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Original Article

Effects of Intercropping and Fertilizer Types on DM Yield and Medicinal Metabolites of Chicory and Fenugreek

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ABSTRACT

Intercropping of medicinal plant can increase the diversity of farming systems. It also protects the environment, water, soil and plays an important role in healthy agricultural production and human consumption. In order to investigate the effect of intercropping and fertilizer types on dry matter (DM) yield and medicinal metabolites of chicory and fenugreek, a factorial experimentwas carried out based on a randomized complete block design with three replications in an experimental farm located at Behbahan, Khuzestan province, Iran, during 2019-2020. The first factor was different fertilizer sources (Chemical, Organic and Integrated) in three levels; chemical fertilizer (urea + triple super phosphate),Vermicompost, and integrated fertilizer (50% chemical fertilizer+nitroxinbiofertilizer + fertile phosphate 2); while, the second factor was five levels of intercropping patterns, including: sole chicory (S_C), sole fenugreek (S_T), one row of chicory plus one row of fenugreek (C_1T_1), one row of chicory plus two rows of fenugreek (C_1T_2) and two rows of chicory plus one row of fenugreek (C_2T_1). The highest root dry weight (487 Kg/h), inulin content (1%) and inulin yield (4.87 Kg/h) of chicory root was obtained in the sole cultivation of chicory coupled with integrated fertilizer. The highest trigonelline content (0.48%) of fenugreek was obtained in C_2T_1 pattern coupled with chemical fertilizer application; while, the highest trigonelline yield (13.14 Kg/h) were obtained in sole cultivation of this plant. Considering the total DM yield of the two plants, the extent of medicinal actives in both plants and land equality ratio (LER) higher than one, intercropping patterns of C_1T_2, C_1T_1 treated with combined fertilizer andvermicompost were more beneficial than the sole cropping.

INTRODUCTION

Intercropping is an effective strategy for sustainable agriculture via increasing and stabilizing the yield [1]. Actually, in mixed culture, the optimal use of environmental resources such as water, light, soil, and food is attributed to the plants' height differences the way that plants' diverse aerial and underground organs are located [2]. Medicinal plants' cultivation has an essential role in farming systems' diversity, their profitability improvement, and has an important contribution to human health [3].

Chicory (*Cichorium intybus* L.) is one of the important medicinal plants of the Asteraceae family. Chicory contains polysaccharide drug compounds such as inulin, cesquiterpen, lactones, coumarins, flavonoids, shikoric acid, and vitamins with different medicinal usages [4]. Inulin has tremendous effects on human growth and health. Chicory is known as the richest source of inulin, which is cultivated in most countries of the world for this purpose [5]. Chicory can be also a pleasant and nutritious food source for livestock [6]. Feeding the Livestock with chicory does not cause bloating. Furthermore, high levels of

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minerals, water-soluble carbohydrates and the presence of condensed tannins and phenolic compounds in chicory could reduce the intestinal parasite population in livestock [7].

Fenugreek (*Trigonella foenum-graceum* L.) is an annual herb plant of the leguminous family as medicinal, forage and spice applications. Many advantages have been mentioned in traditional medicine for the fenugreek such as analgesic, anti-inflammatory, anti-cancer effects, lowering the cholesterol and blood lipids for treating the diabetes [8]. Trigonelline and nicotinic acid are the most important metabolites of fenugreek and are very effective in treating diabetes and lowering the blood cholesterol. Fenugreek grains are also used to increase animal milk and are considered as the forage plant due to their high nutritional quality [9].

Indiscriminate use of chemical fertilizers reduces the soil fertility and increases environmental pollution [10]. Nowadays, biofertilizers have been presented as suitable alternatives for chemical ones in order to increase soil fertility [11]. Microorganisms used in bio-fertilizers directly or indirectly participate in plant nutrition [1]. These microorganisms are able to increase the plant growth, root growth, shoots dry weight and grain yield through the production of hormones [12-13]. The results of previous research have revealed that biological or chemical fertilizers alone can not be useful for sustainable production of crops and in most cases; they can be used as a supplement to chemical fertilizers [14].

Vermicompost application in sustainable agriculture, in addition to providing micronutrients and consumed elements, could also lead to increasing the activity of useful soil microorganisms, organic carbon, microbial biomass and enzymatic activity, porosity and water retention capacity, plant growth hormones and organic acid production in soil and crops' growth and yield's improvement [15]. Considering the nutritional and medicinal importance of chicory and fenugreek plants, this research was carried out to investigate the effects of nitrogenous fertilizer types (biological, chemical, integrative) and mixed cropping on DM yield and medicinal metabolites of these plants in replacement intercropping system.

MATERIAL AND METHODS

Experimental Site

A field experiment was performed in growing season 2019-2020 at Agricultural Research Station at Behbahan (30º36´N latitude, 50 º14´E longitude and altitude of 320 m above sea level) , Iran. The maximum and minimum temperatures at the site were 50.6 °C (on June) and 4.33 °C (on December), respectively. The total annual rainfall during the growing season was 356.6 mm, all of which falling from November to May.

