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Domestication and nutrient management of *Monarda citriodora* Cer.ex Lag. in Sub Tropical region of Jammu (India)

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Abstract

Monarda citriodora Cer. ex Lag. grows wild in the rocky upland prairies and pastures of the south eastern tall grass Missouri and ranges from Texas and Mexico North. It is an aromatic annual herb and is one of the important sources of Thymol. The crop has been domesticated by our Institute not only in subtropical but also in temperate region in Jammu and Kashmir. Growth, Yield and yield attributes performance under varying levels of nitrogen were examined in a field experiment. Forty five day- old plants were supplied with varying levels of N, i.e. 0, 30, 60, 90,120 and 150kg N/ha in soil. Maximum biomass was recorded at 90 Nitrogen kg/ha (T4) but at par with 120 and 150Nkg/ha. Herbage yield was increase 14.40, 16.73 and 27.95 percent higher with 30, 60 and 90kg N/ha., respectively, over 0 kg N/ha. Significantly influenced the total number tillers/plant and branches at 60 DAT, pre- flowering and 50% flowering highest in T4 (90kgN/ha) treatment. Nitrogen levels significantly influenced the plant height at 60 DAT, pre-flowering and 50% flowering. Significantly the highest plant height (40, 63 and 79 cm) was recorded by T4 (90kgN/ha.) treatment but at par with T3 (60kgN/ha.). Nitrogen levels 90kg/ha. is best suitable for commercial cultivation and higher yield.

Keywords: Monarda, thymol, nitrogen, cultivation, essential oil

Introduction

Lemon mint, otherwise known as Horse mint, purple horsemint, plain horsemint or lemon bee balm is an easy recognizable old time favorite. The scientific name of *Monarda citriodora*, was given to this wild flower in honor of Nicholas Monarda a Spanish physician in the 1500's who discovered several medicinal properties of plants found in the America's. The name citriodora refers to the citrus like scent that the plant has when the leaves are crushed. *Monarda bradburiana* Beck and *Monarda fistulosa* L. essential oils showed good mosquito repellent activity with minimum effective dosages (MED) of 0.055 ± 0.036 and 0.078 ± 0.027 mg/cm², respectively (Nurhayat *et al.* 2013) [13]. This scent makes it deer resistant and if you rub leaves on skin it make a fair insect repellent also. The essential oil from *Monarda citriodora* flowers have medicinal properties as antibacterial activity (Lu Zhan-gou *et al.* 2011) [10], and suitability perfuming and flavoring of food industries. Due to high demand of its essential oil, there has been increasing interest in the cultivation of this plant for commercial production. *Monarda citriodora* Cer. Ex Lag. An alternate rich source of thymol (Verma and Chandra, 2013) [19]. Nitrogen is one of the most important nutrient as well as expensive input in agricultural production. An adequate supply of nitrogen is closely associated with growth and development of crop plants. It plays an important role in plant metabolism by virtue of being an essential constituent of structural cell and many metabolically active compounds. It is also constituent of chlorophyll, which is important for harvest of solar energy (Bray, 1983) [5]. Details of nutrient management and agricultural practices to get optimum oil yield per unit area. Research was carried out to develop an Agrotechnology, packing with chemical composition leading to constituent oriental production package of "Monarda Oil".

Distribution and Natural History**Distribution**

Monarda citriodora (Lemon horse mint) grows wild in the rocky upland prairies and pastures of the south eastern tall grass region.

Its occurs on limestone glades, bald knobs and rocky prairies in Missouri and ranges from Texas and Mexico North to Missouri and Kansas. It is an aromatic annual herb widely distributed in America (Baily, 1977) [2]. Plants were introduced in India by Scientists of Indian Institute of Integrative Medicine (CSIR), Jammu from Europe.

Climate and Soil

Table 1: Soil physico- chemical properties of experimental area.

