertilizer treatment in both years.

Relative Value Total (RVT): The determination of intercropping benefits is more usefully based on economic factors than simply on yield. Thus, the intercropping of two crop species should yield net incomes that are as good as or better than the monoculture of either crop (Javanshir *et al.*

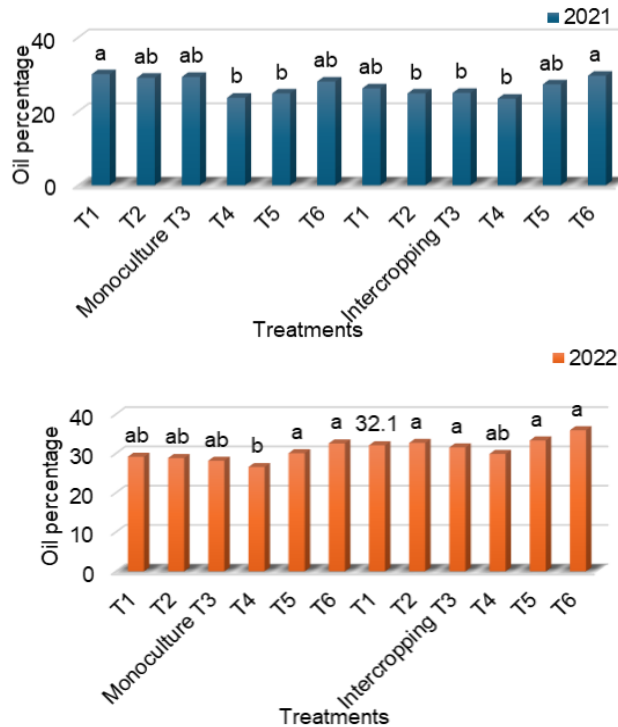


Fig. 7: Changes in safflower seed oil percentage, affected by cropping pattern levels [pure safflower cultivation and mixed safflower and fenugreek cultivation under fertilizer treatments in 2021 and 2022]. Means with at least one letter in common are not statistically significantly different at the 5% probability level using the least significant difference (LSD) method. Results on the left side show safflower flower weights with different fertilizer treatments when grown in monoculture. Results on the right side show safflower flower weights with different fertilizer treatments when intercropped with fenugreek. Treatments include T1 (No fertilizer), T2 (Application of 100% urea), T3 (NPK foliar application), T4 (Application of Barvar 2 phosphate + 100% urea), T5 (Application of Barvar 2 phosphate + 50% urea) and T6 (Barvar 2 phosphate + NPK foliar application)

2000). Based on Table 2, in both the first (2021) and second (2022) years, the RVT values in the mixed cropping treatments with the fertilizer treatment of Barvar 2 phosphate fertilizer + 50% urea were greater than one, indicating the economic profitability of these mixed cropping systems compared to monocultures. The application of nitrogen fertilizer and Barvar 2 phosphate fertilizer in mixed cropping conditions, in most cases, increased the RVT. The highest economic benefits (RVT close to or greater than 1) in the first (2021) and second (2022) years were obtained in the mixed cropping treatment with the fertilizer treatment of Barvar 2 phosphate fertilizer with 100% and Barvar 2 phosphate fertilizer with 50% urea.

Discussion

The results of the comparison of means showed that the maximum safflower seed and oil yields were obtained from

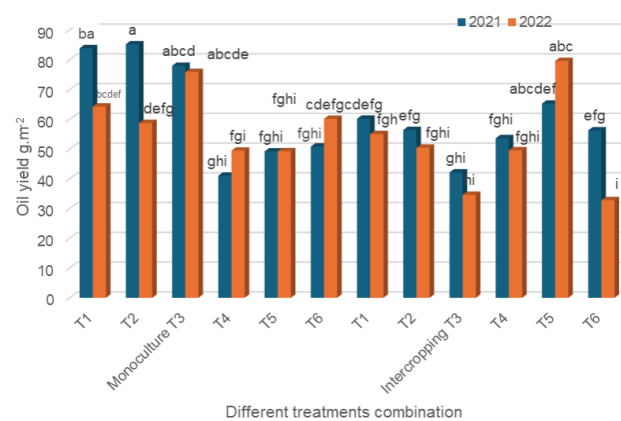


Fig. 8: Changes in safflower oil yield, affected by cropping pattern levels [pure safflower cultivation and mixed safflower and fenugreek cultivation under fertilizer treatments in 2021 and 2022]. Means with at least one letter in common are not statistically significantly different at the 5% probability level using the least significant difference (LSD) method. Results on the left side show safflower flower weights with different fertilizer treatments when grown in monoculture. Results on the right side show safflower flower weights with different fertilizer treatments when intercropped with fenugreek. Treatments include T1 (no fertilizer), T2 (application of 100% urea), T3 (NPK foliar application), T4 (application of Barvar 2 phosphate + 100% urea), T5 (application of Barvar 2 phosphate + 50% urea) and T6 (Barvar 2 phosphate + NPK foliar application)

