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Research Article

DEVELOPMENT OF AN AGRO TECHNIQUE FOR LARGE-SCALE PRODUCTION OF GERANIUM'S PLANTLETS FOR CULTIVATION AND BRINJAL INTERCROPPING

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ABSTRACT

Essential oil-bearing medicinal and aromatic plant *Pelargonium graveolense* is commonly known as rose-scented geranium because of its characteristic rose like smell. Its essential oil possesses medicinal properties such as insect repellent properties and also used in fragrance and food industry. Its demand is very high because of the above use in India and the worldwide market. This experiment is designed to develop an agro technique method for large scale production of geranium plantlets (cultivar CIM-Pawan) at field conditions using simple stem cutting method at North Indian field conditions in Uttar Pradesh, India. And also introduce a new method of intercropping cultivation of geranium in Brinjal (*Solanum melongena*) that is developed. This experiment was designed in such a manner to developed thousand plantlets from one year old a single mother plants. As we know near about 30,000 stem cuttings are required for planting an area of one hectare land. So focusing on large demands of plantlets developed an agro technique that is able to fulfil the requirements. The crops were raised using one month olds plantlets transplanting, *viz*. with intercropping in Brinjal. After harvesting oil was extracted by hydro-distillation using Clevenger apparatus and analyzed by gas chromatography (GC). The Citronellol/Geraniol ratio (C: G), quality determining factor was found to 1.23. The GC and GC-MS analysis results indicated that the essential oil components, quality and quantity were significant. The major constituents of the essential oil were Citronellol (29.81%), Geraniol (24.23%), Linalool (3.12%), Iso-menthone (4.09%), Citronellyl formate (7.51%), Geranyl formate (6.07%), and 10-epi-γ-eudesmol (2.70%).

Keywords: North India, Geranium, Essential oil, *Pelargonium graveolense*, Secondary metabolites, plant growth regulators, Inter cropping.

1. INTRODUCTION

Geranium (*Pelargonium graveolens* L., family Geraniaceae) is one of the important monoterpenoid rich aromatic plants, yielding an essential oil which is highly prised for its very strong and profound rose-like odour for this the plant is also known as rose scented geranium. It has been well characterised phytochemically and its oil constituents are well elucidated [1-3]. Geranium essential oil is used commercially in various food, pharmaceutical, and cosmetics industries. It is also used as an important ingredient in medicinal preparations for acne, bruises, broken capillaries, burns, cuts, poor circulation, dermatitis, eczema, lice, oily complexion, ringworm [3-6], ulcers, and wounds etc. [7-8]. On the skin, rose geranium oil helps to balance the secretion of sebum and clears sluggish and oily skins, while the antiseptic property makes Geranium oil an effective aid to help with burns, wounds, ulcers and other skin problems [4-9]. Its oil has insecticidal properties and used for insect repellent [10-12]. Hydro distillation of leaves and tender shoots of the plants are used for the extraction of rose scented essential oil. The major oil constituents are

geranial and citronellol. The oil also contains α-pinene, β-pinene, α-terpinene, limonene, cis-rose oxide, trans-rose oxide, methone, geranyl acetate, nerol and geraniol etc. Geranium is shallow-rooted crop and, as such, it requires well drained porous soil. The crop is found to perform better with soils with a pH of 5.5-8.0, though a calcium rich porous soil is the best. It can be grown in temperate, subtropical and tropical climates at various altitudes from 1,000 to 2,200 m. It thrives best in subtropical climates with a temperature ranging from 5°C to 23°C. However, a temperature below 3°C kills the plant. Warm winters coupled with mild summer temperatures and, well-distributed annual rainfall ranging from 100-150 cm is ideal. However, heavy rainfall results in water-logging, causing root-rot and stunted growth. It has been observed that it grows equally well at much lower altitudes and tolerates higher temperatures up to 43°C in the plains when grown under irrigated conditions. Geranium is easily propagated by cuttings [1], since there is no seed in geranium, vegetative propagation is the only viable option for its propagation. Terminal cutting about 20cm long and consisting of about 6-8 nodes are the best suited material for propagation, as these give 80% rooting even without any treatment.