Parameters	Analytical value	Method employed
Mechanical properties		
Sand (%)	66.4	Bouyoucous Hydrometer method (Piper, 1966) [17]
Silt (%)	18.0	
Clay (%)	15.6	
Textural class	Sandy loam	
Chemical properties		
pH	8.10	Suspension of soil and water 1:2.5 with glass calomel electrode (Jackson, 1973) [6]
EC (dS m ⁻¹)	0.24	Salt bridge measurements from the suspension used for pH determination (Jackson, 1973) [6]
Organic carbon (%)	0.56	Dichromate oxidation of organic matter (Jackson, 1973) [6]
Bulk density (g cc ⁻¹)	1.41	Core method (Black, 1965) [4]
Available N (kg ha ⁻¹)	211.0	Alkaline permanganate method (Subbiah and Asija, 1956) [18]
Available P (kg ha ⁻¹)	17.20	0.5 N Sodium bicarbonate (pH 8.5) (Olsen <i>et al.</i> , 1954)
Available K (kg ha ⁻¹)	130.0	Ammonium acetate extraction method (pH 7.0) using flame photometer (Jackson, 1973) [6]

Morphology

Its attains a height of 50-100 cm. Several stems grow from the base and are lined with pairs of 3 to 8 cm long, soft textured, lance shaped leaves. Leaves have a very strong and pleasant *Trichyspermum ammi* (ajwain) like fragrance when crushed. It grows quickly during spring and bloom it white, purple and pink colored whorled flower head attract humming birds, bee and butterflies from May through July, continuing to bloom even later in the year if given water. Test weight (1000 Seeds) varies from 0.50 to 0.60 gram.

Pictures of Seed, Nursery, Vegetative, 50% flowering and full blooming stage of *Monarda citriodora*.



Seed

Nursery

Vegetative Stage



50% Flowering Stage

Full Blooming Stage

Uses

Edible Use

Edible part of plant is leaves- raw and cooked. They are used as flavoring and garnishing agent in Salad, summer punches

Monarda citriodora Cer. L is *Rabi* Season Crop. It requires 20-30 temperature for higher herb and oil yield. Soil with a clay loam or loam texture, good structure and moderate water holding are ideal for monarda cultivation. It can tolerate dry soil and requires little water and direct sunlight. Soil physico-chemical properties of experimental area as under (Thesis Ph.D. SKUAST, Chatha, Jammu 2011) [7].

and as condiment in cooked foods and also used to make a refreshing lemon tea. It makes a lovely aromatic tea and flowers are edible.

Medicinal Uses

Uses in colds, coughs, fevers, flue, bronchial problems, colic, flatulence nausea, stomach cramps, menstrual irregularity, bowl ailments to expel worms, induce sweats, headaches etc., most often as tea especially for headaches, other medicinal treatment methods for bronchial problem include inhalation of the extracted essential oil or vapor therapy. The essential oil used as an insect repellent and in perfumery.

Biological Properties

To evaluate the cytotoxic potential of essential oil of *Monarda citriodora*. It has been tested for the MTT Cell viability assay in human leukemia HL-60 Cells. Essential oil of *Monarda citriodora* has significant cytotoxic potential in HL-60 Cells, with IC 50 value (Concentration of test compound which is required to inhibit 50% Cell Viability) of 22 Ug/ml. (Pathania *et al.* 2013) [16].

Material and Methods

A field experiment was conducted at Field Research Station, Indian Institute of Integrative Medicine (CSIR), Jammu during *Rabi* -2013. This region lies 32°44' latitude (N) and 75°55' longitude (E). The altitude of the area is ≈300 m above sea level. The annual precipitation at this site is 500–700 mm. The experiment was laid out in Randomized Block Design with four replication and six treatment (0, 30, 60, 90, 120 and 150 kg N/ha) Nitrogen used as urea. The forty-five days old seedling were transplanted in each plot (11.4x4.5 m) manually at depth 5-6 cm and irrigated.

The observation regarding plant height (cm) was recorded randomly selected tagged five plant with the help of putting graduated scale from the base of plant, total number of tillers per plant, total number of branches in each selected plant was counted was at 60 DAT, pre flowering and 50 % flowering respectively. Fresh herb weight and dry herb weight were

recorded at harvest in kg per plot and converted to quintal per hectare. At harvest, herb sample of *Monarda citriodora* were distilled in a Clevenger type apparatus for essential oil determination. Oil yield kg per hectare was obtained through multiplied oil content and fresh herb yield. The data were analyzed statistically and results were evaluated at 5 % level of significance.