The Soil and vermicompost analysis

For the soil analysis, the soil samples were randomly collected from the depth of 0–30 cm of 10 points. The samples of the soils and vermicompost were air-dried, in the laboratory condition and were sieved to reach the particles less than 2mm, which were used for the physical and chemical analysis. Then, they were uniformly mixed and their characteristics were determined. The physicochemical properties of the soil and vermicompost characteristics are shown in table 1 and 2, respectively.

Research Method

The experiment was carried out using a factorial experiment based on randomized complete block design with three replications. The first factor was different fertilizer sources (Chemical, Organic and Integrated) in three levels; chemical fertilizer (urea+triple super phosphate),vermicompost, and integrated fertilizer (50% chemical fertilizer+nitroxinbiofertilizer + fertile phosphate 2); while, the second factor encompassed five levels of intercropping patterns. These patterns included: sole crop chicory (SC), sole crop fenugreek (S_T) and three intercropping ratios of chicory: fenugreek, (C_1T_2): one row of chicory + two rows of fenugreek, (C_1T_1): one row of chicory + one row of fenugreek and (C_2T_1): two rows of chicory + one row of fenugreek. The chicory and fenugreek seeds were provided by Pakan Bazr Company, Isfahan, Iran. After the field preparations, the land was divided into three blocks and each block was divided into 15 experimental plots. The size of each plot was 12 m^2 and consisted of eight rows. The distance between rows' spacing was 35 cm. According to the results of the soil test, the amount of nitrogen and phosphorus chemical fertilizer (in the form of urea 46% N) and triple superphosphate (44% P_2O_5), respectively, for each plant was determined based on the two stages (time of plantation and 4-6 leaf stage). The amount of vermicompost was calculated based on the nitrogen contents of the soil, the chemical fertilizers and 50% mineralization per year and then were added before sowing. Chicory and fenugreek were sown simultaneously by hand mid November and the seedlings were thinned at the 3-4 leaf stage in order to obtain optimal plant densities. The first irrigation was done immediately after the seed sowing and subsequent irrigations were performed during the growing season according to the rainfall status and water needs of the plants. Weeds were controlled by hand in several stages during the growing season.

Data Collection

DM yield and Land Equivalent Ratio (LER)

At the end of the growing season fenugreek and chicory was harvested at their maturity stages on February 29, and March 31, 2020 respectively. Harvesting was accomplished manually from the middle of each plot in 2m^2 area. In the intercropping treatments, chicory and fenugreek were harvested separately. Then, they air-dried in the shade for 14 days and DM yields were measured as Kg/h.

To evaluate the performance of chicory and fenugreek intercropping in comparison with pure culture, the land equivalent ratio (LER) indices were used according to following equations [16].

$LER = LER_a + LER_b$

$LER_a = Y_1/F_1$

$LER_b = Y_2/F_2$

Where:

LERa and LERb are chicory and fenugreek land equivalent ratio;

Y_1 and Y_2 are forage DMyield of chicory and fenugreek under monoculture conditions; and

F_1 and F_2 are dry forage of chicory and fenugreek under intercropping systems.

Measurement of root dry weight and inulin content in Chicory

Since three traits of dry root yield and inulin content and inulin yield in chicory and two traits of seed yield and trigonelline in fenugreek were not common in both species, So, These traits were separately statistically analyzed and the effects of cropping systems were studied on their variations.

After drying the aerial parts of the plants, the roots were harvested and placed in the shade for seven days and then the roots' dry weight was recorded. Saengkanuk *et al* methods with some modifications were used for anlaysing the Inulin content of the roots [17]. Anthron reagent was used to measure inulin in chicory and was measured by UV-Visible spectroscopy. In this method, the amount of inulin (as active ingredient) was measured using 0.28% anthron reagent in concentrated sulfuric acid, which is a specific carbohydrates' indicator. The formation of a color complex (green) was investigated and standard curve of inulin (absorption in terms of concentration) in the wavelength was calculated as 620 nm. According to inulin analysis, the percentage of inulin content on a dry weight basis and inulin yield was computed by the following formula Puangbut *et al*:

Inulin yield (Kg/h) = inulin content (%) × tuber yield (Kg/h) [18].

Measurement of Seed yield and Trigonelline of Fenugreek

For seed yield determination of the fenugreek, the plants harvested at maturity stage and air dried in shade for 10 days and expressed as Kg/h. The trigonelline was determined in the seeds' samples through using HPLC method of Hassanzadeh *et al* [19]. The seeds were blended with 80% methanol and magnesium oxide (MgO). After the incubation at 60 °C for 30 min, the homogenates were centrifuged and the supernatant was collected. After the evaporation of methanol, the methanol-soluble extracts were dissolved in distilled water. The samples were filtered using a syringe filter unit and the aliquots were used for determination of trigonelline by HPLC. A mixture of methanol:water (50:50 v.v) was used as the mobile phase and pH of the solution was adjusted to 0.5 with50 mM sodium acetate. The elution was made in an isocratic mode at a flow rate of 1 Ml/min and the detection was made at 268 nm by UV detector. The retention time of trigonelline was 4.4 min. Before carrying out the HPLC analysis, the calibration curve was designed using different concentrations of trigonelline in the mobile phase media (Fig. 1 and 2).