Recently, a protocol has been developed at CSIR-CIMAP (Central Institute of Medicinal and Aromatic Plants, Lucknow), for the large scale production of geranium cell clones and plants have been obtained under field conditions with improved oil-yield and quality [13-14]. Geranium (*Pelargonium* sp.) cultivar 'CIM Pawan' was grown from stem cuttings at the experimental farm of CSIR-CIMAP, Lucknow, India. The propagation of Geranium through leaf petioles has also been reported to give a good rooting percentage (75%), which will help to multiply this plant in larger numbers than the traditional method of propagation using 20 cm-long cuttings [15-20]. About 30,000 cuttings are required for planting an area of 1 ha. Before planting, the land should be properly prepared by ploughing and the soil should be mixed with powdered FYM. Ridges and furrows are made, the application of fertilizer and irrigation should be done a day prior to planting. The cuttings are carefully planted at a spacing of 50 cm x 50 cm. They must be irrigated immediately after planting. Irrigation is continued on alternate days for about 10-15 days and then reduced the frequency of irrigation by twice a week [21-22].

Intercropping is one of the most important beneficial methods for farmer especially for short duration crops simultaneously can grow with more than one crop using same inputs of fertilizer and irrigation. Intercropping is also a natural way of pest management process. Intercropping is also reported to control weed [23]. Several intercropping experiments related to geranium with mints [17] and potatoes [18] have been successfully conducted in CSIR-CIMAP Lucknow by Verma *et al*. Intercropping of *Vigna unguiculata* or black gram is beneficial during the long phase and they do not affect the geranium crop. It has been experimentally observed that at the CIMAP Lucknow, polyhouse cultivation reduces weed infestation, the number of irrigations and produced less weed biomass. It has been observed at the CIMAP, Lucknow, that the cultivation of geranium in association with marigold (*Togetes minuta*) improves the survival of geranium plants over the monsoon time in the North Indian plains. Roots knot nematodes, *Meloidogyne incognita* and *M. hapla*, have been found to damage the geranium crop. Application of Aldicarb 20 kg ha^{-1} to the soil reduces the incidence of root-knot. With this background information in hand, the proposed plan on *Geranium* was undertaken. A field experiment was conducted at the experimental purpose in the village Mohammadpur, District, Barabanki, Uttar Pradesh. The study was laid out in a plot size 3x2m using randomized complete block and four replications. The soil was sandy loam with pH 6.6. Treatments were geranium and intercrops spaced at 50.00 cm between plants with two rows of Brinjal (*Solanum melongena*) were sown /planted already. The number of plants of geranium was kept at constant in all the treatments.

This study explores a protocol for large scale production of geranium cutting for cultivation purpose in district of U.P. (North India). The developed geranium cultivation technology is transfer to the rural areas farmer. This cultivation technology will be solves the problems at rural areas faced by farmers engaged in geranium cultivation. The farmers of the area are practicing cultivation of other medicinal plant, and expressed their interest for the cultivation of geranium crop because it's high price essential oil crop.

1.1. Challenges face for raising Geranium seedlings

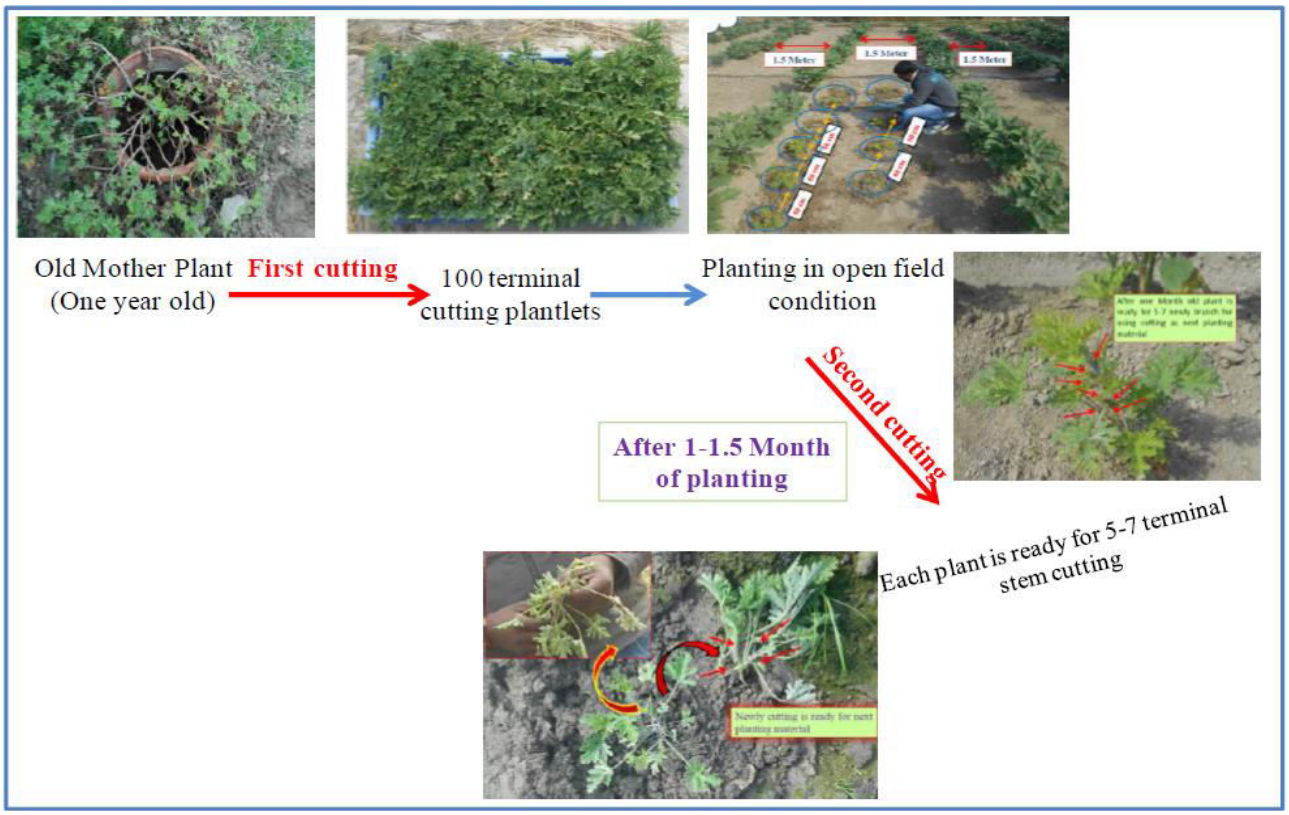
Availability of raw plant material (seedling) at the time of planting is a major concern as there are no seed formation in geranium, hence vegetative propagation is must. Tissue culture and other methods are time taking and expensive processes as well. Planting of cuttings can

