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Effect of Seeding Rate and the Spraying with Licorice Extract in Oil Percentage and Active Ingredients of Caraway (*Carum Carvi* L.)

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ABSTRACT
A field experiment was conducted to study the effect of three levels of seeding rate (4.7 and 10 kg·h^{-1}) and three concentrations of licorice extract (0.3 and 6 g·L^{-1}) the data were analyzed statistically by using a factorial experiment according to the randomized complete block design (RCBD) with three replicates. The results showed that the seeding rate 7 kg·h^{-1} gave a significant increase in seeds yield, volatile oil yield, and limonene ratio, which were 1162.6 kg·h^{-1}, 25.6 kg·h$^{-1,}$ and 30.41%, respectively. In turn, seeding rate 4 kg·h^{-1} gave a higher average of the volatile oil percent and carvone percent, which were 2.3 % and 67.27%, respectively. The results also revealed that spraying of licorice extract with 3 g·L^{-1} gave significantly influenced in seeds yield and volatile oil yield which were (1016.6 and 23.3 kg·h^{-1}) respectively, whereas the spraying of licorice extract with 6 g·L^{-1} gave the highest ratio of carvone and limonene which were 29.71% and 67.54% respectively. The interaction between the seeding rate of 7 kg·h^{-1} and licorice extract with 3 g·L^{-1} had a significant effect on seeds yield and volatile oil yield, which amounted to (1234.2 and 28.4 kg·h^{-1}), respectively.

Keywords: araway; carvone; limonene**;** humic acid; seeding rate.

INTRODUCTION

The growing interest in medicinal plants is due to the high price of pharmaceutical medicine and the reduction of side damage when used. The World Trade Organization expects that the trade in medicinal and aromatic plants will rise to 5 trillion dollars in 2050 (Noorhosseini, 2011). The caraway plant, *Carum Carvil* L, is one of the most important medicinal plants, which belongs to the Umbelliferae family, which includes more than 250 species of important leafy and medicinal vegetable crops, spread in some regions of the world, which differ in their content of volatile oils in quantity and quality. This crop is the most widespread and cultivated in Central and Southern Europe, Siberia, Iran, Turkey, China, India, and South Africa for its medicinal uses, and the essential oil in caraway seeds is 1-6% (Sedlakova et al., 2003). It contains a high percentage of Carvone and Limonene compounds, which give its oil the medicinal importance (Csedo, 1980, Svab, 1992, Bernath, 2000), as well as other biological uses due to its anti-bacterial and anti-fungal properties, and oxidation and mutagenic factors in cells that cause the cancer such as N-Methyl-nitro-N-nitrosoguanidine (Higashimoto et al., 1993, Fang et al., 2010). There are many efforts to reduce the impact of using chemical fertilizers, especially for medicinal and aromatic plants, to obtain a safe and low-cost production, as well as reduce environmental pollution without decreasing the economic return of plants, and the use of organic plant extracts because of their important role in increasing the level of essential oils for crops (Abu-Darwis and Ofir, 2014). Among these extracts is licorice (*Glycyrrhiza Glabray*), one of the widespread herbs in Iraq and a botanical alternative to extracting several natural and manufactured growth regulators that contribute to the growth and production of plants.It contains phenolic compounds, flavonoids, amino acids, vitamins, minerals and organic acids: glycyrrhizic and trihydroxy, which lead to the nourishing and stimulating the formation of the mevalonic acid compound as a bio-initiator for the synthesis of gibberellin hormone in plants (Newall et al, 1996, Shibata, 2000, Moses et al 2002); moreover, it contains vitamin C, one of the most important amino sugars that participate in enzymatic reactions as an assistant factor to form carbohydrate and protein metabolites in the plant and work to regulate cell growth through cell division and expansion (Smirnoff and Wheeler, 2000). Abd El-Azim et al. (2017) found that spraying of licorice extract led to a significant increase in the percentage and oil yield of *Foeniculum vulgare* L, also determining the optimal plant density per unit area is one of the necessary requirements that allow obtaining the highest crop yield to reduce biological competition between plants, which increases the opportunity for plants to benefit from growth inputs such as moisture, light, nutrients, and others more efficiently therefore, determining the plant density is considered one of the important and specific scientific practices for crop production (Sangoi, 2000, Momoh and Zhou, 2001, Ahmed et al., 2010) and directing the biological pathways towards the formation of the isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) compounds, for the synthesis of monoterpenes in plants (Ferandino and Lovisol, 2014, Verma and Shukla, 2015, Griesser et al., 2015, Varitsas et al. 2018). In the absence of studies on the caraway plant in Iraq, this study aimed to determine the effect of seeding rates and spraying with licorice extract on the yield of seeds, oil, and active ingredients in the seeds.

