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Response of *Ruta graveolens* L. to Sowing Dates and Foliar Micronutrients

[1]Naguib Y.N., [1]M.S. Hussein, [1]S.E. El-Sherbeny, [1]M.Y. Khalil and [2]D. Lazari

[1]Cultivation and Production of Medicinal and Aromatic Plants
Department National Research Center- Cairo, Egypt.
[2]Laboratory of Pharmacognosy, School of Pharmacy,
Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece.

Abstract: Two experiments were conducted during two successive seasons of 2004/2005 and 2005/2006 to study the influence of planting dates and/or foliar micronutrients (Mn and Zn) on growth, flowering, and chemical constituents for rue (*Ruta graveolens* L.). Results show that seeds planted on 1st of October, produced significantly the highest plants, more branches and the heaviest weight of leaves and stems, compared to those planted on the 1st of November. The uptake of nitrogen and phosphorus, percentage of coumarin, and rutin as well as essential oil percentage and yield of herb (g/plant), gave higher accumulation at second sowing date. Micronutrients uptake of Fe, Mn, and Zn produced higher percentage with first sowing date. Results; also; indicated that foliar spray with 100ppm Fe was more effective in promoting vegetative growth characters compared with other levels of micronutrients used. Similarly, highest uptake of potassium and zinc percentage was recorded with application of 100ppm Fe, while highest mean values of nitrogen and Mn uptake was produced with 100ppm Zn. Higher percentage of rutin and coumarin were observed with 50ppm Fe and 50ppm Mn respectively. Essential oil yield of herb and flowers gave higher significant values with 100ppm Fe. The interaction effect of sowing date and foliar micronutrients were discussed.

Keywords: Ruta graveolens; essential oil; rutin; coumarin; foliar fertilizers; micronutrients; sowing dates

INTRODUCTION

Ruta graveolens L. (common rue) is a small evergreen sub-shrub or semi-woody perennial. It is native to South Europe and Northern Africa. The medicinal and aromatic properties of rue come from its essential oils, rutin and methyl-nonelcetone. Rutin increases visual sharpness and benefits other visual problems and was used against edema, thrombogensis, inflammation, spasms, and hypertension. It is an antihistamine, a vermifuge, and a rubefacient. Its bitter eupeptic properties make it useful for stomach problems. On moist skin in direct sunlight, it leads to photosensitivity. The essential oil is a central nervous system depressant, and at high doses, a narcotic poison[1,2,3].

A novel treatment for human brain cancer revealed by Pathak *et al.,*[4], who propose that Ruta in combination with $Ca_3(PO_4)$ could be used for effective treatment of brain cancer particularly glioma.

Since, the foliar application of plant nutrient is an additional channel to nutrition, as well as, regulating roots uptake, thus the changing in level of mineral in above ground plant organs are not so much attributable to foliar absorption, but rather to effect of the latter on the uptake of nutrients by root system. In addition, the foliar fertilizer is a beneficial technique to meet the deficiencies of one or more of macro or micro elements, strengthen weak or damaged crops, speed growth and grow better and healthier plants. The promotional effects of micronutrient foliar fertilizer on growth and production of some medicinal and aromatic plants were revealed by several researchers, i.e. on coriander plant using Fe, Mn and Zn9[5]; on sunflower spraying with Zn, Mn, Cu and Fe[6], on *Cymbopogon citratus* applying (B, Mo, Co and Pb)[7] and on *Cineraria maritima* applying (B, Fe and Zn)[8].