be done as soon as the active growing season commences, i.e. in spring, and during most times of the year when soil moisture is sufficient. Planting during very hot times of the year and close to and during winter time is avoided when the plants are usually dormant. At the time of planting, initial temperature should be low (winter) after that high temperatures up to 43°C in the plains can be tolerated by the plant when grown under irrigated conditions. Water logging is also one of the major problems in Geranium cultivation. However, heavy rainfall results in water-logging, causing root-rot and stunted growth. It must be avoided as water logging conditions for geranium cultivation are serious factors in affecting the Geranium crop adversely.

2. MATERIAL AND METHODS

2.1. Preparation of nursery soil

Nursery is prepared under shade conditions in glasshouse of CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India. Geranium (*Pelargonium* sp.) cultivar 'CIM Pawan' was grown from the stem cuttings. Terminal young top shoots cuttings of about 5-7 cm having 3-4 nodes are selected and a slanting cut is made at the 3th or 4th node using a sharp blade and propagated in plastic trays at 5.0 cm spacing. A light watering by hand is also given immediately after planting, followed by regular watering so as to maintain the moisture in the tray. Cuttings are directly planted in field and glasshouse for nursery purpose at the same time. For the experiment perform on the yield of one thousand plantlets were obtained from stem cuttings from a one year old single mother plant (Fig. 1 & 2). Cuttings were made from strong and healthy mother plants which were already grown in glasshouse in earthen pots. Here in this experiment the aims of the study helps provide the seedling availability as raw material for planting. As per estimate, about 30,000-40,000 cuttings are required for planting in an area of one hectare land. For the requirement of seedling availability and cultivation purpose we design such types of above mentioned experiments. The method was optimized to generate the seedlings in short time duration to provide large material for cultivation which is great challenge in Geranium cultivation.



One year old healthy geranium mother plant in which terminal cutting were taken for nursery preparation, 100 terminal cutting plantlets, newly grown one month old plant is also ready for 5-7 terminals cutting for next planting material