MATERIALS AND METHODS

This experiment was carried out in one of the agricultural fields in Baqubah district, Diyala Governorate, Iraq during the winter season of 2018–2019. A factorial experiment was applied according to a randomized complete block design (RCBD) with three replications. The study included two factors, the first included three levels of seeding rate (4,7 and 10 $kg \cdot h^{-1}$), and the second factor included three concentrations of licorice extract (0, 3 and 6 $g \cdot L^{-1}$), after its grinding and soaking in distilled water for 24 hours, as well as filtering (Byan, 2014), 1 ml of soap was added with the plant extract per liter of the solution to reduce the surface tension of water particles on the leaf surface and to cause complete wetting of the vegetative parts. The chemical components of licorice are listed in the Table 1, as reported by (Byan, 2014).

Table 1. The chemical components of the licorice (5 $g \cdot L^{-1}$)

The material	The ratio
Total phenols	405.02 mg/100 g
Total flavonoids	114.91 mg/100 g
Tannins	47.54 mg/100 g
Saponites	27.99 mg/100 g
Carotenoids	11.78 mg/100 g
Vitamin C	1.20 mg/100 g
pH	5. 49
E.c	0 .7 $ds \cdot m^{-1}$
N	0.6 %
P	0.04 %
K	0.3 %
Mg	0.17 %

Caraway seeds were sown homogeneously on October 29th, 2018, within each line, with a depth of 1 cm.The experiment field was fertilized with phosphate fertilizer in the form of triple superphosphate (P_2O_5, 52%) at the rate of 70 $kg \cdot h^{-1}$, and nitrogen fertilizer was added in the form of urea (N, 46%) after the plowing, at the rate of 120 $kg \cdot h^{-1}$ twice, the first time in the first week after germination and the second in the first month after the first time. Soil samples were collected randomly from the field at a depth of (15–30) cm before planting and were analyzed in the central laboratory, College of Science, the University of Baghdad to study their physical

Table 2. The physical and chemical properties of soil

Measurements	Value	Unit of measurement
Organic matter	2.21	$g \cdot kg^{-1}$
CO_3	0.30	%
Ec	2.73	$ds \cdot m^{-1}$
Ph	7.59	-
N	20.6	ppm
P	15.1	ppm
K	187.3	ppm
Clay	45.2	$g \cdot kg^{-1}$
Silt	25.92	$g \cdot kg^{-1}$
Sand	28.88	$g \cdot kg^{-1}$
Texture of soil	clay	
mg	87	ppm
SO_3	0.4	%
Na	240	ppm
Ca	240	ppm

and chemical properties (Table 2). Crop service and irrigation processes were carried out as needed, the plants were harvested on 29/5/2019 after the fruit has ripened and were colored with a brown color and before complete drying.

Studied traits

Seed yield

The total weight of fruits was calculated in all plants of the experimental unit and then converted to $kg \cdot h^{-1}$ unit according to the following equation:

$$\text{Seed yield} = \frac{\text{Experimental unit yield (kg)}}{\text{Experimental unit area } (\text{m}^2)} \times 10000 \quad (1)$$

NPK

Determination of total nitrogen was conducted by using a Microkjeldahl device, phosphorous by using a spectrophotometer device, potassium by using a flame photometer device, and the seed content of these elements was calculated based on dry weight (Jaiswal, 2004, Rayan et al., 2001).

Protein percentage %

Estimation of the protein percentage in the seeds was calculated using the following equation (Egan et al., 1988):

$$\text{Protein percentage} = \\ = \text{Percentage of nitrogen} \times 6.25 \quad (1)$$

The volatile oil

The amount of volatile oil was measured after separating it by sensitive balance and then converting into a percentage. The volatile oil yield ($kg \cdot h^{-1}$) was calculated from the following equation:

$$\text{Oil yield} = \text{percentage of volatile oil \%} \times \\ \times \text{seed yield in the experimental unit } (\text{kg.h}^{-1}) \quad (3)$$

The process of extracting and estimating of oil

The volatile oil was extracted using the water distillation method, according to what was stated in the (British pharmacopeia, 1986) and according to the method mentioned in (Chalechat et al., 1991) and (Akihisa et al 1996). A Clevenger device connected to a beaker of 1 liter was used, and 50 g of dried seeds with the air was ground using an electric grinding machine, then it was placed in a beaker with 1 liter attached to the device and 500 ml of distilled water was added. The distillation process was carried out by heating the beaker continuously at a temperature of 80 °C for a period of two and a half hours for each sample until the amount of volatile oil is extracted from the sample, which consists of two layers are the water and the oil, and these two layers are separated through the separation bath in the oil collection tube, the water is at the bottom and the oil is at the top because it is lighter than water. The quantity of oil for each treatment was measured by a sensitive balance (1260 MP- Sartorius of German origin) and each sample of oil was placed after separation in sealed opaque bottles at a temperature of 4 °C in the refrigerator until the chemical compounds are measured.