Chemical Properties

Gas Chromatography-Mas Apectroscopy

- Physical- chemical properties of *Monarda citriodora* Cerv. Ex lag.
- Physical Properties: (Manual of *Monarda citriodora*, IIM- CSIR, Jammu, 2010)^[11].
Refractive Index nd250c :1049995
Specific Gravity d 25oc :0.927678



GC/MS- GC/MS analysis was conducted on Varion Ion trap MS system (MS-4000), equipped with CP-sil-8 capillary column (length 30m, I.D. 0.25 mm with 0.25 film thickness). GC analysis was carried at oven temperature 50o C/5 -250oC @ 3oC /min. hold time seven minutes Injector temperature 280oC transfer line temperature was maintained at 230oC. Helium was used as a carrier gas with flow rate 1ml/min., Ionization energy in mass was 70ev, mass range 40-500amu and scan rate was 1.92. The constituent were identified at this condition comparison with authentic data library. Mass spectroscopy and RT were matched with library.

Cultivation Practices

Cultivation practices developed by the Institute were followed as described below

Land Preparation

The soil is prepared through disc harrow, ploughing and planking, free from weed infestation to added 15-20 ton/ha. FYM well rotted 15 days before transplanting is well mixed in to the soil.

Seed and Sowing

Monarda citriodora seeds are small size can be grown in nursery beds through broadcasting mixed sand with seeds during 1st week of Oct. to December. Germination takes a time 10-15 day after sowing. One month old seedling is ready for transplanting in well prepared field. Spacing 30x30 cm is

recommended of our institute but 40x40 cm also suitable for good growth and yield attributes. Just after transplanted irrigation of crop. About 300-400 gram seed is sufficient for one hectare.

Manures and Fertilizer

Manures and fertilizer both play important role in *Monarda citriodora* crop cultivation. Its improves physical conditions, structure and water holding capacity of the soil. The crop require 80kg N/ha, 50kg P₂O₅/ha. And 50kg K₂O /ha. In addition to 15-20 tonn/ha. FYM. The full quantity of Phosphorus and potash in the form of Single Super Phosphate and Murate of potash, respectively. Along with half dose of Nitrogen (Urea) is placed in 4-5 cm deep in rows at the time of transplanting. The rest Nitrogen should be divided in two split doses sprayed in top dressing 30 days interval.

Irrigation and Weeding

Adequate soil moisture is required for normal development of all stages of crop. Irrigation is required less compared to other crop. However, 10-15 days interval irrigate in warm weather due to higher production of herbs. Generally, one month after transplanted crop weeds are eradicated with the help of hoe or khurpi and next one month hoeing should be done.

Pest and Disease

There is no major attack of pest and disease was observed.

Harvesting and Distillation

Essential oil is present in leaves and flower parts of the crop where as the stem is devoid of any oil. Therefore, the crop is harvested above the ground at the height where the green are attached to stem. A higher ratio of leaves and inflorescence to stem is desirable for higher recovery. Crop is harvested in May to June at full blooming stage to avoid rain.

The essential oil from the crop is obtained by steam distillation. Green foliage should not be piled in heaps but spread on the floor in shade before distillation. It takes 3-4 hours for completing extract oil in field/ manual distillation unit while in steam operating 2-3 hours only.

Storage of Oil

The oil is stored in stainless steel container. Essential oil undergoes considerable changes in to chemical composition during storage. On longer period of storage, polymerization take place resulting in decrease of thymol content thus adversely affecting the oil quality. To avoid this type of loss the essential oil should be completely dried by using anhydrous sodium sulphate or any other dehydrating material before filling the container. The container should be air tight and filled to the brim and preferably stored in dry, cold and dark conditions (Manual of *Monarda citriodora*, IIM -CSIR, Jammu, 2010)^[11].

Experimentation and Data collection

Result and Discussion

Effect of different treatments on growth attributes

A perusal of data in Table 2 revealed that nitrogen levels significantly influenced the total number of tillers/plant at 60 DAT, pre-flowering and 50% flowering. Significantly maximum number of tillers/plant (3.6, 3.73 and 3.78) was obtained under T4 (90kg N/ha.) treatment but at par with T5 and T6 (120 and 150kg N/ha). Significantly the lowest registered in T1, T2 and T3 (0, 30, 60kgN/ha) treatment.

Various nitrogen levels not influenced significantly on total number of branches/plant at pre-flowering and 50% flowering over the T1(0kg N/ha) treatment but significantly highest no. of branches/plant in T4(90kgN/ha.) treatment at par with T3 (60kgN/ha.) treatment at 60 DAT.

