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Effect of various nitrogen fertilizer doses on essential oil yield and chemical composition of rose-scented geranium (*Pelargonium Graveolens* L.)

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Abstract

Soil is deteriorating in terms of loss of fertility, organic matter, and production potential. The poor management of agriculture, agrochemicals, and other environmental issues, all contribute to the loss of nutrient-rich soil (top layer) and puts agriculture output in jeopardy. This experiment aims to ascertain the appropriate macronutrient (NPK) concentrations that can considerably enhance the development and production of a chosen aromatic plant. Pelargonium graveolens was planted for this study and collected three times throughout the course of the growing season. There are four different nitrogen dosage treatments in the experiment: T1 (control), T2 (80:80:60 kg NPK ha-1), T3 (100:80:60 kg NPK ha-1), and T4 (120:80:60 kg NPK ha-1).The results revealed a substantial relationship between nitrogen dosages and harvesting time in T3 (100:80:60) with maximumincrease of 11.44% in biomass (1130.82 q ha^{-1}), 11.76% in essential oil content (0.19%), and 26.01% in oil yield (219.79kg ha^{-1}). The lowest oil output (173.79 kg ha^{-1}) and herb yield (1014.71 q ha^{-1}) were noted in T_1 (control), which lacked any nutrients. The increased nitrogen dose in T_4 has a favorable effect solely on plant height (103.60 cm), although T4 has the second-lowest herb yield and oil output after control. When compared to control, the nitrogen dose (80:80:60 @ NPK) in T2 has a moderate effect, increasing plant growth, herb production, and oil yield by 6.42, 9.45, and 15.75%, respectively. A considerable impact of nitrogen treatment on the ratio of citronellol and geraniol was revealed by chemical analysis of the essential oil. Therefore, using the right amount of nitrogen fertilizer had an impact on biomass production, the components of essentialoils, and plant development. It also had the potential to maintain the soil's qualities when used in conjunction with improved agronomic management strategies.

Keywords: Aromatic plants, nutrients efficiency, soil degradation, chemical constituents, macronutrients

Introduction

Tons of soilis depleted annually as a result of soil deterioration (or "dying"). Erosion, the loss of organic matter (nutrients), soil infertility, excessive pH, soil pollution, etc. are all signs of degrading soil. Lack of nutrients in the soil, results in decreased crop output which directly affects a farmer's revenue. As with organic or inorganic fertilizer, adequate amounts of nutrients are required to be supplied for maintaining soil fertility.The soil must be capable of supporting root growth for growing crops for being able to absorb a sufficient amount of nutrient supply(white *et al*.2013a)

[1].Due to soil erosion, forest fires, exploitation of agrochemi cals, and other factors, fertile soil (nutrient-
rich soil) found in uphill locations are disappearing at an ast ounding rate. Therefore, farmers are forced to apply excessive amounts of fertilizer in order to improve crop productivity and meet the demand for agricultural products. According to a survey, between 60 to 70 percent of farmers in the western hill regions of Uttarakhand used enormous quantities of nitrogen fertilizer to produce higher-quality crops. The ecology and agricultural output have been both demonstrated to be seriously affected by excessive fertilizer consumption (Acharya *et al*. 2020) [2]. One of the essential element , nitrogen (N), participates in photosynthesis and cell division as well as being a key component of many structural components(Akter *et al*. 2017) [3]. It facilitates the synthesis of nucleic acid, vitamins, hormones, and proteins. Only 40 % of the total nitrogen in urea may be taken up by the plant; the remaining portion may leach, ammonia volatilize, or nitrate denitrifies before dissipating into the soil(Asghari & Cavagnaro, 2011) [4]**.** A required amount of nitrogen is sprayed, which not only improves the activity of photosynthesis but also increases crop production, boosts the content of essential oils, increases yield, and has no adverse effects on the environment (Liu *et al*., 2018; Wei *et al*., 2016) [5, 6]. In addition to nitrogen (N), phosphorous (P) and potassium (K) fertilizers are additional macronutrients that are vitally necessary to plants and aid in certain cell membrane processes and stomata function, respectively. Plants also require them to activate enzymes and maintain the turgidity of the cell. It is an important part of DNA and RNA components. Potassium helps plant to adapt under stress condition