Sowing date is one of the most important limiting factors that influence on plant growth and production. The early sowing date enhanced the growth characters for various plants, such as *Cyamopsis tetragonoloba*[9], *fennel*.[10] and *Hyoscyamus muticus* L.[11]. Meanwhile, the late sowing date was more favorable for promoting the constituents of other plants i.e. *Hibiscus sabdariffa*[12] and *Fagopyrum esculentum*[13]. Meanwhile, Meawad *et al*[14] on *Hyoscyamus muticus* revealed that

Corresponding Author: Naguib Y. N., Cultivation and Production of Medicinal and Aromatic Plants Department, National Research Center, Research St, Dokki, Cairo, P.O box: 12311, Egypt.
Tel: (202)37491417, Fax: 011(202)3337-0931, Mob. 0105098082
E-mail: nabilarateb@hotmail.com

middle sowing date at February 15th resulted the highest values of vegetative growth and root system characters as well as total alkaloid, yield/plant and per feddan and mineral content.

The present investigation was performed to assess the effect of two sowing date and foliar spray with some micronutrient on growth, flowering and chemical constituents of *Ruta graveolens* L.

MATERIALS AND METHODS

Materials: The present study was conducted during two successive seasons of 2004/2005 and 2005/2006 under green house conditions at NRC, Dokki, Cairo, Egypt. Seeds of rue plants were obtained from the Experimental Farm of Pharmaceutical Faculty at Giza. Seeds were sown in earthen were pots No. 30 which was filled with 10 Kg air dried soil. The physical and chemical properties of the experimental soil were determined according to Black[15] and shown in Table (1).

Methods: The seeds of *Ruta graveolens* L. (rue) were sown at two dates, the first at 1st October and the second 1st of November during the two seasons. About twenty seeds were sown per pot and irrigation requirements were regularly fulfilled throughout the experimental periods using tap water. Two weeks after sowing, the seedlings were thinned to two seedlings only. The pots were divided into two groups, representing the two sowing dates. Every group divided to seven subgroups which representing seven treatments (each treatment included ten pots). The treatments were arranged at randomized design and included the following:

- Treatment 1: The plants were sprayed with distilled water (Control)
- Treatment 2: The plants were sprayed with Fe at 50 ppm level
- Treatment 3: The plants were sprayed with Fe at 100 ppm level
- Treatment 4: The plants were sprayed with Mn at 50 ppm level
- Treatment 5: The plants were sprayed with Mn at 100 ppm level
- Treatment 6: The plants were sprayed with Zn at 50 ppm level
- Treatment 7: The plants were sprayed with Zn at 100 ppm level

A basal dose of NPK was applied one month after sowing for all plants using 2g Super phosphate, and 1gm potassium sulphate for each pot. The micronutrients Fe, Mn, and Zinc used as Nervanaid containing (13%) at two levels of 50 and 100ppm sprayed two times, 30 and 45 days after sowing.

Table 1: Physical and Chemical Properties of the Experimental Soil

Properties	First season, 2004	Second season, 2005
Sand %	48.8	50.8
Silt %	28	26
Clay %	23.2	23.2
Soil Texture	Sandy Loam	Sandy Loam
pH	8.2	8.05
E.C.(mmhos/cm)	0.68	0.88
Soluble Ions (Soil Paste) meq / liter		
Ca++	1.1	1.19
Mg++	0.89	0.76
Na+	2.22	2.31
K+	0.18	0.21
Co3-	0.15	0.21
HCO3-	0.68	0.66
CL-	2	2.32
So4-	1.12	1.14
Available Elements (ppm)		
Total N	130	160
P	20.1	38.2
K	222.4	223.8
Fe	80	71
Mn	7.1	8.9
Zn	0.95	1.1
Cu	2.02	1.91

Growth Characters Recorded: During flowering stage, rue plants were collected in two harvesting dates (15th April and on 15th May) for first and second sowing dates in both seasons respectively. The growth measurements were taken as plant height (cm), number of branches/plant, fresh and dry weights of leaves, stems and roots (g/plant) as well as fresh weight of flowers (g/plant).