Table 1 Soil analysis of the experimental site

Potassium (mg/kg)	Phosphorus (mg/kg)	Nitrogen (%)	Organic carbon (%)	pH	EC (ds/m)	Sand (%)	Silt %)	Clay (%)	Soil texture
243	8.09	0.076	0.72	7.6	4.3	8	68	24	Silty-loam

Table 2 Physical and chemical properties of vermicompost

K_2O (%)	P_2O_5 (%)	N (%)	Organic matter (%)	pH	EC (ds/m)
1.8	2.2	1.5	60.2	7.2	6.7

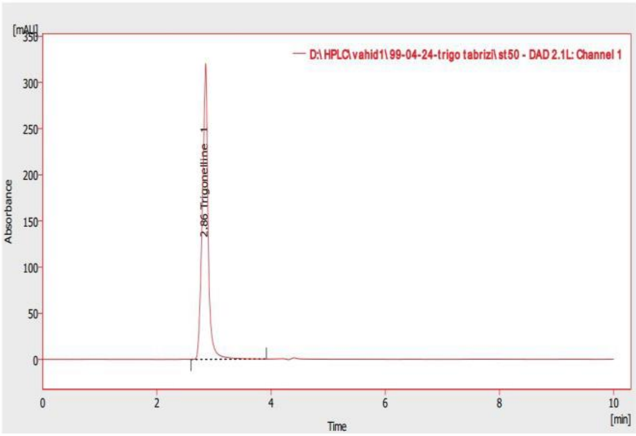

Fig. 1 HPLC chromatograms for standard trigonelline

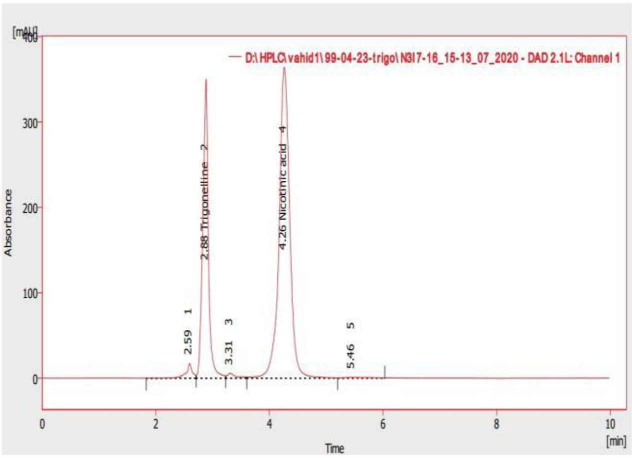

Fig. 2 HPLC chromatograms for fenugreek seeds' samples

Statistical Analysis

Analysis of the variance was carried outusing SAS software (version 9.3; SAS Institute; USA). The data were presented as the mean values ± standard error (S.E.) mean comparisons were made using Duncan's multiple range test at the $P< 0.05$ level and related graphs were drawn by Excel software.

RESULTS AND DISCUSSION

DM Yield Intercropping Patterns

The results of the analysis of variance showed that the main effect of cropping system was significant ($p<0.01$), for DM yield of the chicory, fenugreek and total DM yield; nevertheless, the fertilizer type × cropping system interaction effect, was not significant for DM yield. (Table 3).

Result of mean comparison between intercropping system levels, showed that the highest values of DM yield with average values of 3470 and 1880 kg h^{-1}, were obtained in monoculture of the chicory and fenugreek, respectively (Table 4). Probably, increasing extra-species competition in intercropping compared to monoculture, led to reduced mixture DM yield and therefore, by increasing the share of fenugreek in intercropping, the DM yield of chicory significantly decreased and reciprocally increasing the share of chicory, resulted in reduced yield of DM yield of fenugreek. The highest value of total DM yield (3810 Kg/h) was gained in C_2T_1 treatment, which had not significant difference with other mixed cropping ratios except for monoculture of fenugreek that had the lowest DM yield (1880 Kg/h).The total DM yield in intercropping was increased due to more efficient use of the light allopathic effect on weeds and stabilized nitrogen transfer [20]. Other researchers found similar findings concerning DM yield enhancement in intercropping method [21-22].

Land Equivalent Ratio (LER)

Based on the results of the analysis of variance, the main effect of fertilizer on LER of chicory was significant ($P< 0.05$); however, its effect on LER of fenugreek and total LER was not meaningful. The main effect of cropping system on LER of two plants and total LER was significant ($P< 0.01$), but thefertilizer × cropping system interaction effect was not significant on LER (Table 5).