The Geraniaceae family of plants includes *Pelargonium graveolens* (L'Herit), which produces essential oils (Verma *et al*., 2011) [7]. In this genus, there are more than 280 species, most of which are cultivated for ornamentation(Blerot *et al*., 2016; Lw *et al*., 2012) [8, 9]. Commercial P. graveolens cultivation is done by farmers in order to produce high-quality essential oils, which are then used in the beverage, fragrance, and cosmetics industries (Bergman *et al*., 2021; Džamić *et al*., 2014) [10, 11]. It contains aromatherapeutic essential oil with high concentrations of citronellol, geraniol, linalool, iso-menthone, geranyl propionate, and sesquiterpenes (Peterson *et al*., 2006; Verma *et al*., 2010) [12, 13]. Nutritional deficiencies or overload as well as agro-climatic factors have an impact on crop productivity by lowering herb yield and essential oil content (Esetlili *et al*. 2015) [14]. A surplus of nitrogen can raise input costs of fertilizer and reduce the quality and quantity of biomass as well as the content of essential oils (Chen *et al*. 2017) [15]. This study examined how the geranium plant responded to various nitrogen levels in terms of vegetative growth and essential oil output in order to prescribe real nutrition administration for commercial *P. graveolens*.

Materials and Methods

1. Experimental site

A field experiment of nutrient management on rose scented Geranium was conducted during the growing seasons of 2020 at the nursery of central institute of medicinal and aromatic plant, research center, Pantnagar, US Nagar, Uttarakhand. The experimental site is located between 29º N latitude and 79.38º E longitude and at an altitude of 243 m above mean sea level. The maximum temperature ranges between 35 to 45ºC, and minimum between 2 to 5ºC.

2. Preparation and rising of stem cutting

The uniform terminal cuttings of geranium were taken from the mother plants in uniform size and length (6 inch). The cuttings were immediately planted in polyethylene bags and were kept in greenhouse condition.After 45 days, rooted cutting of the rose scented geranium were transplanted to the experimental field with bed size 3x4 m and 50x50 cm row and intra-row spacing, respectively. The experiment was conductedin three set of replication.

3. Details of treatments

Four different doses of nitrogen T_1- Control, T_2- 80:80:60 @ NPK, T_3-100:80:60 @ NPK, T_4-120:80:60@NPK ha^{-1} was applied to the experimental field.Each treatment was applied after mixing vermicompost @ 3 ton per hectare in soil. Nitrogen in the form of urea was divided into four batches. Firstdose of nitrogen were applied with 80 kg ha-1 P_2O_5 (as triple super phosphate) and 60 kg ha-1 K_2O (as potassium sulphate) at the time of transplanting and rest of thebatches were added at 15, 30 and 45 day of transplanting.

4. Observation and Data analysis

In 2020, plants were harvested three times during the flowering stage, in January (winter), April (spring), and June (summer), by cutting 10 cm above the soil surface. Fresh herb yield (q ha-1) and total oil yield (kg ha-1) were calculated following each harvest. For agro-morphological characterization, the plant's height (in cm), the number of branches on plant-1, and the presence of essential oils were noted. Statistical analysis of variance was performed on all experimental data with a P-value of 0.01 using the calculator. Gas-liquid chromatography was used to determine the essential oil of geranium's chemical composition.