the pure cultivation of this plant along with the application of 100% urea treatment; however, the highest land equivalent ratio was achieved under mixed cropping conditions with the fertilizer treatment of Barvar 2 phosphate fertilizer + 100% urea and 50% urea treatments. These findings showed that biofertilizers may play a very important role in intercropping systems. Except for the oil percentage, which was higher in 2022, the other characteristics were higher in 2021. These variations might be associated with the average rainfall recorded in the study area, which was 243.33 mm in 2021 and 157.22 mm in 2022.

The findings of this study underscore the significant advantages of intercropping safflower with fenugreek in enhancing crop productivity and resource efficiency. Intercropping systems, as demonstrated in this research, not only improve yields but also optimize the use of available resources, such as light, water, and nutrients, thereby promoting sustainable agricultural practices. Similar results have been observed by numerous researchers (Burgess *et al.* 2022; Wang *et al.* 2023; Chelan *et al.* 2024; Mosalman *et al.* 2024).

Previous research has shown that the increase in flower yield in *Rosa damascena* plants due to the application of complete fertilizers, poultry manure, and vermicompost can be attributed to the increased production of phytohormones, especially indole-3-acetic acid (Ahmadabadi *et al.* 2018). Applying macronutrients to plant growth and development, one of the reasons for increased

Table 3: Beneficial indicators of mixed cultivation in safflower plants¹

Year	Fertilizer treatment	Partial LER of safflower	Partial LER of fenugreek	LER	RVT	RYT
2021	No fertilizer	0.76	0.82	1.58	0.90	1.58
2021	Application of 100% urea	0.82	1.37	2.20	0.90	2.20
2021	NPK foliar application	0.48	0.86	1.34	0.82	1.34
2021	Application of Barvar 2 phosphate + 100% urea	1.09	1.07	2.16	1.16	2.16
2021	Application of Barvar 2 phosphate + 50% urea	1.1	0.82	1.90	0.94	1.90
2021	Barvar 2 phosphate + NPK foliar application	0.70	1.1	1.8	0.75	1.8
2022	No fertilizer	0.87	0.89	1.76	0.69	1.76
2022	Application of 100% urea	0.87	1.22	2.09	0.7	2.09
2022	NPK foliar application	0.47	1.05	1.53	0.45	1.53
2022	Application of Barvar 2 phosphate + 100% urea	0.92	0.94	1.86	0.67	1.86
2022	Application of Barvar 2 phosphate + 50% urea	1.38	1.3	2.7	0.97	2.7
2022	Barvar 2 phosphate + NPK foliar application	0.48	1.2	1.6	0.38	1.6

¹LER: Land Equivalent Ratio; RVT: Relative Value Total and RYT: Relative Yield Total

photosynthetic activity can be attributed to the most important photosynthetic part of the plant, namely the leaves. In this way, the use of nutrients increases the leaf area, which leads to greater flower production (Hamedi *et al.* 2019). A study showed that the use of biofertilizers enhanced the flower yield of the chamomile medicinal plant (Sanpizo *et al.* 2006; Fallahi *et al.* 2019). Another study found that the highest flower yield of German chamomile was obtained with biofertilizer inoculation (Salehi *et al.* 2016). The combined application of chemical and biological phosphorus fertilizers, Barvar 2, had the greatest effect on dry flower production in German chamomile (Alijani *et al.* 2011). Other researchers have also reported a reduction in yield compared to pure cultivation in studies on intercropping of amaranth and soybean (Valizadegan 2015), corn and bean (Nazeri *et al.* 2012) and safflower and lentil (Gangadhar *et al.* 2018). In a study on forage vetch and barley, Bingol *et al.* (2007) stated that one of the benefits of this intercropping is the possibility of achieving higher dry matter yield.