Chemical Constituents: Fresh herbage and flowers treatments were separately subjected to water distillation for 3 hours according to Guenther method[16] to determine essential oil content (%). The composition of the volatile constituents was established by GC-MS analyses. GC-MS analyses were performed on a Shimadzu GC-2010 – GCMS-QP2010 system operating in EI mode (70eV) equipped with a split/splitless injector (230^{0}C), a split ratio 1/30, using two different columns: a fused silica HP-5 MS capillary column (30m x 0.25mm (i.d.), film thickness: 0.25µm) and a HP-Innowax capillary column (30m x 0.25mm (i.d.), film thickness: 0.25µm). The temperature program for HP-5 MS column was from 50 ^{0}C (5min) to 290 ^{0}C at a rate of 4 ^{0}C/min and for HP-Innowax column from 40 ^{0}C to 260 ^{0}C (15min) at a rate of 2.5 ^{0}C/min. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. Injection volume of each sample was 1 µL. Retention indices for all compounds were determined according to the Van der Dool approach[17], using *n*-alkanes as standards. The identification of the components was based on comparison of their mass spectra with those of NIST21 and NIST107 Massada[18] and those described by Adams[19], as well as by comparison of their retention indices with literature data[19,20,21]. In many cases, the essential oils were subject to co-chromatography with authentic compounds (Fluka, Sigma).

Dried herb at (70ºC) was used to determine total nitrogen using the method provided by Hach *et al*[22] .

Macro and micro-elements were determined after wet digestion according to the method described by Chapman and Pratt[23].

Phosphorus was determined in digested material using vrandate molybdate method. Potassium was measured by flame photometer (Eppendroff). Micro-element was estimated using Atomic absorption Zeiss – FMD.

Rutin and coumarin content in rue herb were determined according to the method of Zummo *et al*[24] and Harbon[25], respectively.

Statistical Analysis: The determined data of the parameters of both seasons were arranged and the mean values were statistically analyzed as described by Snedecor and Cochran[26] using LSD at level 5%.

RESULTS AND DISCUSSIONS

Growth Characters:

Effect of Sowing Date: A perusal of data (Table 2) indicated that, the earlier sowing date of rue plants (1st October) caused significant promotion for plant height, number of leaves, as well as stems dry weight compared with the later sowing date (1st November). Meanwhile, the significant increment of branches number, fresh weight of leaves and stems were recorded with second sowing date. No remarkable variations were observed with other growth characters as a result of sowing dates. However, the promotion of some growth criteria by various sowing dates may be attributed to the favorable day length and temperature conditions, which produced better growth. In this connection, Mann and Vyas[27] on *Plantago ovata*, revealed that sown crop in 15th November showed significantly higher plant heights, number of leaves per plant and dry mater accumulation compared to the later sowing dates of (25November, 5 and15 December). Similarly, Singh *et al*[28] reported that palmarosa sown directly or transplanted on July 1st and July 21st produced maximum herb, while the delay in planting to August and September resulted in 40 and 55% reduction in yield, respectively. However, Singh *et al*[29] reported that peppermint planted on 30 December, was superior crop growth, comparing with other planting dates and that is attributed to favorable light and temperature conditions.

Effect of Micronutrient Fertilization: The application of Fe, Mn or Zn at different levels improved most of growth characters significantly, compared with the control treatment (Table 2). Generally, Fe at 100ppm was observed to be the most favorable growth characters micronutrient since it produced the highest promotion effect for most of growth parameters.

The maximum increment in plant height and number of branches reached to 21.3% and 23.3 %, respectively, Fresh weight of leaves, stems and roots recorded about 24.2%, 76.3%, 52.5% and dry weight of same organs reached to 36.8%, 75.0% and 24.0% above the control treatments, respectively. In general, the improvements in vegetative growth due to applying different micronutrients were in agreement with the findings of various investigators on different plants such as, on *Coriander sativum*[5], on some radish cultivars[30] and on *Cineraria maritima*[31].

Effect of Interaction Treatments Between Sowing Date and Micro-nutrient Fertilization: Data tabulated in Table (2) showed that sowing date of 1st October combined with Fe at 50ppm caused maximum mean values of leaves number/plant, as well as stem dry weight (g/plant); while, increasing Fe concentration from 50ppm up to 100ppm at the same early sowing date gave the highest mean value of plant height. On the other hand the interaction treatments between the later sowing dates (1st November) with Fe at 100ppm had a superior effect on number of branches/plant, fresh weight of different organs (g/plant) as well as leaves dry weight (g/plant).