5. Chemical composition of essential oil

The essential oil was analysed using GC-FID techniques. Analysis of essential oil byGC-FID technology on Thermo Fisher Trace GC-1300 mixedwith TG-5 fused silica capillary column (30 m × 0.25 mm × 0.25 µm) and flame ionization detector (FID). Nitrogen at flow rateof 1.0 mL min-1 was used as carrier gas. The oven temperature initially risen from 60 to 23 °C at 3 °C min-1. An injector and detector temperature maintained at 250 and 280 °C, respectively. The injection volume was 0.02 µL neat with a split ratio of 1:40. GC-MS performed on Clarus 680 GC coupled with Clarus SQ 8C mass spectrometer of PerkinElmer equipped with Elite-5 fused silica capillary column (30 m × 0.25 mm × 0.25 µm). The oven column temperature program was from 60 to 240 °C at 3 °C min-1 with initial and final hold time of 2 min. Helium was employed as carrier gas at 1.0 mL min-1. And the sample was split using a ratio of 1:30. The injector and detector temperatures were held at 250 °C. Different compounds were ionised using an ionisation potential of 70 eV. Mostly compounds were identified by comparing their mass spectra (MS) data with literature data (Adams, 2007) [16].

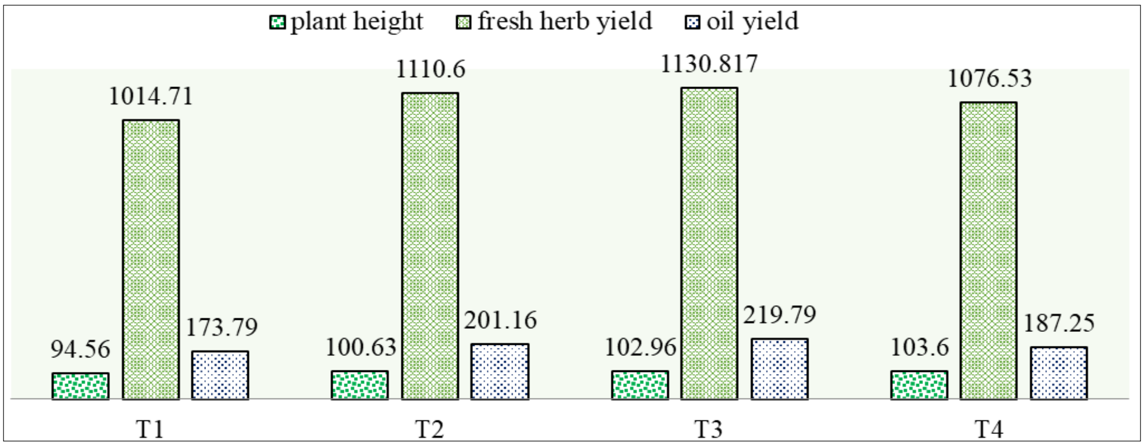
Results

Overall statistical analyses showed significant differencesbetween first, second and thirdharvestsof rose scented geranium for fresh herbage yield, oil contentand oil yieldwith respect to fertilizer treatments shown in Table 1 and Figure 1.

Table 1: Effect of NPK Fertilizer on agronomic traits, fresh herb Yield (kg/ha.) and essential oil yield (kg/ha) of *P. graveolens* at each harvest (H1, H2, H3)

Parameters	Plant Height			No. of Branches			Fresh Herb Yield (q/ha)			Oil content per 100			Oil Yield(kg/ha)		
Treatment	H1	H2	H3	H1	H2	H3	H1	H2	H3	H1	H2	H3	H1	H2	H3
T1	138.67	81	64	5.0	5.0	6.0	186.34	512.74	315.74	0.16	0.17	0.18	29.81	87.15	56.83
T2	152.33	84.44	65.11	5.0	5.0	5.0	210	565.8	334.81	0.17	0.18	0.19	35.70	101.84	63.61
T3	160.33	88.11	60.44	5.0	6.0	6.0	212.35	576.6	341.87	0.17	0.2	0.2	36.10	115.32	68.37
T4	171.33	84	55.44	6.0	6.0	6.0	213.6	544	318.93	0.16	0.17	0.19	34.18	92.48	60.60
Interaction	H	T	H*T	H	T	H*T	H	T	H*T	H	T	H*T	H	T	H*T
SEM	0.48	0.55	0.95	0.205	0.24	0.41	1.33	1.78	5.33	0.001	0.001	0.002	0.36	0.48	1.45
CD**	1.406	1.624	2.812	N/A	N/A	N/A	3.91	5.21	15.64	0.002	0.003	0.008	1.06	1.42	4.26