The use of Barvar 2 phosphate biofertilizer improves seedling germination and establishment, resulting in strong seedlings in the field. On the other hand, the use of urea fertilizer as a starter causes rapid seedling growth in the field and increases the production of lateral branches (Malam and Solanki 2022; Akhtar *et al.* 2023; Makaza and Khiari 2023). The increase in the number of lateral branches of safflower in different mixed cropping ratios can be attributed to having adequate space, especially in the upper third of the plant cover resulting from the mixed cropping of chickpea and safflower. It seems that the safflower plant in the ratios of safflower-chickpea (50:50 and 75:25) was more successful than other planting ratios in increasing the number of lateral branches while utilizing more sunlight. Other researchers have also reported an increase in the number of lateral branches of companion species in intercropping with chickpea (Kumar and Singh 1987).

Rajesh *et al.* (2010), in their research on intercropping cumin and lentil, came to the conclusion that the highest lentil seed yield was obtained from pure cultivation. According to studies, increasing sink potential is achieved by accumulating more dry matter at the post-anthesis stage. In this way, the balance between source and sink ensures the

highest yield of chickpeas. The higher yield of chickpeas is due to a greater number of pods and a higher number of seeds per square meter. Kumar *et al.* (2009), in a study comparing the yield of intercropping corn and beans with pure cropping, concluded that in the strip intercropping pattern, where crops are managed in different strips, an increase in mixed yield was observed compared to the pure cropping of each of the two species separately. It seems that under the conditions of intercropping safflower with fenugreek, increasing plant density in the desired area has increased interspecific competition for available resources, and as a result, has reduced crop yield in intercropping compared to pure cropping. Pandita *et al.* (2013), in their study on intercropping mung bean and corn, stated that the yield of mung bean in intercropping was lower than in pure cropping. In this experiment, increasing the plant's accessibility to nutrients, especially nitrogen and phosphorus, and the simultaneous use of chemical fertilizers along with biological fertilizers can lead to increased growth and photosynthesis due to the increase in leaf number, leaf area, and leaf area duration of the plant, which are factors that increase yield in fertilizer treatments compared to the no-fertilizer treatment. Research has shown that the use of biological fertilizers alone is not enough to meet the needs of the plant. However, if chemical and biological fertilizers are used together, the yield of these plants often increases significantly (Naghizade *et al.* 2012).

The results of this study showed that the application of chemical urea fertilizers increases seed yield. The study highlights the critical role of fertilization strategies in optimizing the performance of both crops in intercropping systems. The application of Barvar 2 phosphate biofertilizer, combined with nitrogen sources such as urea, significantly enhanced the yield and oil content of safflower. Our findings are consistent with earlier studies (Luo *et al.* 2023; Shokrani *et al.* 2024).

Moreover, the results show that the highest seed yield and oil percentage were achieved with the application of Barvar 2 phosphate with NPK in mixed cropping conditions. This emphasizes the synergistic effect of biological and chemical fertilizers, which has been supported by research indicating that biofertilizers can

improve nutrient availability and enhance plant growth by promoting beneficial microbial activity in the soil (Mahanty *et al.* 2017). This finding is consistent with the results reported for safflower by Fard and Naseri (2013). The application of nitrogen fertilizer can increase seed yield by enhancing vegetative growth, expansion and the longer duration of leaf and stem area (Zhou *et al.* 2021). In intercropping corn and safflower, it was reported that safflower seed yield in the chemical fertilizer treatment and the combined treatment of bio-fertilizer, phosphorus fertilizer and chemical fertilizer significantly increased compared to the unfertilized control. Another study found that the highest fenugreek yield was obtained from the chemical fertilizer treatment, and compared to other fertilizer treatments, cow manure, poultry manure, sheep manure and compost had relative superiority in the quantitative traits evaluated (Mohammad *et al.* 2011). Yolcu (2011) reported that, in lentil, the use of manure and zeolite improved lentil yield. The results of the experiment on intercropping corn and peanuts showed that the grain yield and biological yield of both species increased with the combined application of chemical fertilizer and manure (Kimaro *et al.* 2009). Kalkhoran *et al.* (2012) found in their experiment that the combined application of biofertilizers—Nitroxin, which contains nitrogen-fixing bacteria, manure, and chemical fertilizers—increased the biological yield of sunflower compared to the separate and control treatments. Khorramdel *et al.* (2013) reported that inoculation with biological fertilizers (Nitragin, Nitroxin and Biophosphorus) in sesame plants significantly increased seed yield. Jahan *et al.* (2010), in a study of the effects of the timing of manure application and the use of different types of biofertilizers on the quantitative and qualitative characteristics of pumpkin squash, showed that the bio-fertilizer Nitragin significantly increased fruit and seed yield.