Result of the means comparison, showed that the obtained (LER) of chicory in all fertilizer types and intercropping patterns were greater than fenugreek LER, indicating that chicory was the dominant species and showed higher competitiveness compared to fenugreek in this experiment. The higher value of LER for chicory (0.85) was obtained by vermicompost application (Table 6); while chemical and integrated fertilizer had lower LER values with a no significant difference. Among the cropping system, the highest LER of chicory and fenugreek was obtained in C_2T_1 and C_1T_1 patterns, respectively. The highest total LER, also was related to C_2T_1 pattern, which was not significantly different from C_1T_1 treatment, and both were higher than unit; however, in the C_1T_2 pattern, the LER was less than unit (Table 6). The LER less than 0.5 indicates a lack of plant superiority based on land use efficiency [23]. In intercropping patterns, chicory had more positive effect than its association with fenugreek, which had increa sed its LER. It is expected that the LER increase higher than 0.5 depends on the supplementary degree of intercropping components [24]. The advantages of the intercrops are higher when the yield of one or both of the respective sole crops is quite low [25]. In addition to the positive cooperation of two plants, the higher value of LER than one, is related to nitrogen stabilization and the availability of high-consumption elements for these two plants. Nutrient exchange has increased the competitive ability for controlling the weeds and nitrogen fixation. The differences in the root system of mixed components and greater radiation uptake are the reasons of increasing LER in intercropping system [26].

Chicory Traits

According to the results of analysis of variance, the main effect of cropping system and fertilizer type and fertilizer × cropping system interaction were significant for root dry weight of chicory ($p<0.01$ and 0.05), respectively (Table 7).

The highest and lowest root dry weight of the chicory (470 and 170 Kg/h) were observed in monoculture of the chicory and C_1T_2 treatment, respectively (Table8). According to the results of the fertilizer× cropping system interaction, showed that the highest dry weight of chicory root was related to pure chicory cultivation coupled with integrated fertilizer and vermicompost application, which had no significant difference with monoculture and chemical fertilizer application and C_2T_1 pattern with application of integrated fertilizer (Fig. 3). The presence of diazotrophic bacteria in inbio-fertilizers through plant hormone production leads to more carbon allocation to the roots and consequently weight gain of the roots would increase, which is the result of the accessibility of appropriate amounts of chemical fertilizers [27]. In an experiment on tomato, it was reported that phosphorus solvent bacteria could increase the phosphorus access in soil and simultaneous application of phosphorus solvent and triple superphosphate fertilizer is able to produce the highest root dry weight in this plant [28]. Other researchers stated that vermicompost could function well to provide the nutrients needed for plants such as nitrogen, phosphorus and potassium which in turn increase the root dry weight [29]. Since all agronomic and climatic traits are involved in the root yield per unit area, the reason for the reduction of root yield in mixed ratios can be related to reducing the number of rows of the chicory cultivation within the fenugreek cultivation [30]. On the other hand, in monoculture conditions due to less competition, the plant has a suitable cellular status and the potential for its cellular development is satisfied, which increases the metabolic activity of growth and development rate. Similar results were obtained regarding the root dry weight in intercropping of sugar beet and wheat and Faba bean [31-32].

Any factor that increases the leaf area, light absorption, and photosynthesis of plants and transports more hydrocarbon materials to the roots is able to increase the root DM yield [33].

Based on the analysis of variance, the effect of fertilizer, cropping system and the irinteraction effect were significant on the root inulin content and inulin yield ($p<0.01$) (Table 7). The highest and lowest inulin content with average values of 0.53% and 0.38% were obtained in the integrated and chemical fertilizer, respectively (Table 8). For cropping system, the highest and lowest inulin content with values of 0.73% and 0.27% were related to the sole cultivation of chicory and C_1T_2 cropping pattern, respectively (Table 8).

Table 3 Results of analysis of variance (MS) for the effect of fertilizer treatments and intercropping on DMyield of chicory and fenugreek

Source of variation	Df	MS		
		Chicory	Fenugreek	Total
Replication	2	1611264 *	219132 ^{ns}	811231 ^{ns}
Fertilizer type (F)	2	68154 ^{ns}	218112 ^{ns}	391323 ^{ns}
Cropping system (C)	4	4816521 **	2172154**	5711123 **
F × C	6	321521 ^{ns}	63211 ^{ns}	121231 ^{ns}
Error	22	351120	115113	510214
CV(%)		21.48	25.54	22.40

^{ns}, * and ** indicate non-significant, significant at 5% and 1% probability level, respectively.

Table 4 Means ± SE (Standard error) of three fertilizer types and different intercropping patterns on DM yield of chicory and fenugreek

Factors	Treatment	Chicory(kg/h^{-1})	Fenugreek (kg/h)	Total(kg/h)
Fertilizer	Chemical	2830 ± 292 a	1390 ± 158 a	3370 ± 237 a
	Integrated	2680 ±302 a	1190 ± 165 a	3100 ± 265 a
	Vermicomost	2730 ± 184 a	1160 ± 197 a	3090 ± 250 a
Cropping system	Chicory:Fenugreek (1:1)	2590 ± 128 b	1160 ± 143 b	3750 ± 147 a
	Chicory:Fenugreek (1:2)	1790 ± 191 c	1220 ± 128 b	3010 ± 260 a
	Chicory:Fenugreek (2:1)	3130 ± 219 ab	680 ± 80 c	3810 ± 190 a
	Pure chicory	3470 ± 281 a	-	3470 ± 281 a
	Pure Fenugreek	-	1880 ± 200 a	1880 ± 200 b

In each column, means with similar letter (s) are not significantly different (p≤0.05) according to Duncan's multiple range test.