Data presented in Table 2 indicated that nitrogen levels significantly influenced the plant height at 60 DAT, pre-flowering and 50% flowering. Significantly the highest plant height (40, 63 and 79 cm) was recorded by T4 (90kgN/ha.) Treatment but at par with T3 (60kgN/ha.) at 60DAT, T3 and T2 (60 and 30kgN/ha) at pre-flowering and T5 and T6 (120 and 150kgN/ha.) at 50% flowering stage. Significantly

shortest plants was observed (33, 34, 32 and 34cm) at 60DAT, (58, 56and 55cm) at pre-flowering and (60,65 and 64 cm) at 50% flowering.

Adequate supply of N promotes higher photosynthetic activity and vigorous vegetative growth and a result, the plant turn in to dark green color. A high N supply favors the conversion of carbohydrate into protein which, in turn, promotes the formation of protoplasm. Protoplasm, being highly hydrated, is conducive for the succulent plant growth (Balasubramaniam and Palaniappan 2005)^[3].

Effect of different treatments on herb and oil yield

Herbage yield of *Monarda citriodora* increase with increase in Nitrogen levels but economically suitable for particular level. Application of Nitrogen at 90kgN/ha. Produced the highest mean herbage yield (217.11qtls./ha.), which was statistically equal with T5 and T6 (120 and 150kg N/ha.) treatment (Table 3). Significantly the lowest yield 156.43, 182.75 and 187.87 qtls./ha. (T1, T2 and T3) treatment respectively. This indicated that herb yield was increased with increased in nitrogen fertilization up to T4 level (90 kg /ha.). Herbage yield was increase 14.4, 16.7 and 27.9 percent higher with 30, 60 and 90kg N/ha., respectively, over 0 kg N/ha. Similar result were obtained in dry weight qtls./ha and oil yield kg/ha in this finding.

It is an established fact that nitrogen is one of the essential constituent required for the synthesis of protein, chlorophyll and other organic compound of physiological significance in the plant system. Since, in the plant system most of the nitrogen accumulated in the reproductive structure is trans located from vegetative parts, the assumption seems to be justifying that nitrogen application lead to increased nitrogen content in the plants right from early stage of crop growth. The faster growth of plants evidenced from increased biomass per plant at successive stage of crop growth with nitrogen subscribe to the views that there was better availability of metabolites and nutrients, which synchronized to the demand for the growth and development of each reproductive structure of monarda plant. Similar results were also reported by Naruka *et al.* 2012^[12], Krishnamoorthy and madalgari, 2000^[8], Krishnamoorthy and madalgari, 2002^[9], Asharf and Noman 2006^[1], Wahab and Mohamed, 2007^[20] and Nath *et al* 2008^[14].

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Table 2: Effect of nitrogen on growth and yield attributes of *Monarda citriodora*

Treatment	No. of tillers/plant			No. of branches/plant			Plant height (cm)		
	N kg/ha	60 DAT	Pre-flowering	50% flowering	60 DAT	Pre-flowering	50% flowering	60DAT	Pre-flowering
0	2.2	2.20	2.25	17	16	18	33	58	60
30	2.5	2.46	2.63	18	20	21	34	60	65
60	2.6	2.80	3.25	20	22	22	37	62	64
90	3.6	3.73	3.78	21	25	25	40	63	79
120	3.6	3.68	3.74	18	22	21	32	56	71
150	3.5	3.55	3.45	18	22	25	34	55	74
SEm±	0.21	0.20	0.20	0.79	2.10	2.17	2.05	2.24	4.55
CD at 5%	0.45	0.44	0.42	1.69	4.48	4.63	4.37	4.78	9.70

Table 3: Effect of nitrogen on Oil content, fresh herb and dry weight of *Monarda citriodora*. Cer.L.

Treatment N kg/ha	Oil content (ml/kg)	Fresh herb (Qtls. /ha)	Dry weight (Qtls. /ha)	Oil yield (kg/ha)
0	3.2	156.43	90.74	44.80
30	4.4	182.75	91.84	71.63
60	4.7	187.87	94.79	78.27
90	4.9	217.11	109.26	94.26
120	4.5	204.68	101.27	81.35
150	3.5	196.27	90.74	61.66
SEm±	0.32	10.77	6.39	7.82
CD at 5%	0.68	22.95	13.61	16.67

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