Other researchers have also reported a decrease in biological yield in the mixed cultivation of always-spring and soybean (Valizadegan 2015) and corn and mung bean (Khorramdel *et al.* 2013) compared to pure cultivation. Miri (2007) observed in his experiment that there was no significant difference in yield between mixed and pure cropping treatments. Despite the significant differences between the treatments, the highest dry weight of vegetative organs in lentils was related to pure lentil cultivation, while the lowest dry weight was related to the strip cultivation of lentils and cumin. Considering that the structure of the plant cover resulting from mixed cultivation plays an important role in the optimal use of sunlight and consequently increases crop yield. The results of the experiment on mixed cultivation of corn and peanuts showed that the biological yield of both species increased with integrated feeding of chemical and animal manure (Zulfiqar *et al.* 2000). Khorramdel *et al.* (2013) reported that inoculation with biological fertilizers Nitragin, Nitroxin and Biophosphate in sesame plants caused a significant increase in seed yield. Jahan *et al.* (2010), in their study on the effects of the timing

of application of animal manure and the application of different types of biological fertilizers on the quantitative and qualitative characteristics of paper gourd, showed that the biological fertilizer Nitragin caused a significant increase in fruit and seed yield.

Zulfiqar *et al.* (2000) conducted a study on the mixed cultivation of beans and barley and stated that light, water, and nutrients may be completely absorbed by the plants in mixed cultivation and stored in different organs. They attributed this absorption to the differences in competitive ability between economic organs and vegetative growth among plants in mixed cultivation. When legumes are grown in mixture with other plants, the nitrogen fixed by these plants in the soil can be transferred to the companion plant in the mixture and consequently lead to an increase in its economic yield (Banik *et al.* 2006). Kushwaha and De (1987) found that the mixed cultivation of mustard and chickpea in a ratio of 66:33 had the highest number of nitrogen-fixing nodules in the soil compared to other planting ratios. Therefore, it seems that increased nutrient uptake, especially nitrogen, can be considered another effective factor in improving yield in different ratios of mixed cultivation of chickpea and safflower.

Snider *et al.* (2014) stated that small seeds usually have a lower percentage of coat than larger seeds; therefore, they have a higher percentage of oil. The increase in oil percentage in mixed cultivation compared to monoculture can be attributed to the increased nitrogen availability through biological nitrogen fixation by fenugreek. This is because nitrogen is one of the nutrients that affect the activity of plant photosynthesis enzymes (Ekrena *et al.* 2012).

Rezaei-Chiyaneh *et al.* (2021) stated that the existence of suitable conditions for the growth of cumin plants, including the availability of nitrogen in mixed cultivation with fenugreek, has led to improved growth and photosynthesis and consequently, an increase in the amount of essential oil compared to pure cultivation. Alizadeh *et al.* (2010), in a study on mixed cultivation of beans and basil, found that there was no significant difference in essential oil percentage between the treatments. Rao (2002) found that in mixed cultivation of fennel and mint, the essential oil percentage of the seed was affected by the phosphorus fertilizer used. The combination of biological and chemical fertilizers led to an increase in the essential oil percentage compared to the control treatment. Additionally, the combined treatment of biological fertilizer with chemical fertilizer produced the highest essential oil yield, while the control treatment produced the lowest. Moradzadeh *et al.* (2021) reported that the highest essential oil content of cumin was obtained in the integrated nutrient management system of chemical fertilizers and biofertilizers. Moghaddam *et al.* (2013), in a study on cumin, showed that the combined treatment of nitragin, phosphate solubilizer, and vermicompost significantly increased the essential oil percentage compared to the control.