Determination of Carvone and Limonene ratio

The active components of the volatile oil were analyzed at the Environmental Research Center / Ministry of Science and Technology / Baghdad by using a gas chromatography/mass spectroscopy device (GC/MS) attached to with an FID detector to separate the components of the volatile oil of the caraway crop. The injection rate was 1 microliter in a capillary column, it is a type of DB-fused silica with dimensions (30 × 0.25 mm, with a thickness 0.25 μm) and a temperature of 40 $°C \cdot min^{-1}$ to 280 °C every 18 minutes at an increment rate (4 °C/min), the helium gas was used as carrier gas with an average flow of 1 $ml \cdot min^{-1.}$ The type of GC/MS is ITS-40 (Finningan MA/USA), the detector type is EV 70, the injector temperature is 220 °C, and the detector temperature is 240 °C. The oil-active compounds were detected in the presence of the electronic library of standard compounds attached to the device.

Statistical analysis

The results were analyzed using the SAS statistical program (2001) and the averages were compared according to Duncan's test at a probability level of 0.05 (Al-Rawi and Khalaf Allah 1980).

RESULTS AND DISCUSSION

The results in Table 3 showed that a significant effect between seeding rates and the spraying with licorice concentrations as well as the interaction between them on seed yield, percentage, and oil yield per hectare, where the seeding rate was 7 kg·h^{-1} gave the highest average in seed yield and oil yield, amounting to 1162.6 kg·h^{-1} and 25.6 kg·h^{-1} respectively, in comparison with the lowest average at a seeding rate of 4 kg·h^{-1}, which reached 757.1 kg·h^{-1} and 17.4 kg·h^{-1} for the same traits, respectively, these results are consistent with his finding (Kizil, 2002, Uliah et al., 2014) that increasing the plant density of *Pimpinella anisum* and *Coriandrum sativum* crops led to a decrease in the seed and oil yield.

The table also showed that the seeding rate of 4 kg·h^{-1} was superior in the oil content in the seeds reaching 2.3% compared to the lowest content of volatile oil 2.1% in the seeding rate of 10 kg.h^{-1}, the decrease in the seed and oil yield with the high plant density may be due to the reduction in the number of leaves and the efficiency of the photosynthesis process in the vegetative growth stages, which reduced the production and formation of oil in the seeds (Bouwmeester et al., 1995).

The spraying with a licorice concentration of 3 g·L^{-1} gave the highest average in the seed yield, percentage, and oil yield, which amounted to 1016.6 kg·h^{-1}, 2.3%, and 23.3 kg·h $^{-1}$, respectively, compared to the no-spray treatments with licorice extract which was 832.5 kg·h^{-1}, 2.1%, and 17.4 kg·h^{-1}, respectively.The increase in the above characteristics is due to the nature of licorice extract and its components from solutes that had the effect in the maintaining on the water potential of the plant cell and contributing in the formation of chlorophyll, which is reflected on the activity of the photosynthesis process as well as increase the growth and yield (Shibata, 2000, Zadeh et al., 2013), these results are consistent with what was found by Abd El-Azim et al. (2017) that the spraying with licorice extract on *Foeniculum Vulgare* plant led to an increase in the seed and oil yield of the plant. In addition to the licorice solution containing glycyrrhizic acid, which it had a role in increasing the activity of plant hormones such as Mevalonic acid, which it had vital importance for the synthesis of monoterpenes and the synthesis of volatile oil as well as increase its percentage in the seeds (Thanaa et al., 2016).

As for the interaction between the seeding rates and the spraying with licorice concentrations, where the treatment of the seeding rate is 7 kg·h^{-1} with the spraying of licorice concentration 3 g·L^{-1} was superior and gave the highest rate in the seed yield and oil yield, which amounted to 1234.2 and 28.4 kg·h^{-1}, respectively, compared to the seeding rate of 4 kg·h^{-1} and the non-spray with the licorice extract, which gave the lowest rates in the above-mentioned characteristics 634.9 and 14.0 kg·h^{-1}, respectively, also the treatment of seeding rate of 4 kg·h^{-1} with the spraying of licorice concentration 3 g·L^{-1} gave the highest rate in the percentage of oil, which amounted to 2.4%, compared to the seeding rate of 10 kg·h^{-1} and the non-spray with the licorice extract, which gave the lowest rate in the same characteristic 2.0%.

Table 3. Effect of seeding rates and licorice concentrations on seed yield, oil percentage, and oil yield

Seed yield (kg·h^{-1})				
The seeding rates (kg·h^{-1})	Licorice concentrations (g·L^{-1})			Mean
	0	3	6	
4	634.9 H	770.8 G	865.7 E	757.1 C
7	1019.8 C	1234.2 A	1234.0 A	1162.6 A
10	842.8 F	1045.0 B	930.0 D	939.2 B
Mean	832.5 C	1016.6 A	1009.9 B	
Volatile oil percentage %				
4	2.2 C	2.4 A	2.3 B	2.3 A
7	2.1 D	2.3 B	2.2 C	2.2 B
10	2.0 E	2.2 C	2.1 D	2.1 C
Mean	2.1 C	2.3 A	2.2 B	
Volatile oil yield (kg·h^{-1})				
4	14.0 H	18.5 F	19.9 E	17.4 C
7	21.4 D	28.4 A	27.1 B	25.6 A
10	16.9 G	23.0 C	19.5 E	19.8 B
Mean	17.4 C	23.3 A	22.7 B	