Flowering Yield and Essential Oil Content:

Effect of Sowing Date: The comparison between both sowing dates revealed the superior effect of the later sowing date on the essential oil content (%) of herb and flowers as well as oil yield for herb (Table 3), which reached to 129.4%, 13.40% and 7.41% over that produced during first sowing date, respectively.

In this connection, Spitzovo[32] on foxglove (*Digitalis purpurea* L.) cited that late sowing (after May) reduced flower in the flowering season to 60-70% and when it was delayed until the end of June the plants didn't flower in the flowering year. Similarly, Lavy and Palevitch[33] on *Papaver bracteatum* found that flowering rate decreased from 92.1% in plants sown on the earliest date (19 August) to 74.2% in those sown on the latest date (13 September).

On the other hand, the first sowing date gave the maximum mean value of oil yield for flowers which reached 33.33% over the 2nd sowing date. The effect of sowing date on essential oil percentage and yield of various aromatic and medicinal plants were studied by several authors i.e. Rao[34] on *Mentha arvensis* revealed that crops planted in August, November and December produced significantly higher total biomass and essential oil yield than those planted in September and January. Meanwhile Muni *et al*[35], found that oil yield of four cultivars of *Mentha arvensis*, generally decreased with the delay in planting from 20th January to 20th February.

Effect of Micronutrient Fertilization: The data in (Table 3) show that the application of the higher level of Fe and Zn were more effective than the lower one in inducing marked increments in flowers fresh

Table 2: Effect of sowing date and micronutrients fertilization on vegetative growth characters of *Ruta graveolens* plants (Average of two seasons).

Treatment ppm	Sowing date	Plant height/cm	No. of leaves	No. of branches	Fresh weight(g/plant)			Dry weight(g/palnt)		
					Leaves	Stem	Roots	Leaves	Stem	Roots
Control	1st date	38.3	58.4	4.3	10.2	4.7	2.7	3.3	2.5	3.3
Fe 50		42.2	68.2	4.9	14.1	9.6	3.8	5.2	4.7	3.2
Fe 100		44.8	58.2	4.7	11.9	8.5	3.3	4.4	4.1	2.8
Mn 50		40. 4	66	4.3	13.5	9.1	3.1	4.9	4	2.6
Mn 100		39.3	61.2	4.4	13.1	8.2	4	5	4	3.6
Zn 50		33.5	56.2	4.1	12.9	7.7	3.1	4.3	3.6	2.8
Zn 100		37.4	51.8	4.3	16.3	10.6	4.3	3.4	2.8	1.7
Mean of 1st date		39.4	60	4.4	13.1	8.3	3.5	4.4	3.6	2.6
Control	2nd date	26.4	57	4.8	16.1	7	5.2	4.3	2.3	2.6
Fe 50		27.4	63.8	5.6	15.4	7.8	5.9	4.9	2.9	2.8
Fe 100		33.8	65.2	5.8	20.8	12.3	8.9	5.9	4.2	3.3
Mn 50		33.3	63	5.7	18.2	10.5	8.5	5.2	3.5	3
Mn 100		28.8	59.7	5.4	14.7	10.4	8	3.9	2.9	2.9
Zn 50		28.8	59.3	5.2	12	6.8	7.1	3.7	2.4	2.7
Zn 100		20.3	44.4	5.2	6.6	3.4	2.1	1.8	0.52	1.2
Mean of 2nd date		28.4	58.9	5.3	14.8	8.3	6.5	4.2	2.7	2.6
Control	Mean value of micronutrient fertilization	32.4	57.3	4.6	13.2	5.9	4	3.8	2.4	2.5
Fe 50		34.8	66	5.3	14.8	8.7	4.9	5.1	3.8	3
Fe 100		39.3	61.7	5.3	16.4	10.4	6.1	5.2	4.2	3.1
Mn 50		36.9	64.5	5	15.9	9.8	5.8	5.1	3.8	2.8
Mn 100		34.1	60.5	4.9	13.9	9.3	6	4.6	3.5	3
Zn 50		31.2	57.8	4.7	12.5	7.3	5.1	4	3	2.8
Zn 100		28.9	48.1	4.8	11.5	7	3.2	2.6	1.7	1.5
LSD Sowing dates		0.6	0.6	0.6	0.6	N.S.	0.6	N.S.	0.6	N.S.
LSD Minerals		0.8	1.1	N.S.	1.1	1.2	1.2	1.2	1.1	N.S.
LSD sowing date X Minerals fertilizer		1.6	1.6	N.S.	1.5	1.7	2.4	N.S.	N.S.	N.S.