The results of some research have shown that the use

of biofertilizers and the availability of nutrients have increased essential oil production. For example, in the research by Alijani *et al.* (2011), the highest essential oil yield of chamomile was obtained from the combined treatment of chemical phosphorus fertilizer and the biofertilizer Barvar 2. Chrysargyris *et al.* (2016) stated that the combination of nitrogen and phosphorus fertilizers has a complementary role in oil production. Eskandar and Ghanbari (2002) reported that chemical fertilizers, especially nitrogen and phosphorus, increase the oil percentage in sunflowers. Most reports support the decrease in oil percentage due to the availability of plant-available nitrogen fertilizer and this is attributed to the negative relationship between oil percentage and protein percentage. Rezaei-Chiyaneh *et al.* (2021) reported that the effect of planting pattern on essential oil yield was significant in the mixed cultivation of cumin and fenugreek. Essential oil yield is a function of seed yield and the essential oil percentage of seed. Therefore, the high essential oil yield in strip mixed cultivation with a ratio of 4 rows of cumin to 2 rows of fenugreek was due to the high seed yield and essential oil percentage in this treatment. Alizadeh *et al.* (2010), in their study of mixed cultivation of beans and sweet basil, found that essential oil yield was higher in pure and mixed cultivation of sweet basil and beans under weed control conditions than in all other treatments. Rao (2002), in mixed cultivation of fragrant geranium and mint, found that the essential oil yield of mint decreased significantly with increasing strip width from 60 to 120 cm due to the decrease in biomass yield of this plant, but the essential oil yield of fragrant geranium was not affected by the strip width.

The morphological differences between safflower and fenugreek, and consequently the creation of different layers and complementary use of resources, better utilization of light, or different soil horizons, can be reasons for the LER being greater than one. Abraham and Sinch (1984) stated that the increase in LER in mixed cropping to more than one is due to increased nitrogen uptake. Agegnehu *et al.* (2006) reported the partial LER of different bean genotypes in mixture with maize hybrid BH 14 as 0.24 – 0.09 and in mixture with maize variety Guto as 0.12 – 0.31 and also the partial LER of hybrid BH 14 and Guto maize variety in mixture with different bean genotypes as 0.82 – 0.94 and 0.77 – 0.94, respectively. According to the equal LER with relative yield total (RYT), the role of morphological differences in achieving higher LER and RYT, and consequently the profitability of mixed cropping, was reported by Yilmaz *et al.* (2008) in maize-legume intercropping, Ghosh (2004) in maize-peanut intercropping, and Hauggaard-Nielsen *et al.* (2009) in barley-pea intercropping. Hiebsch *et al.* (2002), in a study of different densities in the intercropping of maize and two soybean varieties, showed that the amount of land equivalent ratio ranged from 1.1 to 1.4 in the combination of maize with the two soybean varieties tested, and the reason for the increase in LER was fewer weeds in intercropping and stability in

the use of production resources. Hayder *et al.* (2003) also reported the range of land equivalent ratio from 1.39 to 1.52 in maize-soybean intercropping. In terms of competition, it is inferred that different plant species in proximity to each other do not compete for a particular element; in other words, the effect of interspecific competition is equal to or less than intraspecific competition. In such a situation, plants not only do not compete with each other but also complement each other. Due to the effective use of available resources, the quantity and quality of mixed cropping increase. Higher absorption of photosynthetic active radiation, water, and biological nitrogen fixation in mixed cropping can be the main reason for its higher yield than pure cropping. In other words, yield stability in mixed cropping is higher. The results indicate that intercropping safflower with fenugreek leads to a higher Land Equivalent Ratio (LER) compared to monoculture systems. This finding aligns with previous studies that have reported increased yields in intercropped systems due to better resource utilization and reduced competition for nutrients (Willey 1979; Hiebsch *et al.* 2002). Specifically, the complementary growth habits of safflower and fenugreek allow for more efficient light interception and nutrient uptake, which is critical in maximizing overall biomass and seed production.

Conclusion

The results obtained from this research showed that, in the safflower plant, the highest land equivalent ratio was achieved under mixed cropping conditions with the fertilizer treatment of Barvar 2 phosphate fertilizer, 100% urea and 50% urea. In conclusion, the integration of safflower and fenugreek in intercropping systems, coupled with optimized fertilizer applications, presents a promising avenue for enhancing agricultural productivity and sustainability. Future research should focus on long-term assessments of these intercropping systems, exploring their impacts on soil health, biodiversity, and economic sustainability across different climatic and soil conditions. This would provide valuable insights for farmers and policymakers aiming to implement sustainable agricultural practices that address food security challenges while minimizing environmental impacts.

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Author Contributions

Saeid Zehtab Salmasi: Research idea, Initial editing, final

draft and corresponding, Yaghoob Raei: Experimental design, final editing and Parisa Rostami: Experimental data, Initial draft.

Conflicts of Interest

All authors declare no conflict of interest.

Data Availability

All relevant data are within the paper and its supporting information files.

Ethics approval

Not applicable to this work.

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