



Productivity and Phytochemical Composition of caraway (*Carum carvi* L.) plant in Response to *Alternaria alternata* as One of Endophytic Fungi and Nitrogen Fertilization grown under newly Reclaimed Sandy Soil Conditions

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ABSTRACT

The present work was conducted during 2018/2019 and 2019/2020 seasons in newly reclaimed soil at Al-Marashda Agricultural Research Station, Qena, Egypt to study the productivity and phytochemical composition of caraway (*Carum carvi* L.) plant as response to spraying *Alternaria alternata* as endophytic fungus at 0.3% three times (0, 3 and 6 times per season) and three nitrogen levels (0, 30 and 60 kg N/feddan) as ammonium sulfate and their combinations. Obtained data indicated that the vegetative growth and fruit characteristics, essential oil percentage and oil yield/feddan as well as leaf contents of N, P, K and total soluble carbohydrates of caraway plant significantly affected by spraying endophytic fungus and N fertilizer treatments. The most effective for the growth characteristics and productivity was due to spraying 6 times per season of endophytic fungus and using 60 kg N/fed. Compared to the other treatments. Meanwhile, the highest values of total carbohydrates, essential oil percentage and oil yield/fed. were obtained when plants were sprayed 3 times by endophytic fungus and fertilized with 60 kg N/fed. in the two seasons. Mostly, high P and K contents in leaves were resulted from plants sprayed with endophytic fungus at 6 times per season combined with 30 kg N/fed. Compared to the other treatments. Phytochemical composition of the essential oil in caraway fruits using GLC analysis indicated that, the most important compounds are carvone (major compound), limonene, linalool, caryophyllene, β -pinene, p-cymene, α -pinene, carveol and dihydrocarvone. Most of the different treatments of endophytic fungus and nitrogen fertilizer improvement content most of these components and markedly increased quality of essential oil (oil % , carvone content and carvone to limonene ratio) compared to the control (untreated plants). Therefore, it can be concluded that using *A. alternata* as endophytic fungus with 60 kg N/fed. as ammonium sulphate can improve the growth, productivity and quality of caraway plants under the reclaimed sandy soil conditions without the need for excessive chemical fertilization to obtain safe and healthy production for the various purposes of these important plants.

Keywords: *Carum carvi*, endophytic fungi, ammonium sulfate, essential oil, chemical composition.

1. Introduction

In Egypt, there is a tendency to increase the agricultural lands according to the political orientations by increasing the expansion of newly reclaimed soils. Also, due to the scarcity of water and the need to preserve it, there is a tendency to use modern irrigation systems to save the amounts of irrigation that are consumed by the flood irrigation system. Also, an excess of soluble salts in sandy soil leads to a reduction in the plant growth, yield and oil content for some medicinal plants (Ashraf and Orooj, 2006). Many researchers confirmed the effect of newly reclaimed soil on the chemical composition of essential oils for different medicinal plants (Shalan *et al.*, 2006; Matter and El Sayed, 2015).

Carum carvi L. belongs to the family Apiaceae, one of the earliest cultivated herbs in different countries. It is commonly known as caraway, which is grown for its high content of essential oil in their seeds. The essential oil of caraway is obtained by the steam distillation of the dried fruits. The major

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compounds in caraway are carvone, α -pinene, limonene, γ -terpinene, linalool, carvacrol and p-cymene (Kocourkova *et al.*, 1999). Caraway can be introduced into livestock feed, as it increases the excitability of the diet, facilitates digestion, and increases milk production. Caraway fruits have medicinal effects, including stimulating, expectorant and anti-spasmodic effects. They are also used to treat stomach pain, constipation and nausea, and they increase the secretion of gastric juice and bile, which increases appetite and leads to stimulation of the digestive system (Dyduchet *et al.*, 2006; Peter, 2006; Sembratowicz and Czech, 2005).

Endophytic fungi involved in phytoremediation process have shown tremendous potential in improving phytoremediation processes as reducing availability of metals, altering soil pH, releasing chelating agents and improving enzymatic properties (Khan *et al.*, 2009; Wenzel, 2009; Urozet *et al.*, 2009). However, the plant-associated microorganisms play essential roles for agriculture and food safety as well as contribute to maintain environmental equilibrium. These beneficial fungi can improve the growth and nutrient uptake of the plants, fix nitrogen, decrease plant stress and disease incidence that influence on the plant development and yield. Some of them can also be useful for protection against numerous pathogens (Montesinos, 2003). Also, Shebany and Abdel-Kader (2013) concluded that endophytic fungi application especially, *Alternaria alternata* was more effective for growth characteristics and oil %, yield and composition as well as N, P, K and Ca contents in dry leaves of sweet basil plants than non- inoculated plants, which may indicate effective symbiotic potential of this species with basil plants.