T_1- Control, T_2- 80:80:60 @ NPK, T_3- 100:80:60 @ NPK, T_4-120:80:60@NPK ha^{-1}



T_1- Control, T_2- 80:80:60 @ NPK, T_3- 100:80:60 @ NPK, T_4-120:80:60@NPK ha^{-1}

Fig 1: Overall effect of different level of nitrogen fertilizer on total fresh herb (q/ha), oil yield (kg/ha)and other contributing characters of *P. graveolens*

1. Plant height

Significance result was observed in all three harvests with a maximum plant height (103.60 cm) recorded in T_4 (120:80:60 kg NPK ha-1), followed by T_3 (100:80:60) 102.96 cm and T_2 (80:80:60) 100.63 cm (Figure 1). Minimum plant height was observed in T_1 (Control) 94.56 cm. The analysis (Table 1) showed an effective correlation between N fertilizer treatments and plant height. While comparing the results of three harvests, higher plant height (171.33cm) was observed in H_1T_4 (120:80:60 kg NPK ha-1) with 23.55 % increase at the time of first harvest H1 during winter and the height decreases up to 50% in H1and H3 harvest done at spring and summer season, presented in Table 1.

2. Branches per plant

The type of planting materials employed showed non-significant differences in number of branches $plant^{-1}$with respect of treatments. Table 1 showed non-significantly interaction between nitrogen dose and harvest with a 20% increase of branches(6.00) per plant in H_1T_4, H_2T_4 and H_3T_4. The lowest number of branches (5.00) was recorded in H_1T_1.

3. Fresh Herb Yield

Statistical significant results of collected data of fresh herb yield in each harvest was shown in Table 1. Total herb yield collected from all three harvestshas significantly increased by 11.44 % with maximum herb yield (1130.82 q ha^{-1}) in T_3 (100:80:60 kg NPK ha-1). Higher rate of Nitrogen dose in T_4 (120 kg ha^{-1}) had a significant influence on herb yield (1076.53q/ha) with 6.09 % increase as compared to control, but herb yield decreases by 5.04 % while comparing with treatment T_3 (Figure 1). The lowest herb yield (1014.71q ha-1) was observed in T_1 (Control). Meanwhile, the significant relationship has been observed between harvesting and fertilizerdoses when compared to each other. When results of fresh herb yield recorded in H_1 (first harvest) compared with harvest H_2 and H_3results, significantly increase of 169.43% and 59.43% in T_2, 171.53 and 60.99% in T_3 and 154.68 and 40.31 % in T_4 were recorded, respectively (Table 1). Similarly harvest H_2 results were compared with harvest H_3results; decline in fresh herb yield of rose-scented geranium was recorded including control (T_1) with 62.39% in T_1, 68.99 % in T_2, 68.66% in T_3 and 70.57 % decrease in T_4.This decline may be due to increase in temperature during summer (Table 1).

4. Oil content

The data about essential oil content (%) of rose-scented geranium is presented in Table 1. An increase in levels of nitrogen (120 kg/ha) has no significantly effect on essential oil content. Highest essential oil content (0.19) was recorded in T_3 with 11.76 % increase as compared to control. Treatment T_2(80:80:60) also had a modest effect with 5.88 % increase in essential oil content (0.18). 11.76 % decrease in essential oil content (0.17) was recorded in T4120:80:60@NPK ha^{-1} as compared to T3. When results were compared with in the harvest, H2 and H3during spring and summer season, respectively showed the presence of highest essential oil content than Ist harvest with maximum essential oil content 0.20 in H_2T_3 and H_3T_3, while the lowest essential oil content was measured in harvest H1 with 0.16 in H_1T_1 and H_1T_4 (Table 1).