Table 5 Results of analysis of variance (mean squares) for the effect of fertilizer treatments and intercropping on land equivalent ratio (LER)

Source of variation	df	MS		
		LER_a (Chicory)	LER_b (Fenugreek)	LER (Total)
Replication	2	0.094 *	0.0008 ^{ns}	0.067 ^{ns}
Fertilizer type (F)	2	0.101 *	0.0155 ^{ns}	0.061 ^{ns}
Cropping system (C)	2	0.421 **	0.0981 *	0.761 **
F × C	4	0.009 ^{ns}	0.0121 ^{ns}	0.009 ^{ns}
Error	16	0.019	0.0181	0.025
CV(%)		18.81	35.12	14.22

^{ns}, * and ** indicate non-significant, significant at 5% and 1% probability level, respectively.

Table 6 Means±SE (Standard error) of three fertilizer sources and three intercropping patterns on land equivalent ratio (LER)

Factors	Treatment	LER_a (Chicory)	LER_b (Fenugreek)	LER (Total)
Fertilizer	Chemical	0.65 ± 0.08 b	0.34 ± 0.05 a	1.02 ± 0.09 a
	Integrated	0.69 ± 0.08 b	0.42 ± 0.04 a	1.12 ± 0.10 a
	Vermicompost	0.85 ± 0.07 a	0.38 ± 0.06 a	1.18 ± 0.10 a
Cropping system	Chicory:Fenugreek (1:1)	0.77 ± 0.07 a	0.49 ±0.04 a	1.24 ± 0.06 a
	Chicory:Fenugreek (1:2)	0.49 ± 0.05 b	0.38± 0.04 ab	0.77 ± 0.04 b
	Chicory:Fenugreek (2:1)	0.91 ± 0.06 a	0.28 ± 0.04 b	1.30 ± 0.06 a

Data are mean values (± standard error) of three replicates (n= 3in each column and for each treatment)
Means with similar letter (s) are not significantly different (*P*≤ 0.05) according to Duncan's multiple range tests.

Although the genes control the pharmaceutical active ingredients, the amount of their production is significantly affected by the environmental conditions. The most important environmental factors are; soil physical and chemical properties, cultivation date, climatic conditions, micro, and macro nutrients' consumption and cropping pattern [34]. Microorganisms play a major role in producing essential compounds such as the minerals, vitamins, and cytokines that are important factors in the direction and transfer of metabolites [35]. Organic fertilizers are also one of the primary criteria of medicinal compounds' productions due to their macro and micro elements. The presence of these vital elements in organic fertilizers effects on early metabolites of plants. Phosphorus can increase the Rabisco enzyme, and consequently facilitates the photosynthesis and biosynthesis of carbohydrates [36]. Similar results, reported by other researchers that biofertilizers in artichoke increases the dry weight of the roots and inulin content [37]. Other investigators recorded the highest root dry weight and inulin percentage in biofertilizer treatment under non-stress conditions [38]. The results of mean comparisons of the intraction effects of fertilizer × cropping system showed that the highest inulin content (1%) was related to the treatment of the sole cultivation coupled with organic and integrated fertilizer sources and the lowest inulin content (0.20%) was related to monoculture and C_2T_1 treatment with chemical fertilizer application (Fig. 3).