The influence of nitrogen fertilization on the growth and essential oil content in caraway plant was studied by different researches (El-Din *et al.*, 2010; Kozera *et al.*, 2013; Aüimoviü *et al.*, 2015). Also, Baranauskienn *et al.*, (2003) revealed that nitrogen application resulted in an increase of total oil yield of *Thymus vulgaris*. Meanwhile, Swarupa *et al.*, (2003) revealed that the maximum growth parameters and seed yield of *Plantago ovata* plants were obtained when plants treated with 50 kg N ha⁻¹ and 30 kg P₂O₅ ha⁻¹. On the other hand, Paschalina *et al.*, (2006) pointed out that essential oil yield and composition of fennel cultivars did not affected by nitrogen levels (60, 80, 100 kg ha⁻¹). Moreover, Omer *et al.*, (2008) pointed out that application of ammonium sulfate at a rate of 60 kg fed⁻¹ at two equal doses is recommended to produce the maximum volatile oil yield in *Ocimum americanum*. Meanwhile, Kandil *et al.*, (2009) revealed that application of compost (organic fertilizer) affects the composition of essential oil of *O. basilicum* in the same way as the effect of ammonium nitrate as an inorganic fertilization. Adding compost to basil plants not only increases oil yield, but also increases content of linalool in the oil, and at the same time decreases methyl chavicol and 1, 8-cineole content (Taie *et al.*, 2010). Also, Abdel-Rahman *et al.*, (2008) on fennel plant revealed that application of active dry yeast at 4 g/l as biofertilizer with 100 ppm tryptophan as organic nitrogenous source gave nearly equal results to those obtained with the high level of mineral nitrogen fertilizer (100 kg N/fed.) alone. The maximum vegetative and flowering growth, fruit and oil yield as well as leaf nutrients content were obtained from the combined treatment of 50 kg N/fed. + 2 or 4 g/l active dry yeast + 50 ppm tryptophan.

Therefore, the aim of this study was to investigate the influence of endophytic fungus (*Alternaria alternate*) and nitrogen fertilizer as ammonium sulfate on the growth, fruit yield, essential oil percentage, oil yield and its components of caraway plants grown under newly reclaimed soil conditions.

2. Materials and Methods

2.1. Site, experimental description and planting seeds

This study was carried out under drip irrigation during the two successive seasons of 2018/2019 and 2019/2020 at Al-Marashda Agricultural Research Station, Agric. Res. Center (ARC), Qena Governorate, Egypt (which, lies between latitude 26°, 09' N, longitude 32°, 42' E). The main objective was to study the influence of endophytic fungus i.e. *Alternaria alternata* and nitrogen fertilizer as ammonium sulfate (20.5% N) on the growth and production of caraway (*Carum carvi L.*) plants grown under newly reclaimed sandy soil conditions. The fruits of caraway (*Carum carvi L.*) were obtained from the Medicinal and Aromatic Dept., ARC, Ministry of Agriculture, Egypt and sown in newly reclaimed sandy soil at the beginning of November for both seasons. Soil samples were obtained from a depth of 30 cm from the used soil surface in this study and some physical and chemical properties of the soil were done according to the methods described by Jackson (1973) and Black *et al.*, (1982) as

shown in Table (1). The organic fertilization as commercial compost at rate of 5 tons/fed. was applied and incorporated to the top 5 cm layer of the experiment soil before the sowing seeds in each season in only one dose. The chemical characteristics of the used compost are shown in Table (2).

A spilt-plot in randomized complete block design (RCBD) with three replicates was followed in this experiment. The experiment contained of 9 treatments. The three levels of nitrogen fertilizer (control, 30 and 60 kg N/fed.) were presented in the main-plots, while spraying times of endophytic fungus (*Alternaria alternata*) i.e. control, 3 and 6 times per season were assigned in sub-plots. Each sub-plot of 3.0 × 3.5 m contained 7 rows. The planting distance was 25 cm between plants and each row contained 12 hills. After 45 days from planting, the plants were thinned to one plant per hill (84 plants/sub plot) i.e. 33,600 plants/fed.

Nitrogen fertilizer in the form of ammonium sulfate (20.5%) was applied with irrigation water in six equal doses after 45 days from seed sowing until the beginning of flowering. Meanwhile, endophytic fungus (*Alternaria alternata*) was sprayed three times per season. The first time of spraying began after 45 days from sowing seeds and the rest times was applied at 15 days intervals. Stock culture of *Alternaria alternata* fungus (4×10^2 cfu ml⁻¹) was diluted with tap water to produce a 0.3% solution and the prepared solution was used immediately. Watering was applied as drip irrigation according to the need of the caraway plants and the soil condition, water analysis is shown in Table (3). All other agricultural practices were carried out as recommended.