The results of Table 4 indicate that the seeding rate has a significant effect on the seeds content of nitrogen, phosphorus, and potassium, as the seeding rate of 4 kg·h^{-1} gave the highest average in the above-mentioned elements which amounted to (2.631, 0.467 and 1.524%) respectively, compared to the lowest average at seeding rate 10 kg·h^{-1}, which reached (2.458, 0.356 and 1.360%,) respectively; in turn, the seeding rate 7 kg. h^{-1} was superior and gave the highest percentage of protein 16.453% compared to the lowest average at the seeding rate 10 kg·h^{-1}, reached 15.451%. The reduction of plant density per unit area and the lack of the competition between plants in the soil content of nutrients and environmental requirements gave an ideal opportunity for the vegetative and root growth of plants, thus increasing the plant's ability to absorb nutrients and their content in seeds (Mossavi, 2012). These results are in agreement with the findings of Abdul Aziz and Muhammad (2018) that reducing the seeding rate of *Coriandrum sativum* L. led to an increase in the seeds' content of nutrients and protein.

The spraying with licorice concentration 6 g·L^{-1} was superior and gave the highest percentage of nitrogen, phosphorus, potassium, and protein, which reached (2.655, 0.476, 1.541 and 16.599%), respectively, compared to the no-spray treatments with licorice extract, which was (2.379, 0.375, 1.366 and 14.850%) respectively. This may be attributed to the licorice extract containing good proportions of plant hormones that have a vital role in cell division and expansion, thus increasing the proportions of nutrients in plant tissues (Ghaloom and Fava, 2012; Thana et al., 2016); moreover, it contains compounds are similar in their activities to the steroid hormones that stimulate the formation of protein compounds in the plant (El-Muhammadi, 2010).

As for the interaction between the seeding rates and the spraying with licorice concentrations, where the treatment of seeding rate 4 kg·h^{-1} with the spraying of licorice concentration 3 g·L^{-1} was superior and gave the highest rate in the percentage of nitrogen and protein, reached 2.704% and 16.968%, respectively.Moreover, the treatment of seeding rate 4 kg·h^{-1} with the spraying of licorice concentration 6 g·L^{-1} was significantly superior and gave the highest rate in the percentage of potassium and phosphorus in the seeds, reached 0.507% and 1.619%, respectively, compared to the lowest rates were (2.241, 0.291, 1.312 and

Table 4. Effect of seeding rates and licorice concentrations on the ratio of nitrogen, phosphorus, potassium, and protein in caraway seeds

Nitrogen %				
The seeding rates (kg·h^{-1})	Licorice concentrations (g·L^{-1})			Mean
	0	3	6	
4	2.511 BC	2.704 A	2.680 A	2.631 A
7	2.387 CD	2.661 AB	2.665 AB	2.570 A
10	2.241 D	2.513 BC	2.621 AB	2.458 B
Mean	2.379 B	2.626 A	2.655 A	
Phosphorus %				
4	0.431 D	0.467 B	0.507 A	0.467 A
7	0.405 E	0.444 C	0.474 B	0.440 B
10	0.291 G	0.326 F	0.451 C	0.356 C
Mean	0.375 C	0.411 B	0.476 A	
Potassium %				
4	1.399 D	1.566 AB	1.619 A	1.524 A
7	1.366 D	1.497 BC	1.551 AB	1.482 B
10	1.312 E	1.314 E	1.454 BC	1.360 C
Mean	1.366 C	1.459 B	1.541 A	
Protein %				
4	15.541 DC	16.968 A	16.850 A	15.954 B
7	14.851 DE	16.412 AB	16.600 AB	16.453 A
10	14.157 E	15.966 BC	16.230 ABC	15.451 C
Mean	14.850 B	16.409 A	16.599 A	

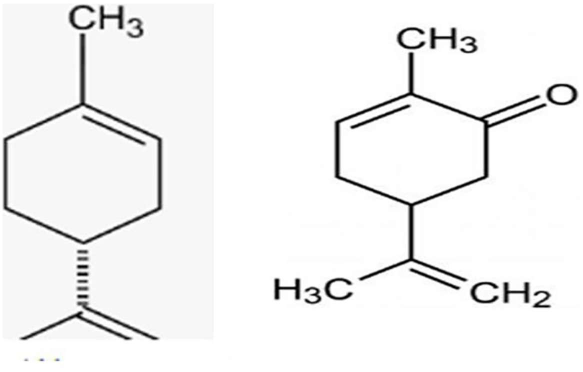
14.157%) respectively, at the treatment of seeding rate 10 kg·ha^{-1} with no spraying with licorice.