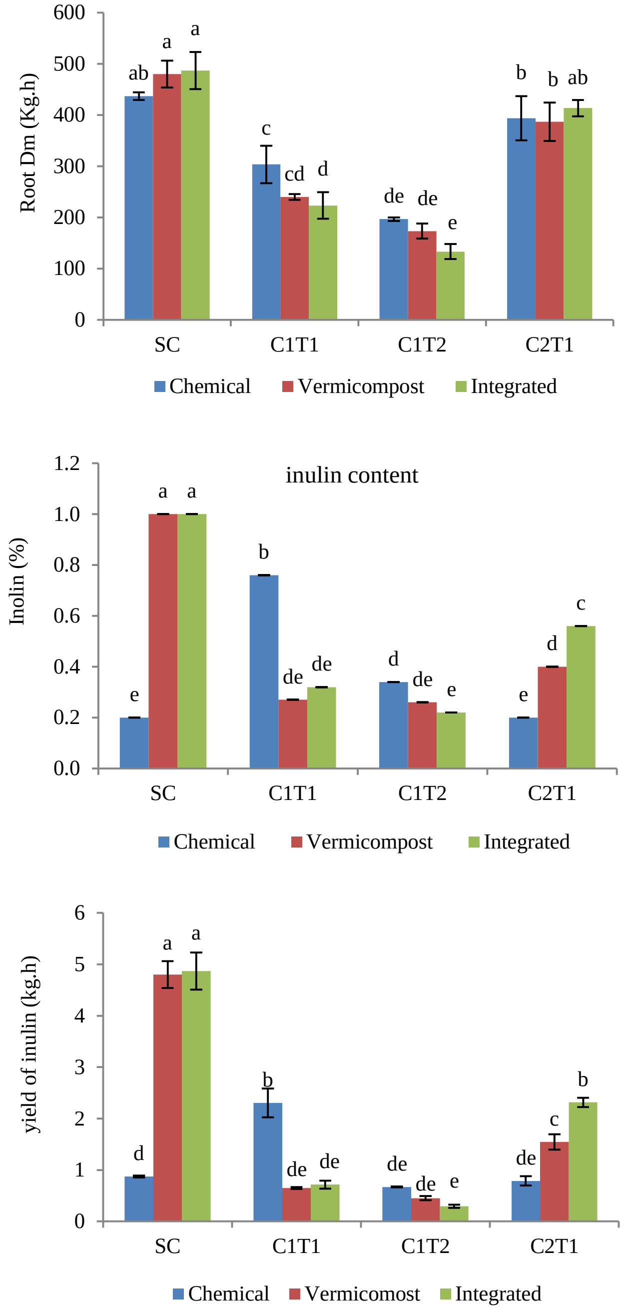

Fig. 3 Interaction effect of fertilizer types and intercropping patterns of root dry weight, inulin content and inulin yield of Chicory

S_C: pure cropping of Chicory, C_1T_1: Chicory1 + fenugreek1, C_1T_2: Chicory1 + fenugreek2, C_2T_1: Chicory2 + fenugreek1 row.

Means with similar letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

In the sole cultivation of chicory, due to lack of interspecies competition of plant, available resources are better used; while, the presence of fenugreek in intercropping can be considered as a kind of stress for plant and considering the food sources' competition the root development and consequently inulin production would be reduced. Similarly, in the intercropping of sugar beet with wheat and corn, the highest sugar extracted from monoculture of sugar beet, and the sugar percentage in Beet roots was decreased along with increasing the share of adjacent plants [32-39]. In this regard, previous researchers reported that due to lack of proper nutrition of shoots, photosynthetic compounds' production under the low light and growth space conditions for the leaf development in mixed culture, the roots' impurities was increased and extraction sugar was reduced [33]. Inulin yield is influenced by the biomass and inulin content; so, any factor that increases these indices can rise inulin yield as well. Based on the mean comparison results, the highest inulin yield of chicory (1.65 kg/h) was obtained in integrated fertilizer application, which was not significantly different from vermicompost treatment and the lowest value (1.25 kg/h) was also related to chemical fertilizer. The highest inulin yield (3.46 Kg/h) and the lowest inulin yield of chicory (0.46 kg/h) were obtained in pure and C_2T_1 treatments, respectively (Table 8). The mean comparison of the fertilizer×cropping system interaction effect, showed that the highest inulin yield of chicory (4.83 kg/h) was obtained in monoculture treatment and integrated fertilizer application, which was not significantly different with monoculture and vermicompost treatment. The lowest inulin yield (0.29 kg/h) however, was achieved using the C_1T_2 treatment with integrated fertilizer application (Fig. 3).

Fenugreek Traits

The main effect of fertilizer and cropping system on grain yield of fenugreekwas significant ($p<0.01$); however, the fertilizer×cropping system interaction effect was not significant (Table 9). The result of mean comparisons showed that the highest and lowest grain yield with average values of 2770 and 1590 Kg/h were obtained from the integrated fertilizer and vermicompost source, respectively. Concerning the cropping system, the highest and lowest grain yield (3260 and 1840 kg/h) were observed in sole cultivation treatments of fenugreek and C_2T_1 pattern (Table 10).

Table 7 Results of analysis of variance (MS) for the effect of fertilizer treatments and intercropping on dry root yield and inulin content of chicory

Source of variation	df	MS		
		Root dry weight	Inulin	Inulin yield
Replication	2	10211 **	0.0013 ns	0.235 *
Fertilizer type (F)	2	1013 ns	0.0711 **	2.595 **
Cropping system (C)	3	165154 **	0.3521 **	14.95 **
F×C	6	3426 *	0.2980 **	5.803 **
Error	22	1322	0.0058	0.067
CV(%)	-	11.12	16.53	15.39

ns, * and ** indicate non-significant, significant at 5% and 1% probability level, respectively.