Table 1: Some of the physical and chemical properties of the experimental soil site during 2018/2019 season.

Texture class	Particle size distribution			CaCO ₃ %	EC (dS m ⁻¹) (1 : 2.5)	pH (1-5)	
	Sand %	Silt %	Clay %				
Sandy	81.3	12.7	6.0	12.55	3.01	8.08	
Na ⁺	Cation (meq L ⁻¹)				Anion (meq L ⁻¹)		
	K ⁺	Ca ⁺⁺	Mg ⁺⁺	CO ₃ ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻
30.02	0.88	12.0	6.2	0.0	0.82	30.6	17.9

Table 2: Chemical analysis of the commercial compost used in the experimental site.

Character	pH (1 :5)	EC (dS m ⁻¹) (1 : 2.5)	O.M%	N%	P%	K%	C/N ratio	Moisture content %	O.C %	Ca %

Table 3: Water analysis of the irrigation water used for the experimental site.

TDS mg/l	pH (1 : 5)	EC (dS m ⁻¹) (1 : 2.5)	Soluble cations (mg/l.)				Soluble anions (mg/l.)			
			Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	CO ₃ ⁻	HCO ₃ ⁻	SO ₄ ⁻	Cl ⁻
225.5	7.3	3.25	28.5	8.8	31.6	6.2	24.7	110.5	41.6	31.2

2.2. Fungal isolation, identification and cultivation

The leaves of *Inga edulis* trees were collected from a private farm in Qena Governorate, Egypt. Leaf samples were washed carefully with tap water, then with sterile distilled water. The surface of the samples was sterilized by sequential immersion in 75% ethyl alcohol for 1 minute, 4% sodium hypochlorite for 3 minutes and finally 75% ethyl alcohol for 30 seconds. Samples were then rinsed using sterile distilled water, and shade-drying (Filip *et al.*, 2003). The leaves were then cut into small pieces and placed on pre-poured glucose-Czapek's agar plates according to Smith and Dawson (1944) and then incubated at 28 ± 2° C for 2-3 weeks in a microbiological incubator until the desired fungus growth i.e. *Alternaria alternata*.

2.3. Growth and yield characteristics

At full ripeness fruit stage in the first of May caraway plants were harvested. Ten plants were randomly chosen from each experimental unit in both seasons and submitted to measure the following plant growth parameters and yield; plant height (cm), branch number/plant, plant fresh and dry weight (g), umbel number/plant, weight of 1000 fruits (g), fruit yield per plant (g) and fruit yield per feddan

(kg). However, total fruit yield/feddan was estimated by multiplying fruit yield/plot by 400 (plot number per feddan).

2.4. Chemical constituents

The chemical constituents in dry leaves of caraway plants were determined at the end of experiments. Nitrogen, phosphorus and potassium percentages were determined according to the method described by Cottenie *et al.*, (1982). Total carbohydrates percentage was determined calorimetrically according to Herbert *et al.*, (1971). The essential oil percentage in caraway fruits was extracted by hydro-distillation according to Guenther (1961). Oil yield/feddan was calculated by multiplying the essential oil percentage by fruit yield/feddan. However, GLC analysis of volatile oil was used for the different treatments using Hewlett-Packard GC. model 5890. Carbowax was a combined 20 m silica capillary column measuring 20 m x 0.32 mm with an inner diameter, a film thickness of 0.17 μm , the temperature was 75 ° C for 5 minutes and increased 4° C/min to 220° C (10 minutes). Helium is the carrier gas and the working flow rate is 1.0 ml/min, and the detector was 9144 hp. The temperature of the detector is 280° C and the injector is 240° C. The separated compounds were determined by matching their retention times with those of the original samples that were injected under the same conditions.

2.5. Statistical analysis

The obtained data were subjected to the statistical analysis of variance by using Statistix 8.1 software (Analytical Software, 2005). The means were compared using the least significant difference (L.S.D.) test at probability level of ≤ 0.05 for comparison according to Gomez and Gomez (1983).