The obtained results from the analysis of the volatile oil in the chromatograph GC/MS in Table (5), and through the diagnosis, it was found that the extracted volatile oil from the fruits of caraway plants is composed of 11–12 terpene compounds, and the ratios of the active compounds carvone and limonene (Figure 1) in the oil differed according to the used experiment factors, namely the seeding rate and the spraying with licorice extract, and Limonene is one of the intermediate compounds for the synthesis of carvone during the growth and development of caraway fruits. The proportions of the compounds differed according to the user variables within the environmental conditions surrounding the plants.

The results of (Table 5, Figure 2 and 3) indicate that the seeding rate of 7 kg·h^{-1} gave the highest content of carvone, reaching 67.27%, followed by a seeding rate of 4 kg·h^{-1} and gave 66.65% compared to the lowest content of the compound at a seeding rate of 10 kg·h^{-1} and was 58.16%, while the seeding rate of 4 kg·h^{-1} gave the highest content of limonene, which amounted to 30.41%, followed by the seeding rate of 7 and 10 kg·h^{-1}, which were 28.16% and 27.47%, respectively. This is attributed to the cultivation of caraway at a seeding rate of 4 kg·h^{-1} led to reducing the factor of competition between plants and provided the opportunity for plants to optimal use of the environmental requirements of light and nutrients to form intermediate compounds and produce more energy for enzyme activity and stimulate the metabolic pathways towards the formation of Terpene compounds in the oil. These results agree with the findings of Bouwmeeste et al. (1995) that shading of plants together in the early stages of caraway fruit growth reduced seed oil and its vital compounds.

The spraying with licorice concentration 3 g·L^{-1} led to an improvement in the content of both limonene and carvone, which reached 29.71% and 67.54% respectively, compared to the lowest content of the above compounds, reached 27.25% and 57.64% respectively when not spraying with licorice extract (Table 5, Figure 4 and 5).

This is definitely due to the content of licorice extract of minerals, sugars, vitamins, and glycyrrhizin compounds (Table 1) that stimulate the mevalonic acid pathway and the necessary enzymes for the synthesis of active compounds (Varitsas et al., 2018). These results are consistent with what was found by Abd El-Azim et al. (2017) and Nasser et al. (2014) that spraying licorice extract on *Trigonella fonum_gracum* and *Foeniculum vulgare* plants improved the content of chemical compounds in



Limonene Carvone

Figure 1. The limonene and carvone compounds

Table 5. Effect of seeding rate and licorice concentrations on the content of limonene and carvone in the volatile oil of caraway seeds

Limonene %				
The seeding rate (kg·h^{-1})	Licorice concentrations (g·L^{-1})			Mean
	0	3	6	
4	29.23	31.54	30.46	30.41
7	27.41	28.63	28.45	28.16
10	25.12	28.98	28.31	27.47
Mean	27.25	29.71	29.07	
Carvone %				
4	62.97	69.72	67.27	66.65
7	65.95	67.17	68.70	67.27
10	44.00	65.75	64.74	58.16
Mean	57.64	67.54	66.90	

Note: each number in Table 5 represents an average of one replication.