Fig. 1: Flow diagram

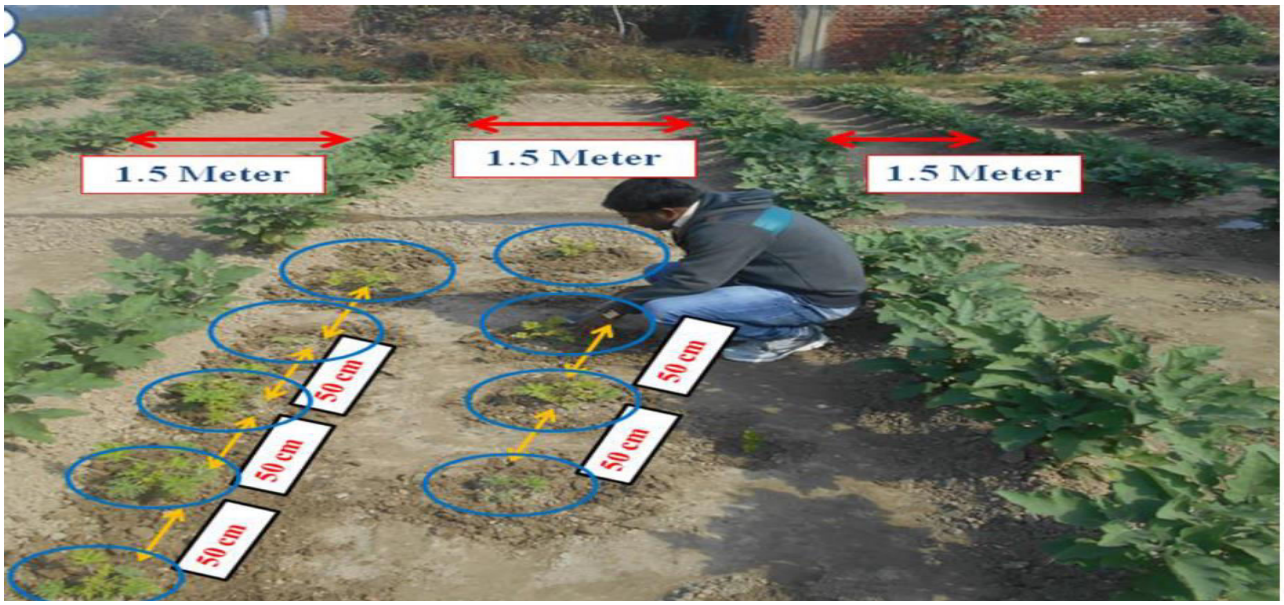


Fig. 2: Farmers' field view of one month old geranium plant intercropping treatments between Brinjal plants

2.1.1. First cutting from newly grown seedlings

Geranium cv. CIM Pawan grown in field the plantlets were obtained from one year old mother plant's stem cutting for first time plantation. After growing one month in the field (total two months plants) the plantlet is ready for first time stem cutting. First time after one month of transplanted old plants is ready for 7-8 terminal stems cuttings of uniform size (5-cm length, 2-4 node and 3-4 terminal leaves) of geranium. Thus first 50 plants will (50x8) multiply into 400 plants after one month.

Remaining 50 plants of geranium were grown in glasshouse from one year old mother plants. Initially our target was production of 1000 plantlets from a single mother plants, having one year old. After one month total 450 plants is ready for planting. Again after one month cutting and propagation, more than thousand seedlings are ready for planting.

2.2. Field trial at north Indian District, in Uttar Pradesh

A short duration study in the rural development Under AcSIR, Ph.D programme was also conducted to fulfil the targets of understanding geranium related cultivation problems at field levels under the supervision of Dr. RP Bansal, who already performed several such rural development programmes. We had chosen a village Mohammadpur at Barabanki district in Uttar Pradesh, where CSIR-CIMAP collaborated with farmers for performing such innovative rural development programme. The name of the lead farmer is Gaya Prasad Maurya in the village Mohammadpur (Fig. 2 & 3) District Barabanki, UP.

Fig. 3: Photograph Shows Dr RP Bansal (scientist) explaining to the villager, how geranium terminal cuttings can be prepared for large scale planting material.

The major problems which farmers were faced for cultivation of geranium that because they have not any technique and method to generate raw material for planting. Because they already doing several medicinal and aromatic plants cultivation for example *Mentha arvensis*, *Ocimum sanctum, Asparagus, rose flower, Palmarosa, Citronella* (lemon grass) *etc.* Because of the high essential oil rate (₹5,000-6000 litter^{-1}) and very high demand from industries of geranium, the farmers are attracted to grow geranium for commercial cash crop.

2.3. Intercropping of aromatic crop *Pelargonium graveolens* with *Solanum Melongena*

Inter cropping is one of the most important beneficial methods for farmers because its short time period simultaneously produce more than one crop using same fertilizer and irrigation. CSIR-CIMAP already did many intercropping experiment of geranium crop but there was not any intercrop with brinjal. A field trial was conducted at the experimental purpose in the village Mohammadpur, District, Barabanki, Uttar Pradesh. The study was laid out in a plot size 6.0 x 2.0 meter using randomized complete block and four replications. The soil was sandy loam. Treatments were sole geranium and intercrops spaced at 50 cm between plants with two rows of Brinjal (*Solanum melongena*) were sown per planted already. The number of plants of geranium was constants in all the treatments.