3. Results and Discussion

3.1. Growth characteristics

The growth characteristics of caraway plants as affected by endophytic fungus (*Alternaria alternata*) and nitrogen fertilizer are shown in Tables (4, 5, 6 and 7) during the two studied seasons. The tabulated data revealed that there are significant differences in the plant height, branch number, fresh and dry weight of caraway plants as a result of endophytic fungus and nitrogen fertilizer treatments during the two seasons. However, the highest values of these parameters were obtained when plants sprayed with *A. alternata* 6 times per season compared to control and the other spraying times. Meanwhile, nitrogen fertilizer at a rate of 60 kg N/feddan was more effective and significantly increased vegetative growth characteristics as compared to the control in both seasons. On the other hand, the interaction effect between endophytic fungus and nitrogen fertilizer treatments was not significant for plant height and branch number in the two seasons, while it was significant for plant fresh and dry weight in both seasons. These results are in accordance with those obtained by Nofal *et al.*, (2001), El-Tohamy and El-Greadly (2007), Kenawy (2010), Matter and El Sayed (2015) and Ahmed (2017). Meanwhile, Ebeid and Shebany (2017) pointed out that treatment of *Chrysophyllum oliviforme* plants with endophytic fungi (*A. alternata*) improved the seedling growth characteristics and leaf mineral content.

Table 4: Plant height (cm) of caraway plants as affected by endophytic fungus (*Alternaria alternata*) and nitrogen fertilizer during 2018/2019 and 2019/2020 seasons.

Nitrogen Fertilizer (A)	Plant height (cm)							
	2018/ 2019				2019/ 2020			
	Spraying times of endophytic fungus (B)							
	Control	3 times	6 times	Mean	Control	3 times	6 times	Mean
Control	83.3	83.5	88.5	85.1	75.4	80.3	84.8	80.2
30 kg N/ feddan	83.2	89.2	93.7	88.7	78.7	83.1	87.8	83.2
60 kg N/ feddan	92.3	95.0	105.5	97.5	90.5	95.6	101.1	95.7
Mean	86.3	89.2	95.9		81.5	86.3	91.2	
LSD at 0.05	A	B	AB		A	B	AB	
	3.5	2.6	N.S		1.5	1.50	N.S	

Table 5: Branch number/plant of caraway plants as affected by endophytic fungus (*Alternaria alternata*) and nitrogen fertilizer during 2018/2019 and 2019/2020 seasons.

Nitrogen Fertilizer (A)	Branch number / plant							
	2018/ 2019				2019/ 2020			
	Spraying times of endophytic fungus (B)							
	Control	3 times	6 times	Mean	Control	3 times	6 times	Mean
Control	8.0	9.8	11.3	9.7	7.1	9.0	10.9	9.0
30 kg N/ feddan	9.2	10.7	12.1	10.7	8.3	10.2	11.6	10.0
60 kg N/ feddan	10.2	12.0	12.9	11.7	9.6	11.9	14.0	11.8
Mean	9.1	10.9	12.1		8.3	10.4	12.1	
LSD at 0.05	A	B	AB		A	B	AB	
	0.6	0.5	N.S		0.5	0.38	N.S	

Table 6: Fresh weight (g/plant) of caraway plants as affected by endophytic fungus (*Alternaria alternata*) and nitrogen fertilizer during 2018/2019 and 2019/2020 seasons.

Nitrogen Fertilizer (A)	Fresh weight (g/plant)							
	2018/ 2019				2019/ 2020			
	Spraying times of endophytic fungus (B)							
	Control	3 times	6 times	Mean	Control	3 times	6 times	Mean
Control	223.3	236.0	246.8	235.4	228.5	237.4	245.0	237.0
30 kg N/ feddan	244.9	256.5	264.3	255.6	247.0	257.1	266.6	256.9
60 kg N/ feddan	253.3	273.1	294.1	273.5	264.2	277.7	293.9	278.6
Mean	240.5	255.5	268.4		246.6	257.4	268.5	
LSD at 0.05	A	B	AB		A	B	AB	
	1.4	2.0	3.5		3.1	2.0	3.5	

Table 7: Dry weight (g/plant) of caraway plants as affected by endophytic fungus (*Alternaria alternata*) and nitrogen fertilizer during 2018/2019 and 2019/2020 seasons.

Nitrogen Fertilizer (A)	Dry weight (g/plant)							
	2018/ 2019				2019/ 2020			
	Spraying times of endophytic fungus (B)							
	Control	3 times	6 times	Mean	Control	3 times	6 times	Mean
Control	98.1	109.8	124.2	110.7	97.7	106.5	125.9	110.0
30 kg N/ feddan	123.6	133.0	143.3	133.3	125.4	137.8	141.5	134.9
60 kg N/ feddan	126.4	137.2	146.8	136.8	128.6	136.9	145.7	137.1
Mean	116.0	126.7	138.1		117.2	127.1	137.7	
LSD at 0.05	A	B	AB		A	B	AB	
	2.7	2.8	4.9		2.0	2.4	4.1	