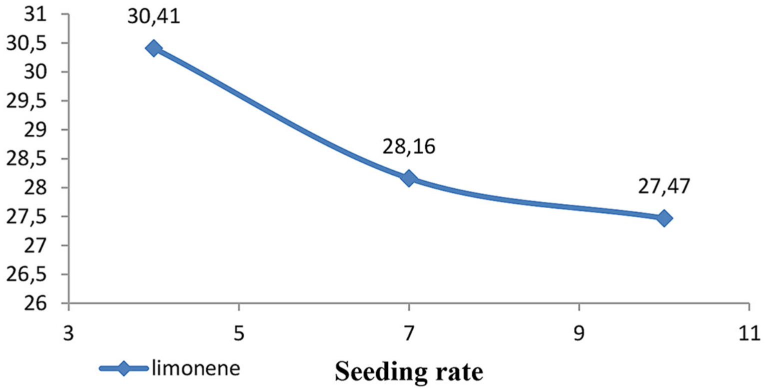


Figure 2. Effect of seeding rate in the content of limonene

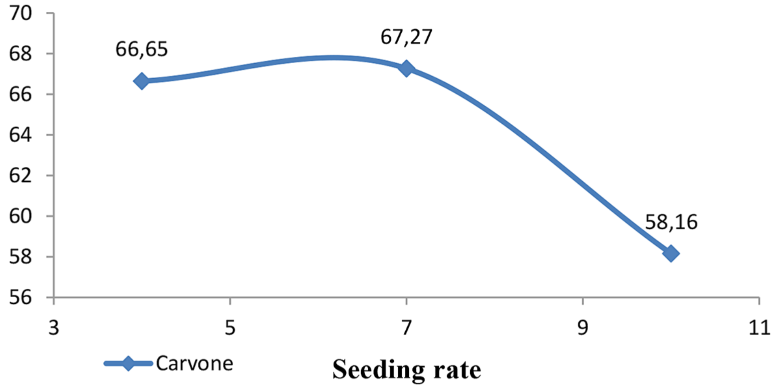


Figure 3. Effect of seeding rate in the content of carvone

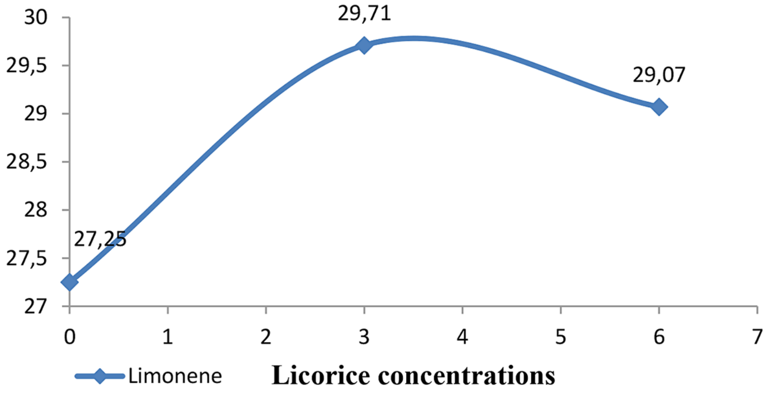


Figure 4. Effect of licorice concentrations in the content of limonene

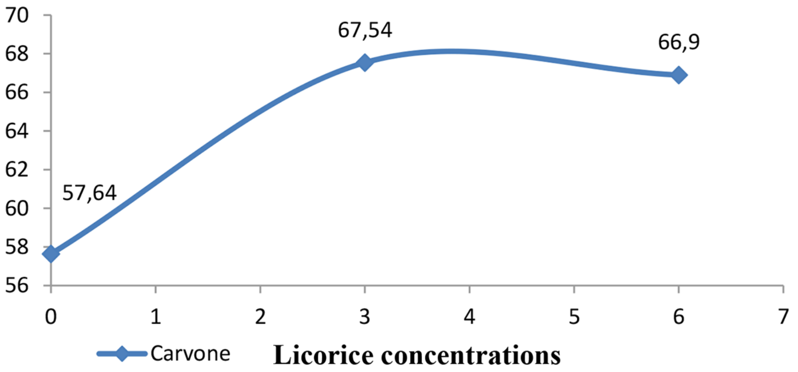


Figure 5. Effect of Licorice concentrations in the content of carvone

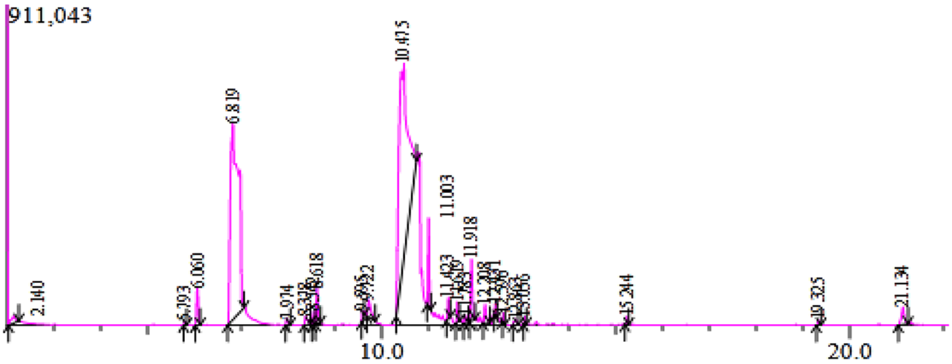


Figure 6. Behavior of the active ingredients at a seeding rate of 10 kg·h^{-1} and no spraying with licorice

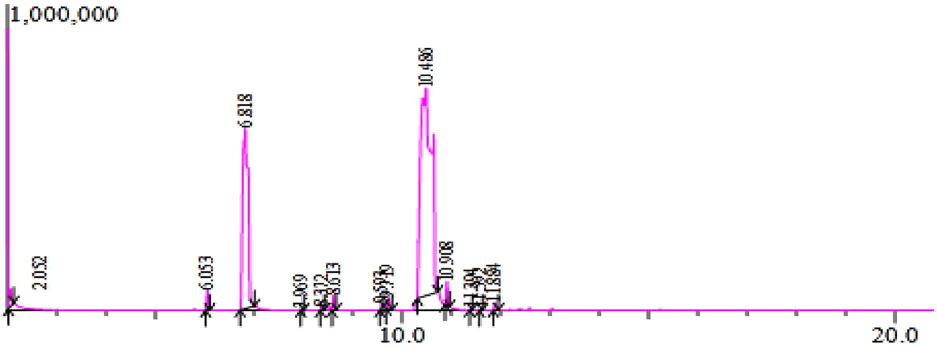


Figure 7. Behavior of active ingredients at a seeding rate of 10 kg·h^{-1}
and spraying of licorice at a concentration of 3 g·L^{-1}

