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Ruta graveolens L. Essential Oil Composition under Different Nutritional Treatments

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Abstract: The use of un-exploited organic industrial by-products and municipal wastes as soil organic amendment has an economic value and environmental interest. However, little is known about their effectiveness on medicinal plants cultivation. An experiment was conducted in this regard to assess the impact of farmyard manure (FYM), composted sugarcane pressmud (CPM) and sewage sludge biosolid (SSB) on volatile oil composition of *Ruta graveolens* L., an important aromatic medicinal herb used frequently in *Unani* system of medicine in India. Volatile oil in the aerial parts of the plant was isolated by hydro-distillation and analyzed by GC-MS. Hydro-distillation of untreated (control) plants yielded 0.32% essential oil on fresh weight basis. The predominant components in the essential oil were n-Hex-4-en-3-one (55.06%), n-Pent-3-one (28.17%), n-Hex-3-en-2-one (14.07%) and n-Hex-5-en-3-one (0.67%). Essential oil obtained from plants treated with FYM amounted to 0.36% of fresh weight and consisted mainly of n-Hex-4-en-3-one (53.64%), n-Pent-3-one (37.82%) and n-Hex-3-en-2-one (7.22%). The hydro-distillation of CPM treated *R. graveolens* yielded 0.37% essential oil on fresh weight basis. The predominant components in its essential oil consisted of n-Hex-4-en-3-one (53.61%), n-Pent-3-one (37.16%) and n-Hex-3-en-2-one (8.12%).The essential oil distilled from SSB treated *R. graveolens* amounted to 0.39% of fresh weight and consisted mainly of n-Hex-4-en-3-one (53.82%), n-Pent-3-one (37.24%) and n-Hex-3-en-2-one (8.37%). Here it is concluded that *R. graveolens* volatile oil percentage is favourably influenced by organic soil amendments where as its composition remains largely unaffected by different organic amendments.

Key words: Farmyard manure · Nutritional factors · Organic farming · Rutaceae · Sewage sludge · Sugarcane pressmud · Volatile oil

INTRODUCTION

Ruta graveolens (garden rue) is a strong-scented, small evergreen sub-shrub or semi-woody perennial which belongs to family Rutaceae. It is well known for its aromatic and medicinal uses. The active principles of the plant are glycosides (such as rutin, a flavonoid), alkaloids (quinolones), furocoumarins (psoralens), essential oil with methyl ketones, alcohols and some other compounds [1]. All parts of the plant contain the active principles, especially the leaves. It is used in many countries as an emmenagouge and as an abortifacient [2]. It is recommended in herbal treatment for insomnia, headache, nervousness, abdominal cramps and renal troubles. The most frequent intentional use of the plant has been for induction of abortion. In whole form, the plant has sedative, antispasmodic, cholagogic, diaphoretic, anthelmintic and emmenagogic properties. According to *Ayurveda*, the plant is bitter, laxative and hot in action. According to *Unani* medicine, rue (*Sudab*) is tonic, digestive, emmenagogue, abortifacient, antiaphordisiac, increases mental activity and useful in treatment of gleet and urinary discharges. Its leaves (barge-sudab), seeds (tukhmesudab) and oil (roghane-sudab) are still used in a number of *Unani* formulations. In *Homeopathy*, a tincture from the fresh leaves is used for varicose veins, rheumatism, arthritis and neuralgia [3].

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A characteristic feature of family Rutaceae is the presence of secretory cavities in the stems, leaves, floral parts and pericarp of the fruits. These cavities contain aromatic volatile oils. Volatile oil of *R. graveolens* is composed of 2-undecanone (50-90%), 2-haptanol, 2-nonanol, 2-nonanone, limonene, pinene, anisic acid, phenol, guiacol and others [1]. It possesses anthelminthical, bacteriostatical and phototoxical properties [4, 5]. The chemical profile of essential oils in general vary in quality, quantity and in composition according to climate, soil composition, plant organ, age and vegetative cycle stage [6]. Essential oils were widely used as fragrances, but now they are largely used in medical industry owing to the presence of a large number of biologically active components. It has therefore become important to develop cultivation protocols of the plants producing them so that the oil of desired chemical profile is obtained without much variation from lot-to-lot. Some studies have reported the effect of agricultural practices on the secondary metabolites in medicinal and aromatic plants [7, 8, 9, 10, 11]. Prediction of germination potential [12] also facilitates medicinal plant research. Fertiliser applications generally affect oil yield by enhancing the amount of biomass yield per unit area. Overuse of fertilizers result in soil deterioration, therefore alternative ways must be adopted to reduce their use in agriculture [13]. A couple of factors including overpopulation and urbanization are a major threat to medicinal and aromatic plants in their natural habitats [14, 15]. Cultivation is the best and most reliable means of conservation as well as secondary metabolite production [16]. Some researchers have pointed out efficacy of organic manures in increasing the growth and yield of medicinal and aromatic plants [17, 18]. We have earlier carried out a study on the composition of rue essential oil under the influence of inorganic and biological fertilizer treatments [19]. Here, we have carried out this experiment to evaluate the impact of un-exploited organic products [farmyard manure (FYM) and composted sugarcane pressmud (CPM)] and municipal wastes [sewage sludge biosolid (SSB)] on the volatile oil composition of *R. graveolens*.