3.2. Flowering and fruiting characteristics

Tabulated results in Tables (8, 9, 10 and 11) show the effects of endophytic fungus and N fertilizer on the yield of caraway plants during 2018/2019 and 2019/2020 seasons. Results revealed that all spraying times of endophytic fungus significantly increased number of umbels/plant, fruit weight/plant, weight of 1000 fruits and fruit yield/feddan compared with untreated plants (control). However, spraying plants with endophytic fungus 6 times was the most effective treatment in increasing flowering and fruiting characteristics during both seasons. Also, there was a significant difference in yield parameters as a result of nitrogen fertilizer levels in the two seasons. It could be concluded that increasing the N fertilizer level up to 60 kg N/feddan resulted in the highest flowering and fruiting characteristics for both seasons. Apparently, the interaction between endophytic fungus and nitrogen fertilizer was positively affected caraway yield. The highest values of yield parameters were recorded with plants spraying with endophytic fungus 6 times per season combined with 60 kg N/feddan. These results are in agreement with those mentioned by Eid (2001), Nofal *et al.*, (2001), Aly *et al.*, (2007), Abd El-Azim and Abd El-Gawad (2008), Hemdan (2008), Ibrahim (2014) and Matter and El Sayed (2015). Also, previous studies indicated that application of these endophytic fungi leads to an increase in the growth and productivity of some plants such as basil (Copetta *et al.*, 2006; Toussaint *et al.*, 2008; Baum *et al.*, 2015; Sabra *et al.*, 2018). The obtained results in the present study revealed that caraway

plant is a very good host for *A. alternata* and hence the fungal spraying might help to improve caraway growth and productivity, especially when planting in conditions like of the experimental conditions with newly reclaimed soils.

Table 8: Umbels number/plant of caraway plants as affected by endophytic fungus (*Alternaria alternata*) and nitrogen fertilizer during 2018/2019 and 2019/2020 seasons.

Nitrogen Fertilizer (A)	Umbels number/ plant							
	2018/ 2019				2019/ 2020			
	Spraying times of endophytic fungus (B)							
	Control	3 times	6 times	Mean	Control	3 times	6 times	Mean
Control	42.3	45.9	55.1	47.8	44.7	48.5	56.1	49.7
30 kg N/ feddan	53.1	56.8	65.3	58.4	53.1	55.7	66.4	58.4
60 kg N/ feddan	64.6	72.1	75.9	70.9	64.9	75.2	77.5	72.5
Mean	53.3	58.3	65.4		54.2	59.8	66.7	
LSD at 0.05	A	B	AB		A	B	AB	
	1.0	1.9	N.S		1.6	1.8	3.1	

Table 9: Fruit yield (g/plant) of caraway as affected by endophytic fungus (*Alternaria alternata*) as spraying and nitrogen fertilizer during 2018/2019 and 2019/2020 seasons.

Nitrogen Fertilizer (A)	Fruit yield (g/plant)							
	2018/ 2019				2019/ 2020			
	Spraying times of endophytic fungus (B)							
	Control	3 times	6 times	Mean	Control	3 times	6 times	Mean
Control	12.0	14.3	17.2	14.5	11.8	13.9	16.3	14.0
30 kg N/ feddan	15.1	17.3	18.9	17.1	15.2	17.2	19.6	17.4
60 kg N/ feddan	18.2	22.6	27.1	22.6	18.7	22.4	27.7	22.9
Mean	15.1	18.0	21.0		15.3	17.8	21.2	
LSD at 0.05	A	B	AB		A	B	AB	
	0.7	0.6	1.0		0.8	0.7	1.2	

Table 10: Weight of 1000-fruits (g) of caraway plants as affected by endophytic fungus (*Alternaria alternata*) and nitrogen fertilizer during 2018/2019 and 2019/2020 seasons.

Nitrogen Fertilizer (A)	Weight of 1000-fruits (g)							
	2018/ 2019				2019/ 2020			
	Spraying times of endophytic fungus (B)							
	Control	3 times	6 times	Mean	Control	3 times	6 times	Mean
Control	5.77	7.10	8.23	7.03	5.47	6.83	8.37	6.89
30 kg N/ feddan	7.00	8.57	9.40	8.32	7.30	8.50	9.30	8.37
60 kg N/ feddan	8.57	9.33	10.50	9.47	8.50	9.57	10.73	9.60
Mean	7.11	8.33	9.38		7.09	8.30	9.47	
LSD at 0.05	A	B	AB		A	B	AB	
	0.44	0.22	0.38		0.55	0.12	0.21	

Table 11: Fruit yield (kg/feddan) of caraway plants as affected by endophytic fungus (*Alternaria alternata*) and nitrogen fertilizer during 2018/2019 and 2019/2020 seasons.