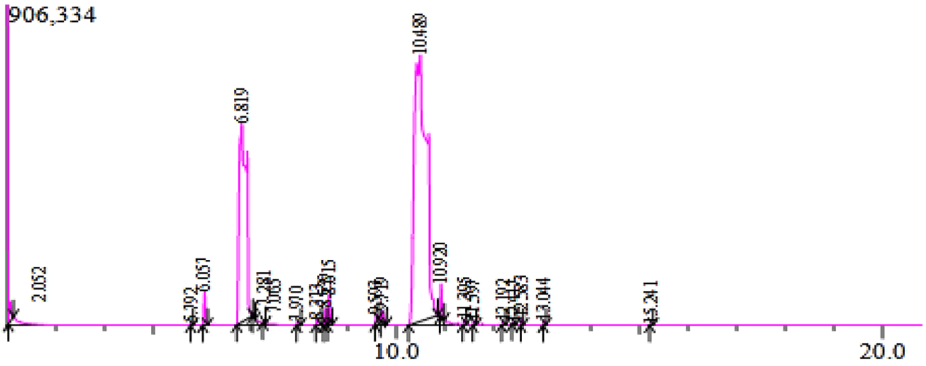


Figure 8. Behavior of active ingredients at a seeding rate of 10 kg·h^{-1}
and spraying of licorice at a concentration of 6 g·L^{-1}

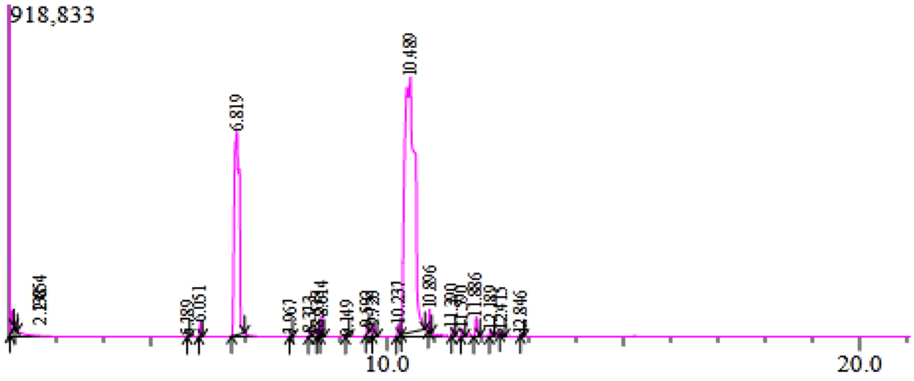


Figure 9. Behavior of the active ingredients at a seeding rate of 7 kg·h^{-1} and no spraying with licorice

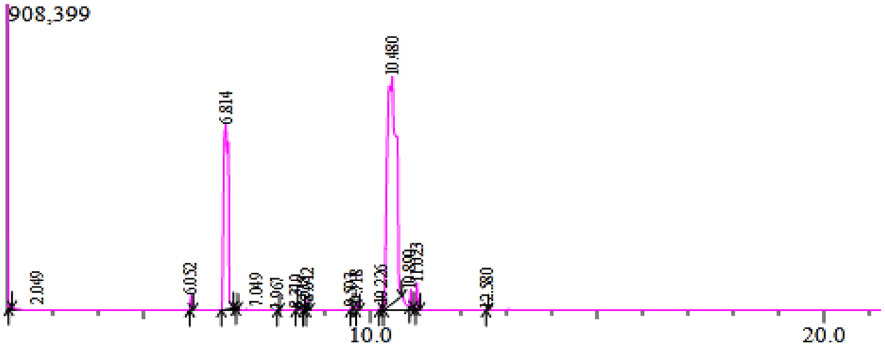


Figure 10. Behavior of active ingredients at a seeding rate of 7 kg·h^{-1} and spraying of licorice at a concentration of 3 g·L^{-1}

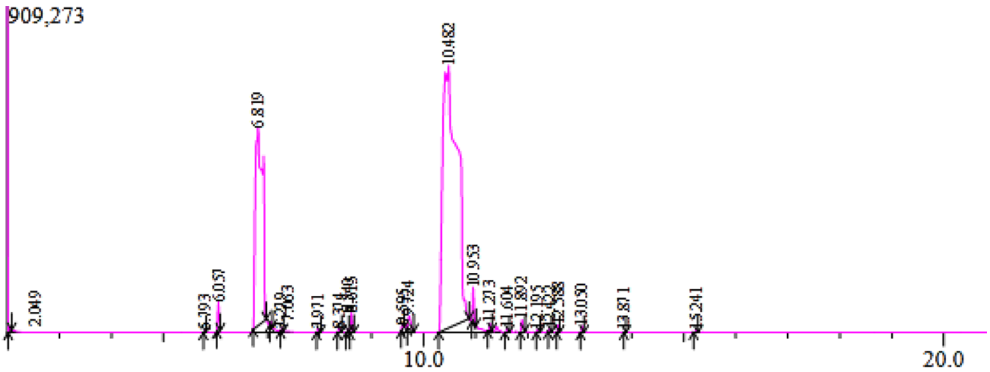


Figure 11. Behavior of active ingredients at a seeding rate of 7 kg.h^{-1} and spraying of licorice at a concentration of 6 g.L^{-1}