2.4. Extraction of geranium essential oil

The aerial parts of geranium hundred gram (100.0g) leaves were collected from farmer's field, grown under field trial intercropping with brinjal plants. Leaves samples were cut into small pieces, collected and stream-distilled in a Clevenger-type apparatus for 120 minutes. The obtained essential oil mix with some small amount of water solution was extracted using diethyl-ether as solvent (1/1, v/v) and dehydrated by the anhydrous sodium sulphate (Na_2SO_4), then finally collected and stored cool and dark place for analysis. After the eliminate solvent, pale yellow coloured essential oil 0.325-0.38% of fresh weight of the leaves was obtained. The oils were collected, measured and kept in 4°C prior to analysis. The essential oil yield was calculated using essential oil content and expressed as mg g^{-1}.

2.4.1. Gas Chromatography-MS analysis

Gas Chromatography (GC) analyses of the geranium (*Pelargonium graveolens*) essential oil collected from field of farmers grown under rural development programme (CSIR-800) was conducted. For GC, oil were performed using Perkin Elmer Auto System XL GC with Equity-5 column of dimension 60 m X0.32 mm with film thickness 0.25µm. Oven temperature was programmed from 70°-250°C at 3°C/min with an initial and final hold of 2 min and from 250°-290°C at 6°C/min with final hold of 5 min. Injector and detector temperature was maintained at 290°C and 280°C, respectively. Hydrogen gas was used as carrier gas (10 Psi column head pressure) with a split ratio of 1:50. The relative amounts of individual components were calculated based on GC peak areas.

2.5. Statistical analysis

All field and glasshouse experiments were successfully conducted more than three times in different three seasons and the average of generated data were used for morphological and other physiological and Biochemical analysis. Five randomly selected plants of each treatment were used for analysis. Results were compared with standard deviation from mean value at level of significance ($p <\_ 0.005$).

3. RESULTS AND DISCUSSION

Essential oil obtained from aromatic plants is extracted from the leaves, flowers and stem of the different crops. Mentha essential oil is obtained from leaves of *Mentha arvensis* [2]. *Artemisia* essential oil is obtained from leaves of *Artemisia annua* [22]. Similarly the glandular trichome of Dioscorea species is a storage house of elemol rich essential oil [11]. Yadav *et al.*, [21] have also reported lower glandular trichome density and size resulted in lower artemisinin and essential oil content in water deficit stress treated *A. annua* plants. Geranium oil is an essential oil which is extracted from the leaves, flowers and stem of the geranium plant. The key chemical constituents of geranium oil include geranic, citronellyl formate, linalool, sabinene, myrtenol, eugenol, methone, geraniol, terpineol, citral, and citronellol [18]. The demand of rose scented geranium essential oil is much more for its huge demands in perfumery, medicinal, and cosmetic industry [15]. Leading company in the global geranium oil market include Penta Manufacturing Company, Fleurchem, Albert Vieille, Ernesto Ventos, Indukern Internacional, and Robertet Group. According to industry sources, demand for rose geranium oil is growing and currently outstripping the growth in supply. World market demand for geranium oils is estimated at 400 tonnes annually. In the coming

years, global demand is expected to grow between 8.4% and 11.3% annually until 2024-2025, reaching between $14.0 and $15.8 billion. Where in India is importing more than 20 t of this oil from other countries to meet the local demands of the Indian perfumery industries, in addition to an indigenous production of only about 20 t of oil annually. There are lots of difficulties and cost issue for the production of geranium plantlets grown *vis* tissue culture approach [16]. For shortening of such problems in our study we had developed a planned simple stem cutting method for the production of geranium plantlets in very easy and simple manner and also try to develop it with intercropping system. As we know that Geranium is vegetatively propagated through propagules raised from cuttings [1]. For this our study is also focused that how to produce one thousands propagules from one year old single mother plants (Figs 1-3). There are lots of crop already grown use intercropping system for time and disease managements. Our study first time introduces that the geranium crop grown into brinjal intercropping. Intercropping is one of the most important beneficial methods for farmer for short duration crops in this both crop can grow simultaneously and using same recourses as fertilizer and irrigation [17-18]. Intercropping is also a natural way of pest and time management process. Intercropping is also reported to control weed [23]. Several intercropping experiments related to geranium with potato and mints have been successfully conducted [14-18]. Intercropping of cowpea or black gram is beneficial during the long phase and they do not affect the geranium crop. It has been observed, that the cultivation of geranium in association with marigold (*Togetes minuta*) improves the survival of geranium plants over the monsoon time in the North Indian plains. Roots-knot nematodes, *Meloidogyne incognita* and *M.hapla*, have been found to affect the geranium plant. Application of Aldicarb 20 kg/ha to the soil reduces the incidence of root-knot. With this background information in hand, the proposed Geranium experiments have successfully achieved the targets.