weight (g/plant). However, Fe at 100ppm recorded maximum mean values which reached to 78.1% more than control treatment. These results were in agreement with the findings by Khalil *et al*[36] on *Tagets erecta* L., who reported that micronutrient application (Zn, Mn and Zn + Mn) induced significant increments in flowers fresh weight. They added that Mn treatment was more effective for this character than Zinc.

The highest herb essential oil percentage resulted with foliar application with higher zinc level which reached almost 1.7% over the control treatment. For the herb essential oil content (ml/plant), iron at the two levels caused the highest significant stimulation which recorded more than double the values of control plants.

On the other hand, the application of various microelements fertilization caused significant essential oil accumulation in flowers. The content of flower essential oil (ml/plant) recorded highest significant mean values by foliar spray with the two Fe levels used. The promotive effect of micronutrients on

Table 3: Effect of sowing date and micronutrients fertilization on weight of flowers and essential oil content in herb and flowers of *Ruta graveolens* L. plants (Average of two seasons).

Treatment ppm	Sowing date	Essential oil/herb		Flowers fresh	Essential oil/ flowers	
		Oil %	ml/plant	weight(g/plant)	Oil%	ml/plant
Control	1st date	0.070	0.032	3.00	0.108	0.003
Fe 50		0.056	0.025	3.39	0.111	0.006
Fe 100		0.079	0.040	7.50	0.117	0.009
Mn 50		0.066	0.020	4.60	0.136	0.006
Mn 100		0.054	0.012	2.21	0.113	0.002
Zn 50		0.074	0.023	3.30	0.037	0.001
Zn 100		0.074	0.035	5.10	0.060	0.003
Mean of 1st date		0.068	0.027	4.2	0.097	0.004
Control	2nd date	0.107	0.022	3.4	0.110	0.004
Fe 50		0.198	0.050	3.7	0.136	0.005
Fe 100		0.136	0.040	2.8	0.143	0.004
Mn 50		0.128	0.033	3.2	0.065	0.005
Mn 100		0.167	0.020	2.6	0.156	0.002
Zn 50		0.134	0.019	1.9	0.085	0.002
Zn 100		0.219	0.020	1.8	0.075	0.002
Mean of 2nd date		0.156	0.029	2.9	0.110	0.003
Control	Mean value of	0.089	0.027	3.2	0.109	0.004
Fe 50	micronutrient	0.127	0.038	3.5	0.124	0.006
Fe 100	fertilization	0.128	0.040	5.7	0.130	0.007
Mn 50		0.097	0.027	3.9	0.146	0.006
Mn 100		0.111	0.016	2.4	0.089	0.002
Zn 50		0.104	0.021	2.6	0.061	0.002
Zn 100		0.147	0.027	3.6	0.068	0.003
LSD Sowing dates		0.003	N.S.	1.10	N.S.	0.001
LSD Minerals		0.006	0.005	1.30	N.S.	0.001
LSD sowing date X Minerals fertilizer		0.008	0.008	2.40	N.S.	0.002

essential oil percentage of herb and flower yield (ml/plant) may be attributed to their effect on enzymes activity and metabolism improvement. Similar results were reported by Tarraf *et al*[37] on *Rosmarinus officinalis* L. and Khalil *et al*[36] on *Tagetes erecta*.

