Effect of Gibberellic acid on some qualitative traits of Patchouli (*Pogostemon patchouli*)

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Abstract:To study the effect of Gibberellic Acid (GA_3) on chlorophyll content, dry matter accumulation and essential oil content of patchouli (*Pogostemon patchouli*), an experiment was conducted at the northern part of West Bengal, India during 2020-21. GA_3 was applied at 0, 50, 100, 150, 200, 250 and 300 ppm as foliar spray. Treatment with GA_3 at the rate of 200 ppm recorded the highest dry matter content in leaves of patchouli whereas GA_3 application @150 ppm showed highest total chlorophyll content in leaves. Maximum percentage of essential oil content was obtained in plants sprayed with GA_3 @150 ppm which was at par with GA_3 @ 100 and 200 ppm treatments.

Key words: Patchouli, Gibberellic acid, Hormone, Essential oil.

Introduction

Patchouli [(*Pogostemon cablin* Benth.) syn. (*Pogostemon patchouli*)] is one of the important aromatic plants in India belonging to the family Lamiaceae. It was originated from Philippines and Indonesia is the leading producer of patchouli oil. In terms of bulk, it is approximately the tenth most important essential oil (Lawrence 2009). India produces around 10-15 ton patchouli oil per annum (Chakrapani *et al.* 2013). Shade dried leaves of patchouli produced around 2.5% -3.5 % of essential oil (Vijaykumar 2004). The essential oil is found in the glandular trichomes on the adaxial surface of the leaves and also present in the stem, flowers and roots but of inferior quality. The patchouli oil actually consists of twenty-four different sesquiterpenes (Buré & Sellier 2004; Dung *et al.* 1990). The major component in patchouli oil is patchouli alcohol (patchoulol – 35-40%) although nor-patchoulenol (the true odor carrier), ß-patchoulene and λ-guaiene, ß-bulnesene, seychellene, caryophyllene are the minor components which add up to its aroma. These sesquiterpenes are reportedly biosynthesized from farnesyl-diphosphate (FPP) by sesquiterpene synthases such as epi-cedrol synthase, (E)-ß-farnesene synthase, pentalenene synthase, germacrene C synthase and patchoulol synthase (Deguerry*et al.* 2006). Patchouli is known for both of its aromatic and medicinal values, which expands its potential uses in aromatherapy, cosmetics, perfumery, therapeutic, pharmaceuticals, flavourings etc. The oil can be blended well with other essential oils like lemongrass, sandalwood, bergamot, which complement its exclusive value in the global market. Thus, its exquisite fixative properties have raised the demand of patchouli in many leading perfume brands like *Tabu*, *Miss Dior*, *Opium*, *Picasso* etc. (Singh *et al*.2002). The oil is also used in making of incense, scented candles as well as flavouring alcoholic and non-alcoholic beverages. It is widely used in the traditional preparation of medicines in India and China (Agarwal 1985; Akhila & Tiwari 1984). Patchouli is well-known for its antiseptic properties to treat skin and scalp diseases. It is also used as an insect repellant and an antidote to snakebites (Kirthikar & Basu 1991).

Realizing the importance of the crop, researches have been initiated in different aspect of crop management and their impacts on quality parameters including essential oil content. Among the crop management inputs, plant growth regulators play a major role in the production of primary and secondary metabolites within the plant body. They influence the plant growth, number of essential oil storage structures and biosynthesis of essential oil which conclusively can alter and improve the quality and quantity of essential oil. There are many types of phytohormones discovered namely, auxins, cytokinins, abscisic acid, gibberellins and ethylene (Rivier & Crozier1987). Gibberellins are isoprenoid compounds (mainly diterpenes) synthesised by the mevalonic acid (MVA) pathway from acetate units of acetyl coenzyme A. They are synthesised in the apical shoot buds (young leaves), root tips and developing seeds. Gibberellic acid (GA_3) is a natural hormone with tetracyclic, diterpenoid compounds. Misra (1995) reported that the increase in photosynthetic pigment (chlorophyll a and b and carotenoid) contents and essential oil yield of a shade grown patchouli cv. Johor, with the application of GA_3 @ 10-500 mg l^{-1}. On the other hand, a dose of 250 mg l^{-1} was the optimum concentration of GA_3 recorded for least growth pigment content and oil yield. According to Kumar *et al.* (2012) gibberellic acid when sprayed @ 75ppm GA_3 yielded 0.85% and 1.94% menthol oil during winter and summer season respectively in mentha (*Mentha piperita* L.). In view of the reports on beneficial effects of gibberellic acid, the present experiment was laid out with the aim to study on the impact of GA_3 on chlorophyll, essential oil and dry matter content of the final product.