5. Oil Yield

All collected data showed in Table 1 has a significant effect on oil yield. Overall collected oil yield from three harvest T_3 treatment showed maximum oil yield (219.79 kg/h) with 26.01 % increase as compared to control recorded 173.79 kg/ h oil yield (Figure 1). Increase in nitrogen dose in T4 120:80:60@NPK ha^{-1} showed 7.74% increase in essential oil yield (187.25kg/h) as compared to control but 17.38% decrease in oil yield when results was compared with treatment T_3(100:80:60 kg NPK ha^{-1}).T2 also had a significant increase of 15.75% in essential oil yield (201.16 kg/h)as compared to control. When results were compared within harvest (H1, H2 and H3), maximum essential oil yield was recorded in harvest H2 with 193.5% increase in control (87.15kg/h), 185.3% in T2 (101.84 kg/h), 219% in T3 (115.32 kg/h) and 170.56% in T4 (92.48 kg/h), which are twice time higher then harvest H1 (Table 1).

6. Chemical composition (%) of Essential oil of *P. graveolens*

Essential oil of rose scented geranium was analyzed by GC-FID and chemical profiles studied at different treatment are listed in Table 2 with respect to their retention indices. Identified major key compounds were citronellol, geraniol, geranial, linalool, isomenthone, geranyl formate, 10-epi-γ-eudesmol, and geranyl tiglate out of 22 compounds comprising 97.54% of the essential oil in treatment T_3. Minor quantities detected were geranyl valerate, citronellyl acetate, menthone, geranyl acetate, etc. shown in Table 2. Result showed variation in chemical compound ratio at different nitrogen treatments. Higher dose of nitrogen in treatment T_4 (120 kg ha^{-1}) has significantly lower citronellol and geraniol ratio (21.71: 24.10 %) out of total 92.06 percent of chemical compounds present in essential oil. Major compound found in T_3 were citronellol (28.14%), geraniol (26.57%) geranial (8.46) and Geranyl formate (5.03%) shown in Figure 2. Second highest (25.07) percent of geraniol was recorded in treatment T2 with 22.37 % of citronellol. Control showed total 91.28 percent of chemical components out of which citronellol (25.10), geraniol (21.88%), linalool (7.40%) and geranial (7.26) were identified as a major compound.

Table 2: Quality analysis and Chemical profile of essential oil of *P. graveolens*under different fertilizer treatments

S.No	Compounds	Content (%)			
		T_1	T_2	T_3	T_4
1	α-Pinene	0.26	0.42	0.26	0.22
2	β-Pinene	0.10	nd	nd	nd
3	*cis*- Linalool-oxide	1.05	1.07	0.92	1.34
4	*trans*- Linalool-oxide	0.67	0.68	0.53	0.86
5	Linalool	7.40	9.29	6.33	9.42
6	Menthone	1.04	1.19	1.23	1.13
7	Isomenthone	4.66	6.12	5.61	5.46
8	α-Terpineol	0.50	0.58	0.37	0.75
9	Citronellol	25.10	22.37	28.14	21.71
10	Neral	1.32	1.32	1.41	1.34
11	Geraniol	21.88	25.07	26.57	24.10
12	Geranial	7.26	6.50	8.46	6.64
13	Citronellyl formate	0.62	0.51	0.29	1.23
14	Geranyl formate	3.91	4.47	5.03	4.34
15	Citronellyl acetate	1.54	0.76	0.57	0.59
16	Geranyl acetate	1.63	0.62	0.53	0.60
17	β-Caryophyllene	0.99	0.41	nd	nd
18	Germacrene-D	0.69	0.24	nd	nd
19	2-Phenyl-ethyl tiglate	1.50	1.42	1.64	1.65
20	10-epi-γ-Eudesmol	5.04	5.04	5.11	5.52
21	Geranyl valerate	1.84	1.71	1.70	2.32
22	Geranyl tiglate	2.28	2.30	2.84	2.84
Total identified		91.28	92.09	97.54	92.06

nd-not detected; T_1- Control, T_2- 80:80:60 @ NPK, T_3- 100:80:60 @ NPK, T_4-120:80:60@NPK ha^{-1}