Effect of the Interaction Treatments Between Sowing Date and Micronutrients Fertilization: Data presented in Table (3) show that rue plant sowed at first date and treated with higher does of Fe or Zn produced the highest flower yield per plant which reached 7.5 and 5.1 g/plant, with increment 150% and 70% over control, respectively.

The Interaction between date of sowing and micronutrient fertilization significantly increased essential oil percentage and production as compared with control. The highest mean value was recorded with lower iron levels at the second date of sowing. On the other hand, the flower essential oil percentage did not reach any significant value with different interaction treatments, while the highest iron level enhanced the production of essential oil in flowers at the second date of sowing.

Table 4: Qualitative and quantitative composition (% v/v) of volatile oil compounds at late of sowing date for *Ruta graveolens* L.

	Compound	Rt[1]	Herb Cont	Herb Zn 100ppm	Herb Fe50ppm	Flower Cont	Flower Fe100ppm	Flower Mn100ppm
1	2-Nonanone	1092	7.75	4.87	12.36	1.29	1.85	0.78
2	2-Nonanol	1100	0.03	0.08	0.08	0.04	--	--
3	n-nonanal	1104	0.03	0.06	0.09	--	--	--
4	Geijerene	1142	0.30	0.15	0.45	0.09	0.09	--
5	2-Decanone	1192	1.25	0.61	1.15	0.26	0.33	0.19
6	Octyl acetate	1211	0.01	--	--	0.02	--	--
7	Ascaridole	1237	3.97	2.70	4.91	0.39	0.59	0.25
8	Pregeijerene	1287	0.38	0.16	0.49	0.11	0.10	--
9	2-Undecanone	1293	82.06	85.2	73.94	91.31	93.21	96.92
10	2-Undecanol	1300	0.06	0.62	0.11	0.61	0.30	--
11	Nonanyl acetate	1311	0.42	0.31	0.41	0.18	0.14	--
12	Undecanal-2-methyl	1364	1.05	1.39	1.53	0.71	0.72	0.44
13	2-Dodecanone	1393	0.85	0.99	1.15	0.55	0.40	--
14	2-Acetoxydodecane	1432	1.03	1.68	1.83	2.87	1.35	0.76
15	2-Tridecanone	1494	0.50	0.90	0.83	1.57	0.84	0.66
Total			99.69	99.72	99.33	100.0	99.92	100.0

Table 5: Effect of sowing date and micronutrients fertilization on rutin and coumarin percentage of *Ruta graveolens* L. plants (Average of two seasons).

Treatment ppm	Rutin % 1st date	Rutin % 2nd date	Rutin % Mean	Coumarin % 1st date	Coumarin % 2nd date	Coumarin % Mean
Control	0.895	0.945	0.920	0.018	0.20	0.019
Fe 50	1.010	1.190	1.100	0.020	0.25	0.023
Fe 100	1.016	1.080	1.048	0.020	0.026	0.023
Mn 50	0.965	1.170	1.068	0.024	0.027	0.026
Mn 100	0.945	1.020	0.983	0.022	0.020	0.021
Zn 50	1.015	1.100	1.058	0.023	0.022	0.023
Zn 100	0.940	1.070	1.005	0.025	0.024	0.025
Means of sowing dates	0.964	1.082		0.022	0.023	

Essential Oil Component: From the obvious results, most of the micronutrients application under the second sowing date gave the highest essential oil percentage, comparing with first one. Moreover, the applied Zn at 100ppm level and Fe at 50ppm level produced highest herb essential oil percentage, while for flower it was produced with applying the highest level of Fe and Mn. Thus, these treatments were identified.