MATERIALS AND METHODS

Plant Materials and Field Experiments: Seeds of *R. graveolens* (NJSSN-1670) were obtained from National Bureau of Plant Genetic Resources (NBPGR), Regional Station, Niglat, Bhowali, Uttaranchal, India. These were sown in 1m × 1m nursery beds in the middle of October, 2008. Two-month old seedlings were transplanted to the main field in a randomized block design. Twenty plants were transplanted in each block of 3m × 3m size with plant to plant distance of 50 cm and row to row spacing of 60 cm. The soil of the experimental field was sandy loam with neutral pH. The organic carbon content of the soil was 0.27% (w/w). The soil had 123 kg/ha available N, 23 kg/ha available P and 110 kg/ha available K. At the time of transplantation, the treatments were applied. Each treatment was replicated three times. Three treatments consisting of FYM, CPM and SSB (each at a rate of 20 tons/ha) were applied at the time of transplantation. The crop without any treatment was taken as control. The crop was irrigated on alternate days for the first ten days and then throughout the whole process of growth and development, irrigation was carried out at times dependent upon the rainfall. In the case of long-term drought, watering was carried out.

Essential Oil Isolation and Identification: For the isolation of essential oil from aerial parts, the plants were harvested at full bloom stage of development in the second growth season. Aerial parts of each sample (500 gm) was collected and subjected to hydro-distillation in a Clevenger-type apparatus for 4 h. The oil after extraction was collected in screw capped glass vials and analyzed by gas chromatography mass spectroscopy (GC-MS) on an HP-6890 GC system coupled with a 5973 network mass selective detector and equipped with a HP5-MS capillary column packed with fused silica (60 m, 0.25 mm i.d.; 0.25 μm film thickness). The oven temperature program was initiated at 40°C, held for 1 min then raised at 3°C min^{-1} to 250°C, held for 20 min. Other operating conditions were as follows: carrier gas, helium at a flow rate of 1ml/min; injector temperature, 250°C; split ratio, 1:50; injection volume was 0.1 μl. Mass spectra were recorded at 70 eV. Volatile oil components were identified by comparing their retention indices and mass spectra fragmentation patterns with the literature values [20, 21, 22, 23, 19, 24].

RESULT AND DISCUSSION

All organic soil amendments substantially raised essential oil production, represented by increase in oil percentage and fresh weight of leaves of *R. graveolens* as compared to the control (unfertilized treatment). In all cases, the total yield of leaves on fresh weight basis increased as compared to the control variant. Hydro-distillation of untreated (control) plants yielded 0.32% essential oil on fresh weight basis. The predominant components in the essential oil were

Table 1: Comparative statement of the volatile oil composition of *R. graveolens* treated with different organic residues

COMPONENT	RT	Samples % Control	FYM	CPM	SSB
n-Pent-3-one	15.97	28.17	37.82	37.16	37.24
n-Pent-3-en-2-one	16.65	0.20	0.21	0.23	0.27
n-Hex-3-one	19.23	0.45	0.43	0.47	0.48
n-Hex-3-en-2-one	20.54	14.07	7.22	8.12	8.37
n-Hex-4-en-3-one	22.35	55.06	53.64	53.61	53.82
n-Hex-5-en-2-one	23.43	0.33	0.44	0.43	0.48
n-Hex-5-en-3-one	24.33	0.67	0.51	0.53	0.50
n-Hept-4,6-dien-3-one	24.87	0.12	0.13	0.17	0.26

Retention time (RT), farmyard manure (FYM), composted sugarcane pressmud (CPM), sewage sludge biosolid (SSB).