Table 8 Means±SE (Standard error) of fertilizer types and intercropping patterns on dry root yield and inulin content of chicory

Factor	Treatment	Root dry weight (kg/h)	Inulin (%)	Inulin yield (kg/h)
Fertilizer	Chemical	330 ± 30.00 a	0.38 ± 0.07 c	1.25 ± 0.21 b
	Integrated	310 ± 43.68 a	0.53 ± 0.09 a	1.65 ± 0.54 a
	Vermicompost	32 ±37.50 a	0.48 ± 0.09 b	1.54 ± 0.53 a
Cropping system	Chicory:Fenugreek (1:1)	260 ± 17.88 c	0.45 ± 0.08 b	1.15 ± 0.28 b
	Chicory:Fenugreek (1:2)	170 ± 11.02 d	0.27 ± 0.02 c	0.4 ± 0.05 c
	Chicory:Fenugreek (2:1)	400 ± 17.60 b	0.39 ± 0.05 bc	1.54 ± 0.23 b
	Pure chicory	47 ± 15.14 a	0.73 ± 0.13 a	3.43 ± 0.67 a

Means with similar letter (s) are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Considering the role of nutrients such as nitrogen and phosphorus in plants' physiological and biochemical activities, it was underlined that better access of plants to these elements, improves the plant's growth, and increases its photosynthesis, material production, and ultimately its yield [40]. Other scientists concluded that integrated application of biofertilizer and chemical fertilizers produces the highest grain yield [41]. In this study, the presence of microorganisms increased the supply of nitrogen and phosphorus for fenugreek and improved the plant growth and photosynthetic material production due to application of nitroxin fertilizer and fertile phosphate 2 in the root environment.

Application of biofertilizer and organicfertilizer has various advantages such as increasing the plant growth and development by creating a suitable environment for nutrients and increasing the water holding capacity, which would ultimately increase plant's economic yield [42]. Enhancing yieldby integrated fertilizer (chemical and biological fertilizers) leads to more absorption continuity compared to chemical fertilizer and better coincidence between adsorption rate and available nitrogen content [43]. On one hand, in monoculture of fenugreek, due to lack of interspecies competition, all available resources were provided to fenugreek, and each plant had the most utilization of available resources which resulted in yield's advancement per unit area. On the other hand, Nitrogen fixation by fenugreek plant increased the grain yield. Similar results have been reported by others that grain yield of bean is decreased in mixed culture [44]. In addition, in an experiment, the grain yield of three plants, including black seed, chickpeas, and beans in pure cultivation was higher than the mixed treatments [45].

Trigonelline is the most important alkaloid component found in fenugreek seed, which plays a key role in its medicinal effects. For this, secondary metabolite, the researchers believe different physiological roles such as an active factor in the leaf movement, and resistance to biological and non-biological stresses [46-47].

The effect of fertilizer and cropping system were significant on trigonelline content and trigonelline yield in the seed of fenugreek ($P<0.01$). The fertilizer × cropping system interaction effect was significant on trigonelline content ($P<0.01$); while, they had no significant effect on trigonelline yield (Table 9). The mean comparisons of the main effects fertilizer, revealed that the highest amount of trigonelline (0.44%) was detected in the integrated fertilizer application; was not significantly different from other chemical fertilizer and vermicompost sources (Table 10). Since the secondary metabolites are strongly affected by the primary metabolites (carbohydrates, proteins and chlorophyll, etc.), any factor that increases the plant photosynthesis, can also increase secondary metabolites [48].

Trigonelline have been synthesized from the complicated nitrogen compounds ($-NH_2$). The decisive role of nitrogen compounds in increasing alkaloids is due to the presence of main molecule nitrogen in the structure of amino acids and their metabolites [49]. On the other hand, fenugreek requires phosphorus to develop the root system and to provide the energy needed for nitrogen stabilization. Azotobacter and azasperillium bacteria in biofertilizers provide more nitrogen by stabilizing air nitrogen and increase the medicinal metabolites of the plant. According to another research, the phosphorus of chemical fertilizer and soluble phosphorus, due to fertile phosphate2 activity, has led to the root development and nitrogen uptake increase, and as a result, the highest concentration of trigonelline with biofertilizer application and 50% chemical fertilizer (nitrogen and phosphorus) have been obtained [50]. Furthermore, Dadrasan *et al* reported the highest trigonelline with integrated of bio and 50% chemical fertilizer [51]. In our study, among the cropping system, the highest amount of trigonelline (0.44%) was observed in the C_1T_1 pattern, which was not significantly different from other mixed patterns; while, the lowest amount of trigonelline (0.40%) was measured in the treatment sole cultivation of fenugreek (Table 10). The mutual effects of fertilizer×cropping system indicated that the highest amount of trigonelline (0.48%) was gained in the C_2T_1 and the lowest value (0.39%) was achieved into the soil cultivation of fenugreek (Fig. 4). Similarly, Salehi *et al* reported that the content of trigonelline in intercropping systems in two consecutive years was higher than the sole cultivation of fenugreek [52].