Data in Table (4) revealed that total identified compounds in rue herb and flowers oil amounted about 99.3-100%. The main compound in the herb essential oil was 2-Undecanone which formed about 73.94 to 82.06%, the second major compound was 2-Nonanone which reached between 4.87 to 12.36%, the third order of compound was Ascaridole which formed 2.70 to 4.91. This agree with El-Sherbeny *et al*[38], who's found that 2-undecanone was the major compound for leaves and flowers.

Moreover, 2-Undecanone was also the main compound in flowers essential oil, where its percentage reached to 91.31% in the control, while the second one was 2-Acetoxydodecane formed from 0.76 to 1.29% followed by 2-Nonanone which accounted 0.78 to 1.29%. This means that the main compound in flower was more than in herbs.

Foliar application by Fe 100ppm caused an increased in major compound content of herb essential oil which recorded 85.2%, while Fe 50ppm recorded opposite trends. On the other hand, the main constituents of flower essential oil was raised by applied the highest level of both Fe and Zn reach to 93.21 and 92.92% respectively.

Pino[39] indicated that leaf oil of *Ruta graveolens* L. grown in Cuba identified 32 components and the major constituents was 2-undecanone (48.67%) followed by curcuphenol (8.18%) and hexadecanonic acid (5.68). Stashenko, *et al*[40] found that the main constituents of extracts from leaves, flowers, stems and roots were 2-nonanone (8.9%), 2-undecanone (13.4%0, chalepensin (13.0%) and geijerene (19.3%), respectively.

Rutin and Coumarin Content:

Effect of Sowing Date: Data presented in Table (5) illustrated that the second date of sowing was more suitable for more accumulation of rutin and coumarin in ruta herb. Thus, the increment in this date reached to 12.2% and 4.5%, respectively, more than that produced at first date. In this spite, Omidbaigi and Mastro[13] on buckwheat tested seven sowing dates from 5th April to 5th October, they found that the highest rutin content (0.6 g/plant in dry weight) was recorded with those sown in July, while the lowest content (0.31 g/plant in dry matter) was with those sown in August. On the other hand Tremblay *et al*[41] concluded that the highest two coumarin studied for *Angelica archangelica* were obtained at Autumn planting compared with spring date.

Effect of Micronutrients Fertilization: The results shown in Table (5) indicated that the application of various micronutrients levels caused a pronounced increment in rutin and coumarin percentage of *Ruta graveolens* herb than untreated plants. Moreover, the lowest level of the three micronutrients produced more rutin than higher level. The maximum rutin percentage was recorded by applying 50ppm Fe. This increment reached 19.6% above the control one. In addition, the maximum coumarin percentage was recorded with 50ppm Mn which gave 36.8% over the control treatments.

Effect of the Interaction Between Date of Sowing and Micronutrient Fertilization: It is clearly noticed as shown in Table (5) that both rutin and coumarin percentage were produced more with applying different levels of micronutrients used at second date of sowing. Thus, the highest rutin percentage (1.19%) was recorded with application of 50ppm Fe for plants sown at second date compared to 0.945% for control plants, with increase reached to 23.8%. At the meantime, the maximum coumarin percentage produced was 0.027% recorded with application of 50ppm Mn in compared to 0.020 for control treatment, with an increment reached 25%.

Nutrient Content:

Effect of Sowing Date: Data indicated in Table (6) showed that the nitrogen and phosphorus content of *Ruta graveolens* herb were increased markedly with second date of sowing compared to the first one. The increment reached up to 102.4% and 28% for N and P respectively. Meanwhile, Potassium content recorded small decrement reached 2.4% below the first date.

For the micronutrient uptake Fe, Mn and Zn data revealed that the early sowing date produced the highest accumulation for the three elements. The increment in content reached to 29.1, 15.9 and 18.5% for Fe, Mn and Zn respectively, In this respect, Shadia *et al*[42], on *Nigella sativa*, Mann and Vyas[27] on *Plantago ovata* reported that N., P. and K uptake by seed was significantly greater under the earliest sowing date (1 November) for coriander and 15 November for (Isabgol). Meanwhile, Lopez Camello *et al*[43], revealed that, no significant differences in nutrient accumulation due to sowing date (27th May or 19th August) of *Coriandrum sativum*.