Nitrogen Fertilizer (A)	Fruit yield (kg/feddan)							
	2018/ 2019				2019/ 2020			
	Spraying times of endophytic fungus (B)							
	Control	3 times	6 times	Mean	Control	3 times	6 times	Mean
Control	453.1	483.1	503.2	479.8	462.0	478.2	493.2	477.8
30kg N/ feddan	564.5	604.5	654.9	608.0	563.1	613.7	673.3	616.7
60 kg N/ feddan	6.17.5	672.8	715.5	668.6	629.5	684.6	734.4	682.9
Mean	545.0	586.8	624.5		551.6	592.2	633.6	
LSD at 0.05	A	B	AB		A	B	AB	
	3.5	2.3	4.0		4.01	1.93	3.34	

3.3. Phytochemical composition

3.3.1. Essential oil percentage and yield

The presented data in Tables (12 and 13) indicate the response of essential oil percentage and oil yield/feddan of caraway plants as affected by endophytic fungus and nitrogen fertilizer during 2018/2019 and 2019/2020 seasons. The results revealed that endophytic fungus treatments significantly affected essential oil percentage and oil yield/feddan during the two seasons. Generally, spraying plants 3 or 6 times with endophytic fungus resulted in the highest values compared to the control. On the other hand, the maximum values of essential oil percentage and oil yield were obtained from plants treated with 60 kg N/feddan in both seasons. The interactions between the two studied factors showed significant differences in essential oil percentage and oil yield/feddan for the two seasons. However, the highest essential oil percentage and oil yield/feddan were obtained mostly when plants sprayed 3 times with endophytic fungus combined with 60 kg N/feddan during both seasons. These results are in harmony with those reported by Hemdan (2008), Eid and Kassem (2009), Shebany and Abdel-Kader (2013), Sabra (2014) and Matter and El Sayed (2015). In this respect, Sabra *et al.*, (2018) revealed that beneficial fungi can be used to increase the quantity and quality of sweet basil. In addition, Kang *et al.*, (2019) pointed out that the host plant exposed to stress absorbs the required amounts of minerals from the soil after endophytic association.

Table 12: Essential oil percentage in caraway fruits as affected by endophytic fungus (*Alternaria alternata*) and nitrogen fertilizer during 2018/2019 and 2019/2020 seasons.

Nitrogen Fertilizer (A)	Essential oil percentage							
	2018/ 2019				2019/ 2020			
	Spraying times of endophytic fungus (B)				Spraying times of endophytic fungus (B)			
	Control	3 times	6 times	Mean	Control	3 times	6 times	Mean
Control	2.06	2.53	2.55	2.38	2.16	2.50	2.71	2.46
30 kg N/ feddan	2.61	2.75	2.89	2.75	2.53	2.82	2.89	2.75
60 kg N/ feddan	2.67	3.04	2.76	2.82	2.63	3.13	2.84	2.87
Mean	2.45	2.73	2.73		2.44	2.82	2.82	
LSD at 0.05	A	B	AB		A	B	AB	
	0.07	.06	0.1		0.06	0.07	0.13	

Table 13: Oil yield (L/feddan) in caraway fruits as affected by endophytic fungus (*Alternaria alternata*) and nitrogen fertilizer during 2018/2019 and 2019/2020 seasons.

Nitrogen Fertilizer (A)	Oil yield (l/feddan)							
	2018/ 2019				2019/ 2020			
	Spraying times of endophytic fungus (B)				Spraying times of endophytic fungus (B)			
	Control	3 times	6 times	Mean	Control	3 times	6 times	Mean
Control	9.35	12.21	12.85	11.47	9.98	11.94	13.37	11.76
30 kg N/ feddan	14.75	16.64	18.91	16.77	14.23	17.31	19.48	17.00
60 kg N/ feddan	16.49	20.45	19.75	18.90	16.54	21.45	20.88	19.62
Mean	13.53	16.53	17.17		13.58	16.90	17.91	
LSD at 0.05	A	B	AB		A	B	AB	
	0.47	0.33	0.58		0.42	0.52	0.73	

3.3.2. Leaf mineral content

As shown in Tables 14, 15 and 16, contents of N, P and K in caraway leaves were significantly varied according to endophytic fungus spraying and nitrogen levels. Generally, plants sprayed with endophytic fungus 6 times and treated with 60 kg N/feddan resulted in the highest N content compared to the other combined treatments in both seasons. Meanwhile, combined treatment of endophytic fungus 6 times per season and 30 kg N/feddan resulted in the highest values of P and K contents compared to the other combined treatments in most cases. The current findings are similar to those reported by Abdou *et al.*, (2004), Shebany and Abdel-Kader (2013), Sabra (2014) and Matter and El Sayed (2015). In this respect, Strobel *et al.*, (2004) on tall fescue and Ren *et al.*, (2008) on ryegrass and studied the impact of N fertilizer and endophytic fungi on the mineral content in the and revealed that endophytic fungi increased the mineral concentrations in roots and shoots.