Materials and methods

Experimental site and plant materials

The research experiment was carried out during 2019-2020 at the *Terai* zone of West Bengal, India at the instructional farm of Department of Plantation Crops and Processing, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal. The experimental site was situated at 26^0 22`N latitude and $89^0$ 29`E longitude with an altitude of about 48 meter above mean sea level. The maximum mean daily temperature recorded was 34.34 ^{0}C in August, 2019 while the minimum mean daily temperature was 9.86 ^{0}C during January, 2020. The field experiment was conducted in the experiment station having acidic soil and sandy loam to clay loam in texture. No crop was cultivated in the recent previous years. The soil pH ranged from 5.97 to 6.50 while the organic carbon varied from 1.51 to 2.29 % (Sarkar *et al.* 2017).

Stem cutting of 4-5 nodes of 10-15 cm long from the semi-woody portion of the plant was taken with 2-3 leaves kept intact, were dipped in a rooting hormone (IBA 1500 ppm) for early establishment or rooting of the plant. The rooted cuttings were transplanted in the main field after 30 days. The design of the experiment opted was RBD (Randomized Block Design) with 7 treatments replicated thrice, thus covering 21 plots of 2 m × 1 m separated by an irrigation channel of 50 cm. The treatments comprised of: T_1 (control) i, e. no application of GA_3, T_2 (GA_3 @ 50 ppm), T_3 (GA_3 @ 100 ppm), T_4 (GA_3 @ 150 ppm), T_5 (GA_3 @ 200 ppm), T_6 (GA_3 @ 250 ppm) and T_7 (GA_3 @ 300 ppm). GA_3 was applied as foliar spray at monthly intervals. Farmyard manure (FYM) was given at the time of planting and vermicompost (5 t ha^{-1}) was applied at a later stage of @ 2 $plant^{-1}$ encircling the base of the plant. Trichoderma (500g) in addition with 30 kg of vermicompost was applied in three splits i.e., 10 kg each at 15 days interval. Harvesting was done 7 months after transplanting. For determining the total dry matter and herbage yield, the plants were uprooted entirely from the soil and dried in hot air oven at 50 ^{0}C until they attained a constant weight. For chlorophyll estimation, harvesting of leaves was done at three intervals. In addition, the leaves for estimation of oil extraction were harvested after 7 months of transplanting and were shade dried for a period of 3 days (72 hours) under room temperature. Leaves were manually plucked and only matured; disease-free leaves were selected for chlorophyll estimation as well as for oil extraction purpose.

Observations recorded

Estimation of leaf chlorophyll content was done according to the method of Sadasivam and Manickam (1991). 1 g of fresh plant leaf was extracted in 100 ml of 80% acetone. Absorbance of the extract were recorded at 663 nm and 645 nm in a UV-Vis spectrophotometer. Chlorophyll content in leaf was calculated as,

Chlorophyll a content (mg/g) = $[12.7(A_{663}) – 2.69(A_{645})] \times(V/1000\times W)$

Chlorophyll b content (mg/g) = $[22.9(A_{645}) – 4.68(A_{663})] \times(V/1000\times W)$

Total chlorophyll content (mg/g) = $[20.2(A_{645}) + 8.02(A_{663})] \times(V/1000\times W)$

Where, A = absorbance at specific wavelength, V = final volume of chlorophyll extract in 80% acetone which in this case is 100 ml and W = fresh weight of tissue extracted which is 1 g.

The essential oil content in patchouli leaves was estimated by using the soxhlet extraction method as suggested by Barros *et al.* (2013). 2.5 g of crushed dried leaves were filled into the thimble and a volume of 100 ml of solvent (hexane) was introduced into the solvent cup. The isomantle (heat source) was set just above the boiling point of the solvent (hexane i.e., 69 ^{o}C). The process was completed in four hours and the solvent (hexane) was removed by using a rotary evaporator at 40 ^{o}C in order to separate the oil. The essential oil extracted from all treatments of each plot was summed up in average values and the percentage of oil was calculated by multiplying the oil extracted per gram of sample to 100 and expressed in %. The moisture content percentage of leaves, stem and branches were calculated by using of the formula given by Clara *et al.* (2010). The dry matter percentage was calculated in leaves, stems and branches, total herbage (leaves + stems and branches) and roots which had a constant weight after drying by using the following formula.

(i) Dry matter in leaves (%) = (Dry weight of leaves / Fresh weight of leaves) x 100
(ii) Dry matter in stems and branches (%) = (Dry weight of stems and branches / Fresh weight of stems and branches) x 100
(iii) Dry matter in total herbage (%) = (Dry weight of total herbage / Fresh weight of total herbage) x 100
(iv) Dry matter in roots (%) = (Dry weight of roots / Fresh weight of roots) x 100.