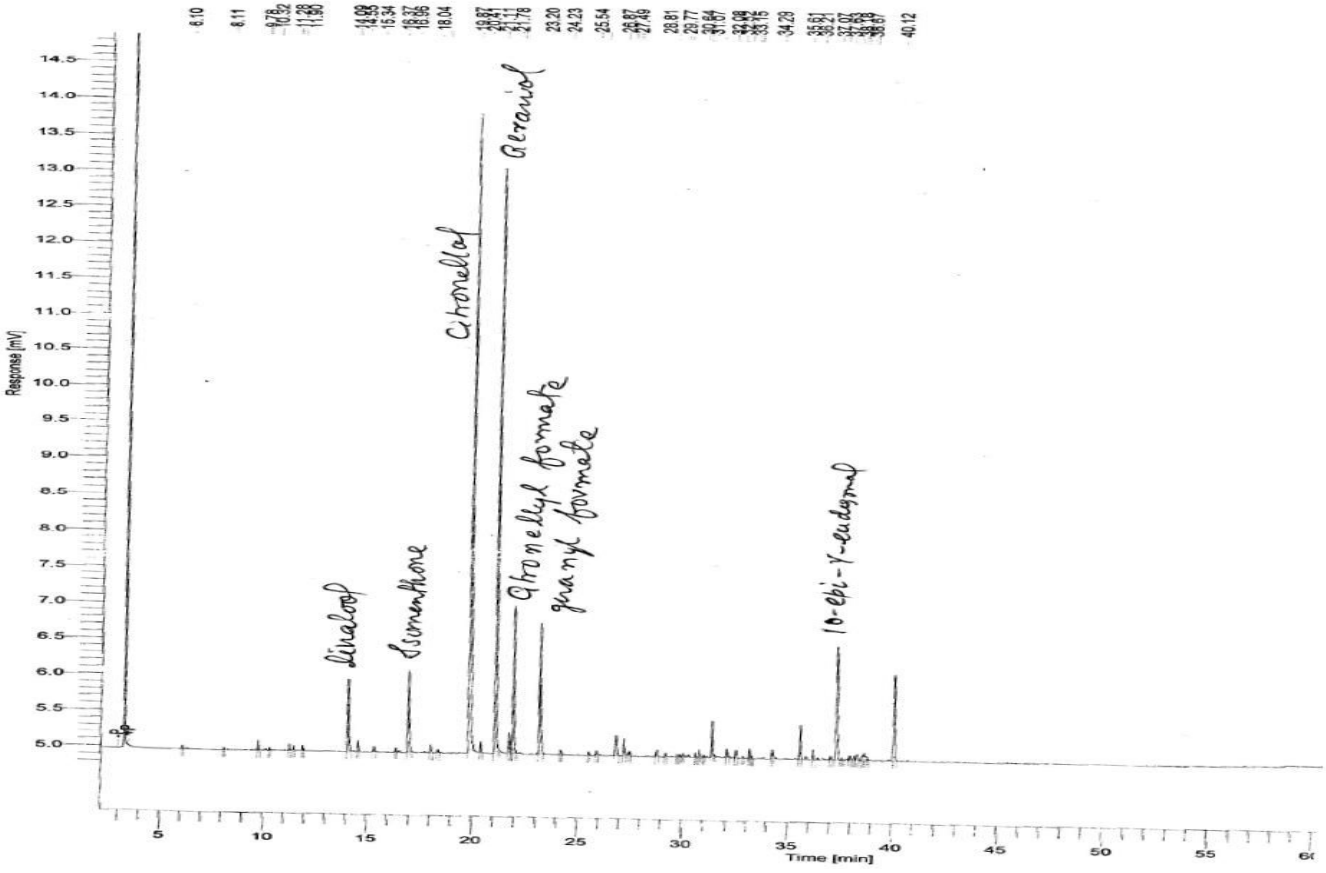