Table 2: Percentage composition of major constituents of volatile oil from aerial parts of *R. graveolens* as per recent studies. (constituents less than 1% have been omitted from the list)

S.No.	Major components	% Composition El-Sherbeny *et al*. [10]	Malik *et al*. [20]	Soleimani *et al*. [30]
1.	n-Pent-3-one	----	28.17	----
2.	n-Hex-3-en-2-one	----	14.07	----
3.	n-Hex-4-en-3-one	----	55.06	----
4.	2-Octanone	1.60	----	----
5.	2-Nonanone	10.15	----	8.8
6.	2-Nonanol	----	----	1.1
7.	Geijerene	----	----	1.6
8.	Geyrene	1.26	----	10.4
9.	1-Nonene	2.45	----	----
10.	2-Nonene	3.07	----	----
11.	Undecane	3.51	----	----
12.	Anthracene	1.21	----	----
13.	3,4-Dihydrobenzo [b] fluoranthene	2.63	----	----
14.	Teteradecanal	1.22	----	----
15.	Dodecanal	2.00	----	----
16.	2-Decanone	1.73	----	1.9
17.	2-Heptanol acetate	----	----	17.5
18.	*cis*-Piperitone oxide	----	----	1.2
19.	2-Undecanone	51.00	----	33.9
20.	2-Methyl-undecanal	----	----	1.1
21.	*trans*-Piperitone oxide	----	----	1.4
22.	2-Dodecanone	2.77	----	1.1
23.	1-Dodecanol	----	----	11.0
24.	12-Methyl-oxa-cyclododec-6-en-2-one	2.36	----	----
25.	Elemol	1.98	----	1.1

n-Hex-4-en-3-one (55.06%), n-Pent-3-one (28.17%), n-Hex-3-en-2-one (14.07%) and n-Hex-5-en-3-one (0.67%). Essential oil obtained from plants treated with FYM amounted to 0.36% of fresh weight and consisted mainly of n-Hex-4-en-3-one (53.64%), n-Pent-3-one (37.82%) and n-Hex-3-en-2-one (7.22%). The hydro-distillation of CPM treated *R. graveolens* yielded 0.37% essential oil on fresh weight basis. The predominant components in its essential oil consisted of n-Hex-4-en-3-one (53.61%), n-Pent-3-one (37.16%) and n-Hex-3-en-2-one (8.12%). The essential oil distilled from SSB treated *R. graveolens* amounted to 0.39% of fresh weight and consisted mainly of n-Hex-4-en-3-one (53.82%), n-Pent-3-one (37.24%) and n-Hex-3-en-2-one (8.37%) (Table 1).

Our results establish that FYM, CPM and SSB treated *R. graveolens* showed enhanced oil percentage. Enhancement in essential oil content by nutritional treatments has also been reported previously [25, 26]. This result may be due to the effect of nutrients present in the manures in accelerating the metabolism reactions as well as stimulating enzymes. The quality and yield of essential oils has been previously reported to be influenced by varied environmental factors [27, 28, 29]. Our earlier reports have also established the influence of fertilizers on the quality and yield of essential oils in crops like *Artemisia annua* [22, 23], *Achillea millefolium* [24] and *R. graveolens* [19]. Edris *et al*. [30] reported higher percentage of essential oil of marjoram plants by the application of composted manure as compared to chemical fertilizers.

Gas-chromatographic analyses of the composition of these essential oils revealed little variation of active constituents. In this study, eight components were characterized in the essential oil from aerial parts of the plant. Previously, 20-33 compounds have been identified in rue oil [25, 26, 30]. Literature survey reveals that *R. graveolens* volatile oils are mainly composed of hydrocarbon ketones. In our study, n-Hex-4-en-3-one was the major component present in all the four samples. n-Pent-3-one was found in considerable quantity in all samples. Contrary to our investigation, undecan-2-one and nonan-2-one were found as major components in other studies on the essential oil of *R. graveolens* [31, 25, 26, 32, 33] (Table 2).

Fluctuations in the concentrations and composition of the oil may be due to the environmental conditions of the field site that may have influenced the anatomy of the oil glands or altered the biosynthetic pathways leading to essential oil production. However, further research is needed to ascertain the possible changes, if any.

CONCLUSION

Nutritional treatments had a significant effect on essential oil content but there was little variation in its composition. Five compounds were identified in the oil consisting mainly of hydrocarbon ketones. n-Hex-4-en-3-one was the major compound followed by n-Pent-3-one and n-Hex-3-en-2-one. Application of various nutrient fertilizers does not increase the total identified compounds in *R. graveolens* essential oil [34-36].

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