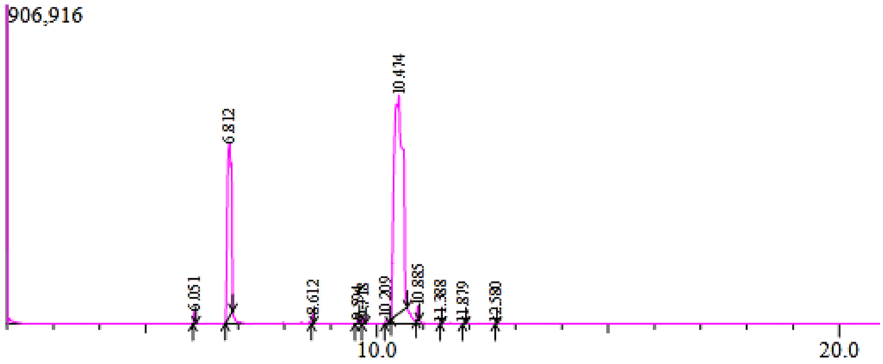


Figure 12. Behavior of the active ingredients at a seeding rate of 4 kg·h^{-1} and no spraying with licorice

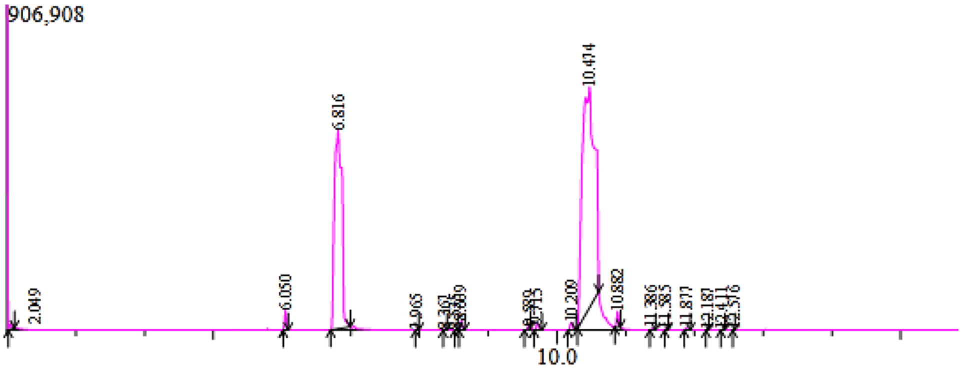


Figure 13. Behavior of active ingredients at a seeding rate of 4 kg·h^{-1} and spraying of licorice at a concentration of 3 g·L^{-1}

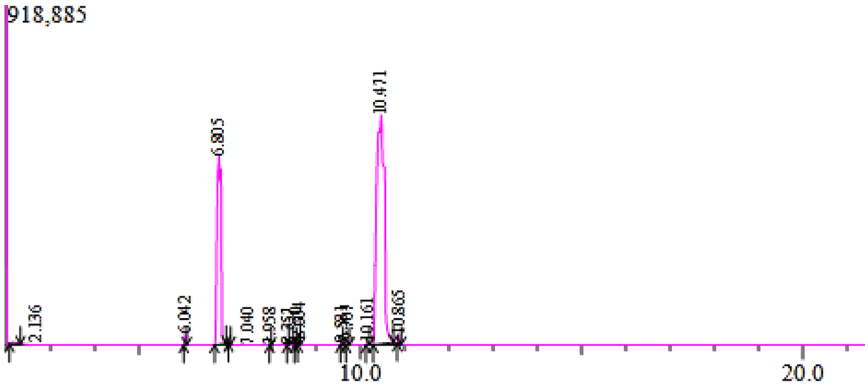


Figure 14. Behavior of active ingredients at a seeding rate of 4 kg·h^{-1} and spraying of licorice at a concentration of 6 g·L^{-1}

their oil. Figures (6–14) illustrate the behavior of the active ingredients with seeding rates and the spraying with licorice concentrations.

CONCLUSIONS

The results of the current study on a crop of Caraway show that using a seeding rate of 7 kg of seeds h^{-1} had a significant effect on the characteristics of seed yield, volatile oil, and the proportion of limonene ratio by reducing competition between plants per unit area on environmental and nutritional requirements, while the use of licorice extract at a concentration of 3 g·l^{-1} had a significant effect on seed yield and volatilization oil from on the vital role of the nutrient in securing the requirements of plant growth from ready-made elements. Therefore, further studies are needed to determine the role of licorice root extract and its mechanism of action towards other plant densities and different species in the central region of the country.

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