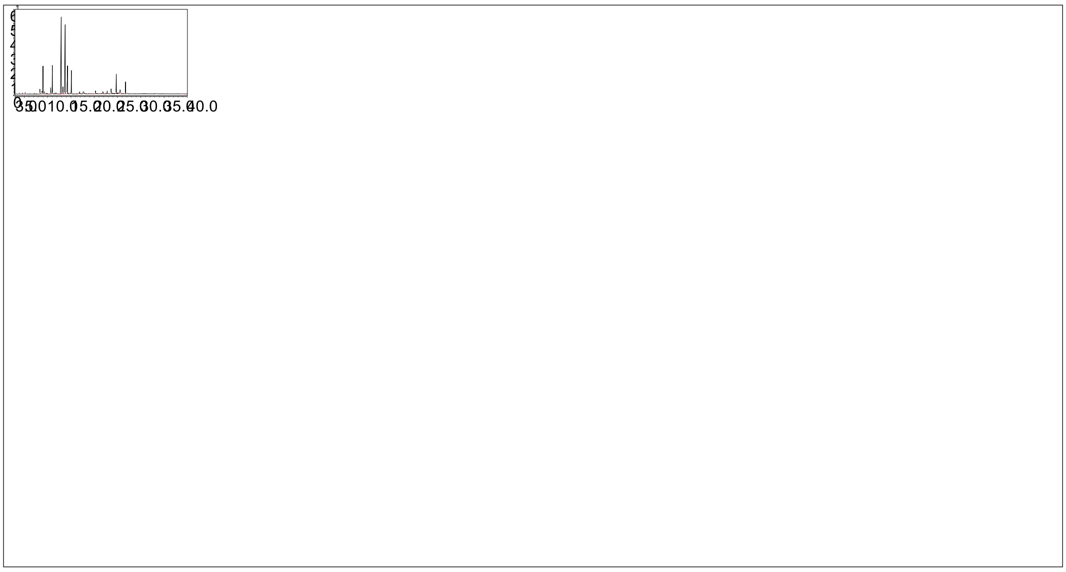


Fig 2: Chromatogram of T3(100:80:60 @ NPK) showing highest ratio of Citronellol: Geraniol compound of *P. graveolens*