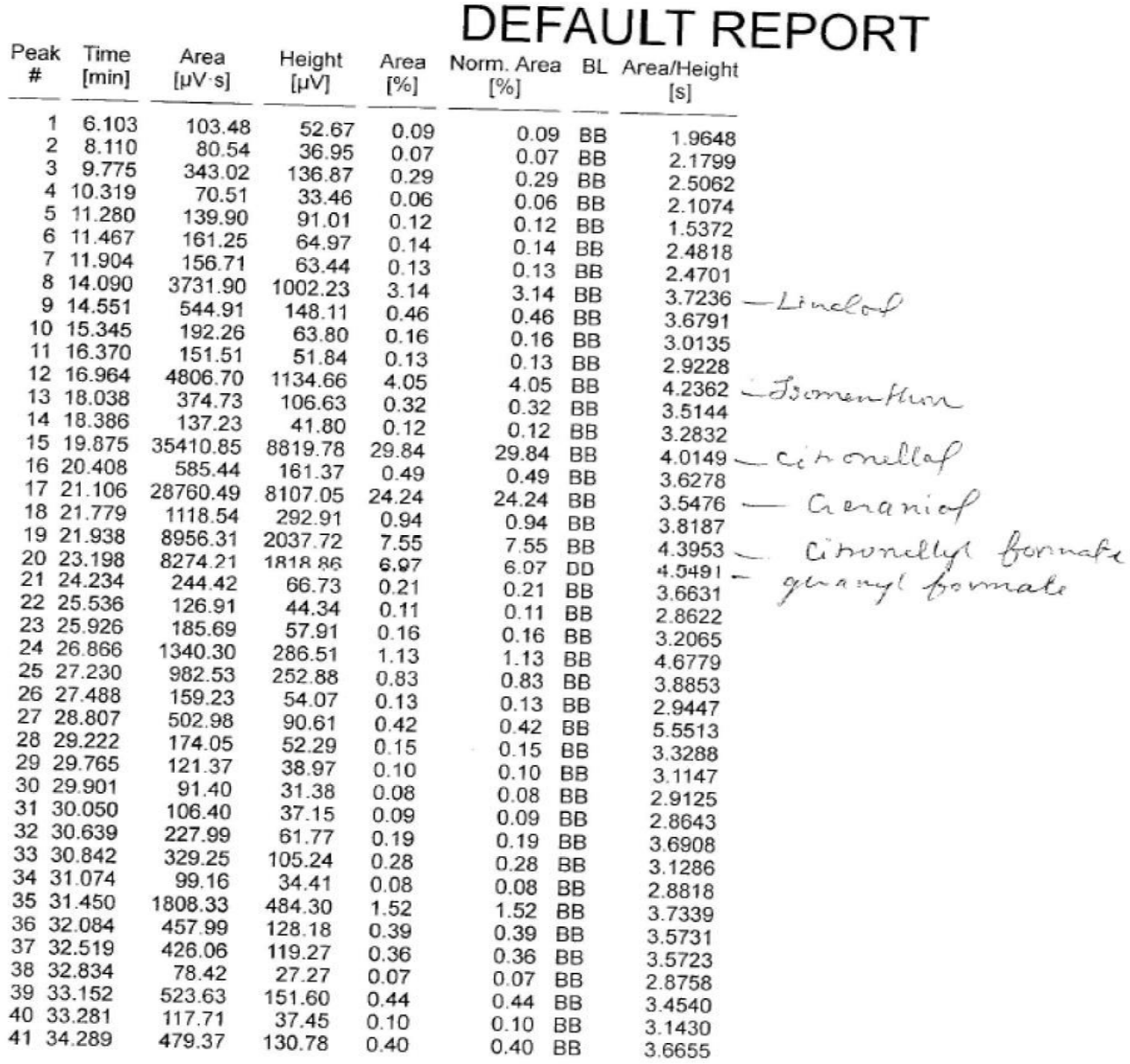