Table 14: Nitrogen percentage in caraway leaves as affected by endophytic fungus (*Alternaria alternata*) and nitrogen fertilizer during 2018/2019 and 2019/2020 seasons.

Nitrogen Fertilizer (A)	Nitrogen %							
	2018/ 2019				2019/ 2020			
	Spraying times of endophytic fungus (B)							
	Control	3 times	6 times	Mean	Control	3 times	6 times	Mean
Control	1.37	1.58	1.64	1.53	1.32	1.47	1.71	1.50
30 kg N/ feddan	1.55	1.75	1.78	1.69	1.54	1.64	1.73	1.64
60 kg N/ feddan	1.74	1.78	1.85	1.79	1.71	1.81	1.88	1.80
Mean	1.55	1.70	1.76		1.52	1.64	1.77	
LSD at 0.05	A 0.06	B 0.04	AB 0.07		A 0.04	B 0.03	AB 0.05	

Table 15: Phosphorus percentage in caraway leaves as affected by endophytic fungus (*Alternaria alternata*) and nitrogen fertilizer during 2018/2019 and 2019/2020 seasons.

(A) Nitrogen Fertilizer	Phosphorus %							
	2018/ 2019				2019/ 2020			
	Spraying times of endophytic fungus (B)							
	Control	3 times	6 times	Mean	Control	3 times	6 times	Mean
Control	0.30	0.34	0.40	0.35	0.28	0.36	0.43	0.36
30 kg N/ feddan	0.36	0.48	0.53	0.46	0.41	0.45	0.53	0.46
60 kg N/ feddan	0.34	0.36	0.40	0.37	0.35	0.38	0.40	0.38
Mean	0.33	0.39	0.44		0.35	0.40	0.45	
LSD at 0.05	A 0.03	B 0.02	AB 0.04		A 0.03	B 0.02	AB 0.04	

Table 16: Potassium percentage in caraway leaves as affected by endophytic fungus (*Alternaria alternata*) and nitrogen fertilizer during 2018/2019 and 2019/2020 seasons.

Nitrogen Fertilizer (A)	Potassium %							
	2018/ 2019				2019/ 2020			
	Spraying times of endophytic fungus (B)							
	Control	3 times	6 times	Mean	Control	3 times	6 times	Mean
Control	1.27	1.37	1.45	1.37	1.37	1.39	1.50	1.42
30 kg N/ feddan	1.49	1.57	1.63	1.56	1.45	1.57	1.71	1.58
60 kg N/ feddan	1.50	1.65	1.60	1.58	1.51	1.67	1.64	1.61
Mean	1.42	1.53	1.56		1.44	1.54	1.61	
LSD at 0.05	A 0.03	B 0.03	AB 0.06		A 0.07	B 0.03	AB 0.06	

3.4. Total carbohydrates percentage

The obtained results in Table (17) revealed that all treatments of endophytic fungus and nitrogen fertilizer caused significant differences in total carbohydrates percentage in caraway leaves during both seasons. However, the highest total carbohydrates percentages resulted from the individual treatment of either endophytic fungus 6 times per season or 60 kg N/feddan in both seasons. The interaction between endophytic fungus and N fertilizer caused significant differences in total carbohydrates percentage. The highest values were obtained from the combined treatment of spraying endophytic fungus 3 times and 60 kg N/feddan for both seasons. These results are in parallel to those obtained by Hemdan (2008), Eid and Kasseem (2009), Sabra (2014) and Matter and El Sayed (2015). In this regard, several investigators reported that some endophytic fungi are among the biologically active species. They added that it involves in the production of chemically diverse metabolites compounds. Besides, endophytic fungi have been reported to increase carbohydrate concentrations in host plant (Hunt *et al.*, 2005; Rasmussen *et al.*, 2007; Ebeid and Shebany, 2017).

Table 17: Total carbohydrates percentage in caraway leaves as affected by endophytic fungus (*Alternaria alternata*) and nitrogen fertilizer during 2018/2019 and 2019/2020 seasons.