Discussion

The combination of nutrients (N:P:K) has significant benefits for plants, including reproductive and vegetative development, an increase in the production of primary and secondary metabolites, and better soil nutrients.Adverse soil conditions that alter soil characteristics including soil pH and basic nutrients typically limit nutrient delivery and plant growth.The pH of the soil solution, which regulates the availability of nutrients and exposure to harmful substances, frequently restricts crop production (rengel, 2015; ryan, 2018) [17, 18]. When nutrients are supplied in excess of what a crop actually needs, residual nutrient reserves are increased. If these reserves are not used up or recovered in succeeding crops, they may cause off site transport and deterioration of environmental quality (Havlin and Heiniger, 2020) [19]. Excessive fertilizer use has reportedly been linked to decreased crop production by compromising plant nutrients (Chen *et al*. 2018) [20]. Maintaining soil fertility is significant for plants' nutrition because it allows them to receive nutrients at the proper time and in the right amount (withers *et al*. 2018) [21].The results of this study show that Treatment T3, which supplies NPK at an optimum ratio of 100:80:60 nutrients, has the greatest impact on agronomical parameters (plant height and branches), biomass, oil content, and yield, followed by Treatment T2 (80:80:60 @ NPK) as compared to control (Table 1 and 2). Results of a fertilization experiment carried out by vanderBom *et al*. (2018) [22] that involved applying a sufficient dose of NPK fertilizers and increasing high as well as sustainable crop production. In light of a reduction in wheat production, Asif *et al*. (2018) [23] came to the conclusion that sufficient supplies of nutrients (N, P,K) were required for maintaining agricultural production of wheat in a climate that was changing due to an increase in the average global temperature and CO2 levels.In this study, harvesting at various seasons has also had an impact on the crop's fresh herb and oil yield. Harvest H2 was carried out in the spring with the highest production of fresh herb and essential oil yield of rose-scented geranium after Harvest H1 was completed in the winter. Crop growth and development had increased quickly since Harvest H1. This increase could be a result of changes in environmental conditions that boost metabolic activity and enhance plant sensitivity to nitrogen sources. The high temperature, humidity, and extensive fertilizer application in T4 (120:80:60@NPK ha-1), may all be significant reasons for the drop in fresh herb yield of roughly 70.57% at the time of harvest in H3 in the summer (Table 1). These outcomes were consistent withUcar *et al*.(2017) [24] findings, showed that increasing the nitrogen rate increased the yield of *Stevia rebaudiana* herbs up to the optimal nitrogen rate, after which the yield of the herbs decreased. The findings of this study are aligned with Alhasan *et al*. (2020) [25] work, which found that *Ocimum* plants grow more slowly and produce less essential oil when nitrogen fertiliser (100 kg and 120 kg N/ ha) is applied above the recommended level.Our findings did not agree with those of Pal *et al*. (2016) [26], who used a larger dose of nitrogen and phosphorus and discovered the best herbal yield.The findings of his investigation showed that fertilizing *T. sepyllem* with N, P, and K had a positive effect on its growth, yield, and effectiveness as a medicinal and herbaceous plant.Findings supported those of Rollon*et al*. (2021) [27] for *Oryza sativa*,Gag *et al*. (2019) for *Moringaoleifera*, Chen *et al*. (2018) [20] for *Triticumaestivum* and Omer *et al*.(2014) [29] for *Artemisia annua*,showing that nutrient administration boosts growth herb yieldand essential oil yield. The variation in chemical composition and agricultural productivity of plants with various fertilizer dose applications was also documented by Holland *et al*. (2018) [30].

The information in Table 2 is consistent with a study Verma *et al*. (2013) [31] that found citronellol and geraniol to be the main ingredients in rose-scented geranium oil. When compared to earlier findings published byBoukhatem *et al*., (2013) [32] and Boukhris *et al*.(2015) [33], that there were substantial alterations in the chemical composition ratio of rose-scented geranium oils. These differences may have resulted from outside influences such as changes in the weather, the timing of harvest, or the changing seasons.The optimal standard for *P. graveolens* essential oil in industrial sectors for the creation of perfume, cosmetics, etc. depends heavily on the citronellol/geraniol ratio. The percentage of citronellol increased, according to certain research, from late winter to April.In the current investigation, treatment T3 had the highest citronellol/geraniol ratio at the time of H2's springtime harvest. When the findings of R. S. Verma *et al*. (2013) [31] were compared to the results, it was clear that the spring season had the largest percentage of geraniol and citronellol content, whereas summer harvest H3 saw a decline. This increase may be due to appropriate temperature which improve metabolism, photosynthetic activity.Changes in chemical composition were observed during leaf growth, including the ratio of geraniol to citronellol. In a study, the quantities of geraniol and citronellol in *P. graveolens* leaves were shown to be highest in the young or mature leaves, respectively(Fekri *et al*. 2021) [34]. Thus, Fertilizer application needs to be adjusted in order to reduce fertilizer costs, increase crop and oil output, improve the quality of essential oils, and conserve the environment.

Conclusion

The findings showed that the application of nutrients at a rate of 100:80:60 kg/ha had a beneficial impact on the development of rose-scented geranium plants. It is evident from the data that applying 100 kg nitrogen ha^{-1} enhanced herb yield, geranium essential oil content, and quality compared to other treatments. It may also assist replenish lost nutrients in the soil.The rose-scented geranium's growth, biomass output, and chemical makeup are not greatly improved by the administration of a higher rate of nitrogen (120 kg/ha), which also raises farmers' expenses. The crop's nutritional requirements, the soil's current fertility, and the target yield all determine how much fertilizer should be administered.

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