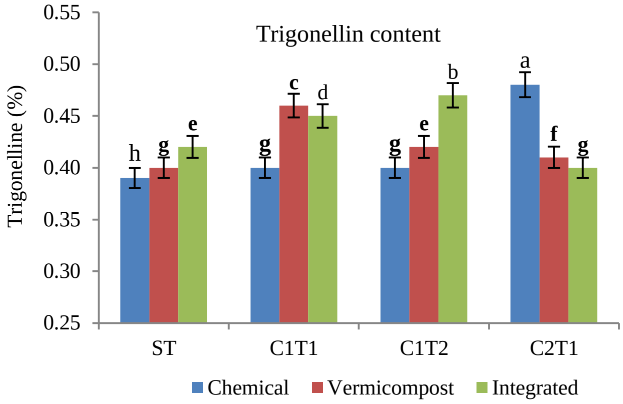

Fig. 4 Interaction effect of fertilizer types and intercropping patterns on trigonelline of fenugreek

Other researchers reported that the accumulation of secondary metabolites in fenugreek seeds is likely to occur under the stress conditions to prevent the production of active oxygen species and light damage [53]. Therefore, the reason for this increase could be because of increased interspecific competition in intercropping, which enforce a kind of stress to the plant and as a result the percentage of secondary metabolites increases with a mild stress. The results of mean comparison revealed that the highest and lowest amount of trigonelline yield (11.40 and 6.70 kg/h) were observed in the integrative and chemical fertilizer. Despite greater concentration of trigonelline in mixed cropping compared to monoculture, the highest and lowest amount of trigonelline yield (13.14and 7.91 kg/h) were observed in sole cultivation and C_2T_1 treatment of fenugreek (Table 10). The reason is that the trigonelline yield is a function of the seed yield and trigonelline concentration. It is worth mentioning that, greater concentration of secondary metabolites in plants is often offset by the lower biomass or seed yield [53].

Table 9 Results of analysis of variance (MS) for the effect of fertilizer treatments and intercropping on seed yield and trigonelline of fenugreek

Source of variation	df	MS		
		Seed yield	Trigonelline	Trigonelline yield
Replication	2	1671124 ^{ns}	0.12121**	28.996 ^{ns}
Fertilizer type (F)	2	5421231**	0.00097**	96.825 **
Cropping system (C)	3	3491321**	0.00201**	47.363 **
F×C	6	961121 ^{ns}	0.00411**	12.623 ^{ns}
Error	22	561123	0.00002	9.194
CV(%)	-	31.81	3.51	29.11

^{ns}, * and ** indicate non-significant, significant at 5% and 1% probability level, respectively.

Table 10 Means±SE (Standard error) different fertilizer sources and different intercropping patterns on seed yield and trigonelline of fenugreek

Factors	Treatment	Seed yield(kg/h)	Trigonelline(%)	Trigonellineyield (kg/h)
Fertilizer	Chemical	2730 ± 387 a	0.42 ± 0.011 b	11.40 ± 1.40 a
	Integrated	2770 ± 270 a	0.44 ± 0.008 a	12.04 ± 1.14 a
	Vermicompost	1590 ±138 b	0.42 ± 0.007 b	6.70 ± 0.58 b
Cropping system	Chicory:Fenugreek (1:1)	2270 ± 394 b	0.44 ± 0.009 a	9.90 ± 1.54 b
	Chicory:Fenugreek (1:2)	2080 ± 257 b	0.43 ± 0.010 a	8.94 ± 1.23 b
	Chicory:Fenugreek (2:1)	1840 ± 227 b	0.43 ± 0.013 a	7.91 ± 0.85 c
	Pure Fenugreek	3260 ± 421 a	0.40 ± 0.004 c	13.14 ± 1.69 a

Data are mean values (± standard error) of three replicates (n = 3In each column and for each treatment, means with similar letter (s) are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

S_C: pure cropping of Chicory, C_1T_1: Chicory1 + fenugreek1, C_1T_2: Chicory1 + fenugreek 2, C_2T_1: Chicory2 + fenugreek1 row.

Means with similar letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

CONCLUSION

The findings of this experiment demonstrate that the bioactive compounds of the chicory root and fenugreek seeds can be significantly affected by agronomic and environmental conditions, including the cropping system and the management type such as the fertilizer type. According to the results of this experiment, the highest DM yield was obtained in C_2T_1 treatment, which was not significantly different from other mixed cropping ratios except the sole fenugreek. In chicory, the highest dry root yield, inulin content and inulin yield were obtained in the pure culture treatment using integrated fertilizers and vermicompost, followed by the mixed ratios of C_2T_1 and C_1T_1. In fenugreek, however, the highest DM yield and trigonelline yield were obtained in a pure cultivation of this plant; nonetheless the total DM yield was much lower than other treatments. Considering the LER in C_2T_1 and C_1T_1 mixed cropping pattern, were more superior to DM yield than the soil cultivation of theseplants. Finally, if the purpose of cultivating these plants is the performance of the inulin content in chicory root or trigonelline content in fenugreek, the pure cultivation of chicory coupled with application of integrated fertilizer or vermicompost in chicory as well as pure cultivation coupled with application of integrated fertilizer in fenugreek are recommended. In a different manner, if the aim of cultivating these plants is to increase the DM yield per unit area, intercropping patterns of C_1T_2, C_1T_1 treated with vermicompostand integrated fertilizer are suggested rather than the sole cropping system.

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