Nitrogen Fertilizer (A)	Total carbohydrates percentage							
	2018/ 2019				2019/ 2020			
	Spraying times of endophytic fungus (B)							
	Control	3 times	6 times	Mean	Control	3 times	6 times	Mean
Control	10.70	12.95	14.93	12.86	11.88	13.74	15.49	13.70
30 kg N/ feddan	13.88	16.03	18.00	15.97	14.74	16.47	17.73	16.31
60 kg N/ feddan	15.96	18.63	17.52	17.37	15.49	18.35	17.31	17.04
Mean	13.51	15.87	16.81		14.03	16.19	16.84	
LSD at 0.05	A	B	AB		A	B	AB	
	0.64	0.26	0.45		0.26	0.34	0.59	

3.5. Essential oil constituents

The presented data in Table (18) show the different chemical compounds in essential oil of caraway fruits in response to endophytic fungi and N fertilizer treatments. The results indicated that the most important chemical compounds in essential oil are carvone, limonene, linalool, caryophyllene, β -pinene, p-cymene, α -pinene, carveol and dihydrocarvone. The mean values of carvone (major compound) in the essential oil showed gradually increase due to increasing nitrogen level from 0 to 30 then 60 kg N/fed. The same trend was noticed with spraying times of *Alternaria alternata* fungus. Most of the different treatments for nitrogen fertilizer and spraying times of *Alternaria alternata* fungus showed an increase in some of these component especially, carvone, linalool, α -Pinene and carvone to limonene(C/L) ratio compared with the control (untreated plants) which, increased limonene content. The highest level of caryophyllene compound (2.90%) recorded when plants sprayed 6 times endophytic fungi without nitrogen fertilizer, followed by linalool, which recorded the maximum value in essential oil (2.22%) when sprayed 3 times combined with 60 kg N/feddan. The maximum carvone content (73.8 %), the minimum limonene level (13 %) and the highest ratio (4.93 %) of carvone to limonene(C/L) were resulted from spraying caraway plants 3 times per season of endophytic fungus and using 60 kg N/ feddan compared to the control and other treatments. These results are in accordance with the findings of Hendawy and Khalid (2011), Sabra (2014), Matter and El Sayed (2015) and Omar (2020) on caraway. Interestingly, there is evidence that biosynthesis of carvone and limonene occurs via the same pathway. This implies that limonene in both intermediate in the biosynthesis of carvone, as well as the end product (Bouwmester *et al.*, 1995).

Table 18: Chemical composition of the essential oil in caraway fruits as affected by endophytic fungus (*Alternaria alternata*) and nitrogen fertilizer during 2018/2019 and 2019/2020 seasons.

Compound %	Nitrogen fertilizer (kg N/feddan)								
	Control			30			60		
	Spraying times of endophytic fungus								
	Cont.	3 times	6 times	Cont.	3 times	6 times	Cont.	3 times	6 times
α -pinene	0.37	0.55	0.70	0.45	0.80	0.92	1.00	1.2	1.08
β -pinene	1.08	0.71	0.81	0.71	0.88	1.05	0.9	1.08	0.83
Limonene	25.4	22.60	20.60	21.00	19.80	17.5	19.00	17.00	17.2
P-cymene	0.55	0.65	0.89	0.68	1.00	1.10	0.78	0.97	0.80
Linalool	1.82	1.83	2.00	1.81	1.98	2.10	1.92	2.22	2.08
Caryophyllene	1.82	2.05	2.90	1.70	1.17	1.19	1.00	0.92	0.78
Carvone	65.22	67.22	69.5	68.11	71.3	73.2	71.43	73.8	73.7
Dihydrocarvone	0.17	0.24	0.26	0.23	0.38	0.35	0.27	0.42	0.38
Carveol	0.33	0.40	0.45	0.38	0.63	0.88	0.40	0.82	0.80
Total	96.66	96.25	98.11	95.07	97.94	98.29	96.7	98.43	97.65
Unidentified compound	3.34	3.75	1.89	4.93	2.06	1.71	3.30	1.57	2.35
Carvone/Limonene ratio	2.6	2.97	3.37	3.24	3.60	4.18	3.76	4.93	4.28

Results of our study may be due to the role of nitrogen fertilization in increase the growth and development of the caraway plants; where the use of endophytic fungi as *A. alternata* was found to release certain of phytohormones and auxins which could enhance plant growth and yield of caraway through improvement uptake of nutrients and enhancing photosynthesis process in plants (AbouAlhamed and Shebany, 2012). In this respect, Hawaka (2000) revealed that microorganisms as bio-fertilizers may affect the growth and production of plants through their actions as nitrogen fixation, promoting substances, organic acids and enhancing nutrients uptake as well as through protection of pathogens.

4. Conclusion

It could be concluded that spraying caraway plant with *Alternaria alternata* at 0.3% as one of endophytic fungus 3 or 6 times per season combined with 60 kg N/feddan as ammonium sulfate under the newly reclaimed sandy soil conditions gave the best vegetative growth and productivity as well as improved the chemical compounds in essential oil of caraway fruits and markedly increased quality of oil (oil %, carvone content and carvone to limonene ratio) compared to the control.

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