Effect of Micronutrients Fertilization: The three micronutrients applied at various levels to *Ruta graveolens* caused in general, an increment in the accumulation of nutrients content, with one exception for potassium content (Table 6). Applied higher level of Fe and Zn was more effective than the low level for nitrogen uptake, while the opposite effect was true for Mn. However, the maximum nitrogen content was recorded when plants were sprayed with 100ppm Zn which reached 2.29%, with the increase over the control reaching 47.7%. For potassium uptake, it is clear that higher dose of three micronutrients was more favorable than the low one. On the other hand, the response of micronutrients Fe, Mn and Zn uptake to foliar nutrients depended on the dose and the element used. Applied Fe and Zn at low dose (50ppm) were more effective than high dose for Fe uptake. Similarly the low dose of Mn and Zn were more favorable than higher ones for Mn accumulation and the maximum content was recorded with applied 100ppm Zn. In the same time, application of 100ppm Fe caused the maximum Zn accumulation (754) comparing with control and other treatments.

Table 6: Effect of date of sowing and micronutrient fertilization on nutrient uptake of *Ruta graveolens* L. (Average of two seasons)

Treatmentppm	Sowing date	N %	P %	K %	Fe ppm	Mn ppm	Zn ppm
Control	1st date	0.81	1.233	5.006	2128	182	736
Fe 50		1.32	0.685	4.551	2450	163	766
Fe 100		1.78	0.710	5.145	2322	191	752
Mn 50		0.97	0.783	4.175	2488	205	782
Mn 100		0.81	1.248	4.670	2693	187	831
Zn 50		1.05	1.101	4.630	2725	215	806
Zn 100		1.86	1.297	4.334	2439	237	793
Mean of 1st date		1.23	1.007	4.644	2464	197	781
Control	2nd date	2.28	0.881	4.611	1720	144	673
Fe 50		2.35	0.954	4.729	1808	156	681
Fe 100		2.40	1.468	4.729	1756	167	755
Mn 50		2.50	1.734	4.215	2100	173	650
Mn 100		2.57	1.444	3.641	2117	170	648
Zn 50		2.64	1.370	4.749	1925	188	627
Zn 100		2.72	1.174	5.066	1933	192	631
Mean of 2nd date		2.49	1.289	4.534	1908	170	659
Control	Mean value of micronutrient fertilization	1.55	1.052	4.809	1924	163	705
Fe 50		1.84	0.820	4.640	2129	160	724
Fe 100		2.09	1.089	4.937	2039	179	754
Mn 50		1.74	1.258	4.195	2294	189	716
Mn 100		1.69	1.346	4.156	2405	179	740
Zn 50		1.85	1.236	4.690	2325	202	717
Zn 100		2.29	1.235	4.700	2186	215	712

The improvement in micronutrients uptake by micronutrients application (Fe, Mn and Zn) would be explained by their role on improving roots that lead to greater absorbing surface of root consequently increasing nutrients uptake and improves transpiration of the nutrients from the soil to plant organs via the roots[44]. The finding of Bandyopadhyay *et al*[45], and Khalil *et al*[36], on *Tagetes erecta* L. are in harmony with these results.

Effect of the Interaction Between Sowing Date and Micronutrient Fertilization: Regarding the interaction between the addition of various levels of micronutrients and dates of sowing (Table 6) the results indicated that in general, the second date of sowing combined with various micronutrient treatments had a promotive effect in producing higher N, P, Fe and Zn content. Meanwhile, the highest mean values of N and Zn were recorded with the application of 100ppm Zn at second date, which gave 2.72% and 5.066% for the two elements, while highest mean values of Fe and Mn were, recorded when the two level of Zn were applied at first date of sowing.

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