Fig. 4(A): Gas chromatograph for geranium oil collected from farmer's field grown under rural development programme (CSIR-800)



DEFAULT REPORT

Peak #	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	BL	Area/Height [s]	
1	6.103	103.48	52.67	0.09	0.09	BB	1.9648	
2	8.110	80.54	36.95	0.07	0.07	BB	2.1799	
3	9.775	343.02	136.87	0.29	0.29	BB	2.5062	
4	10.319	70.51	33.46	0.06	0.06	BB	2.1074	
5	11.280	139.90	91.01	0.12	0.12	BB	1.5372	
6	11.467	161.25	64.97	0.14	0.14	BB	2.4818	
7	11.904	156.71	63.44	0.13	0.13	BB	2.4701	
8	14.090	3731.90	1002.23	3.14	3.14	BB	3.7236	Linalool
9	14.551	544.91	148.11	0.46	0.46	BB	3.6791	
10	15.345	192.26	63.80	0.16	0.16	BB	3.0135	
11	16.370	151.51	51.84	0.13	0.13	BB	2.9228	
12	16.964	4806.70	1134.66	4.05	4.05	BB	4.2362	Isomenthone
13	18.038	374.73	106.63	0.32	0.32	BB	3.5144	
14	18.386	137.23	41.80	0.12	0.12	BB	3.2832	
15	19.875	35410.85	8819.78	29.84	29.84	BB	4.0149	Citronellol
16	20.408	585.44	161.37	0.49	0.49	BB	3.6278	
17	21.106	28760.49	8107.05	24.24	24.24	BB	3.5476	Geraniol
18	21.779	1118.54	292.91	0.94	0.94	BB	3.8187	
19	21.938	8956.31	2037.72	7.55	7.55	BB	4.3953	Citronellyl formate
20	23.198	8274.21	1818.86	6.97	6.07	DD	4.5491	geranyl formate
21	24.234	244.42	66.73	0.21	0.21	BB	3.6631	
22	25.536	126.91	44.34	0.11	0.11	BB	2.8622	
23	25.926	185.69	57.91	0.16	0.16	BB	3.2065	
24	26.866	1340.30	286.51	1.13	1.13	BB	4.6779	
25	27.230	982.53	252.88	0.83	0.83	BB	3.8853	
26	27.488	159.23	54.07	0.13	0.13	BB	2.9447	
27	28.807	502.98	90.61	0.42	0.42	BB	5.5513	
28	29.222	174.05	52.29	0.15	0.15	BB	3.3288	
29	29.765	121.37	38.97	0.10	0.10	BB	3.1147	
30	29.901	91.40	31.38	0.08	0.08	BB	2.9125	
31	30.050	106.40	37.15	0.09	0.09	BB	2.8643	
32	30.639	227.99	61.77	0.19	0.19	BB	3.6908	
33	30.842	329.25	105.24	0.28	0.28	BB	3.1286	
34	31.074	99.16	34.41	0.08	0.08	BB	2.8818	
35	31.450	1808.33	484.30	1.52	1.52	BB	3.7339	
36	32.084	457.99	128.18	0.39	0.39	BB	3.5731	
37	32.519	426.06	119.27	0.36	0.36	BB	3.5723	
38	32.834	78.42	27.27	0.07	0.07	BB	2.8758	
39	33.152	523.63	151.60	0.44	0.44	BB	3.4540	
40	33.281	117.71	37.45	0.10	0.10	BB	3.1430	
41	34.289	479.37	130.78	0.40	0.40	BB	3.6655	

Fig. 4(B): Gas chromatograph for geranium oil collected from farmer's field grown under rural development programme (CSIR-800)

Table 1: Major essential oil constituents of geranium oil sample obtained from farmer's field

Major Essential oil composition	Citronellol	Geraniol	Linalool	Iso-menthone	Citronellyl formate	Geranyl formate	10-epi-γ-eudesmol
Percentage	29.84%	24.24%	3.14%	4.05%	7.55%	6.07%	2.7%

In this experiment essential oil of *Pelargonium graveolens* from the aerial parts and the crop were growing in Mohammadpur and Bisunpur village as well as CSIR-CIMAP field and the essential oil was obtained by hydro distillation process with the help of Clevenger type apparatus and its constituents analyzed by the GC and GC-MS technique (Fig. 4 A & B). Under experiments, the obtained essential oil quality was checked by above GC and GC-MS approach [13-18]. First chopped into small pieces of leaves were used for extraction of rose-scented geranium essential oils and compared for their yield and composition [2-5]. *Mentha arvensis* essential oil

was also isolated and checked [2]. In our geranium experiments leaves oil yields (0.32-0.38%) were recorded. In our field sample total forty-one components representing 98.1% of the total oil were identified (Fig. 4B). Chopped leaf essential oils were richer in Citronellol, Geraniol, Linalool, Iso-menthone, Citronellyl formate, Geranyl formate, and also 10-epi-γ-eudesmol (2.70%) etc. The major constituents of the essential oil were Citronellol (29.81%), Geraniol (24.23%), Linalool (3.12%), Iso-menthone (4.09%), Citronellyl formate (7.51%) and Geranyl formate (6.07%), 10-epi-γ-eudesmol (2.70%). The Citronellol/ Geraniol ratio (C: G), quality determining factor was found to 1.23 (Table 1).

4. CONCLUSIONS

In conclusion, as for as the aims of this field trial-related experiments are concerned, geranium intercropping with Brinjal will improve the economy and opportunities for producers of rural areas farmers in the district of Uttar Pradesh, India. Our study also addresses the geranium cultivation-related major problem of farmers mainly in North India. Seedling availability is one of the major problems faced by prospective farmers. Hopefully, this new technology helps to boost the production of 'Geranium saplings' for farmers' cultivation of Geranium.

5. RECOMMENDATIONS

After the study our research team recommend to the farmers for the cultivation of geranium intercropped with brinjal because it's beneficial for time management farmers will produce more yield and improve their economy. Intercropping is natural way of pest management process as well. Intercropping also controls weed and simultaneously grows more than one crop using same irrigation and fertilizer.

6. ACKNOWLEDGEMENTS

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Conflict of Interest

The authors declare that they have not any conflict of interest about this manuscript and research articles.

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