Statistical analysis

The data generated throughout the course of the study were calculated in average values and were analyzed by Fisher's analysis of variance (ANOVA) method. The significance of differences between the mean variations of different treatments was estimated using critical difference at 5% based on the probability, $P\leq0.05$.

Results and discussion

Moisture content and dry matter percentage (%)

As per the study on moisture and dry matter content (%), it was observed that there existed an inverse relationship between them. Parganiha *et al.* (2016) also showed that when the moisture content in patchouli decreases, the recovery of patchouli oil increases and vice versa. Umesha *et al.* (1991) gave a report on the efficacy of gibberellic acid application that gave maximum amount of dry matter in Clocimum. Faldu *et al.* (2018) disclosed that the foliar application of GA_3 @ 100 ppm increased the total dry matter in groundnut plants and explained the mechanism behind being higher production of photosynthates which led to more dry matter to be transported.

Table 1. Effect of different treatments of gibberellic acid (GA_3) on moisture content (%) and dry matter (%) of patchouli.

Treatments	**Moisture content percentage (%)**				**Dry matter percentage (%)**			
	Leaves	**Stem and branches**	**Total herbage**	**Roots**	**Leaves**	**Stem and branches**	**Total herbage**	**Roots**
T_1 – Control	75.32	84.77	80.89	71.07	24.68	15.23	39.91	28.93

T_2 – GA_3 @ 50 ppm	74.33	81.62	78.49	68.23	25.67	18.38	44.06	31.77
T_3 – GA_3 @ 100 ppm	71.42	83.71	78.10	66.76	28.58	16.29	44.87	33.24
T_4 – GA_3 @ 150 ppm	71.30	79.37	75.92	73.80	28.70	20.63	49.33	26.20
T_5 – GA_3 @ 200 ppm	71.24	81.29	77.44	68.00	28.76	18.71	47.47	32.00
T_6 – GA_3 @ 250 ppm	87.16	81.33	84.07	70.04	12.84	18.67	31.51	29.96
T_7 – GA_3 @ 300 ppm	83.77	78.52	79.68	68.50	16.23	21.48	37.71	31.50
C.D.	**0.94**	**0.52**	**0.42**	**1.13**	**0.94**	**0.52**	**0.96**	**1.13**
SE(m)	**0.30**	**0.17**	**0.13**	**0.36**	**0.30**	**0.17**	**0.31**	**0.36**

Leaf chlorophyll content estimation

The chlorophyll content ('a', 'b' and total) was obtained highest by GA_3 @ 150 ppm (Table 2). Kanjilal & Singh (1998) clarified that increased rate of photosynthesis and increased chlorophyll synthesis were the key functions responsible for development of chlorophyll content on GA_3 treated plants. The results of the present experiment were also similar to Sardoei and Shahdadneghad (2014) who in their experiment, demonstrated various doses of GA_3 and noticed up to 250 mg l^{-1} was most beneficial. Furthermore, Khan (1996) reported that plants applied with GA_3 showed signs of improved carbonic anhydrase (CA) activity, an enzyme which is important in photosynthetic CO_2 fixation and is strictly associated with chloroplast. It leads to high net photosynthetic rate and ultimately increases the dry mass accumulation which was also found in the present experiment.

Table 2. Effect of different treatments of gibberellic acid (GA_3) on Chl. a, Chl. b, Total Chl. and essential oil content of patchouli.

Treatments	**Chlorophyll 'a' content plant^{-1}(mg g^{-1})**	**Chlorophyll 'b' content plant^{-1} (mg g^{-1})**	**Total chlorophyll content plant^{-1} (mg g^{-1})**	**Percentage of essential oil content (%)**
T_1 – Control	1.18	0.45	1.62	1.55
T_2 – GA_3 @ 50 ppm	1.25	0.46	1.71	2.33
T_3 – GA_3 @ 100 ppm	1.64	0.60	2.25	2.79
T_4 – GA_3 @ 150 ppm	1.87	0.71	2.57	2.85
T_5 – GA_3 @ 200 ppm	1.61	0.56	2.17	2.77
T_6 – GA_3 @ 250 ppm	1.46	0.53	1.99	2.39
T_7 – GA_3 @ 300 ppm	1.35	0.49	1.83	2.15
C.D.	0.12	0.08	0.16	0.08
SE(m)	0.04	0.03	0.05	0.03

Percentage of oil content (%)

As per the record (Table 2), the oil yield in percentage was produced highest with GA_3 @ 150 ppm which was at par with GA_3 @ 100 and 200 ppm. Application of 100 mg L^{-1} GA_3 resulted in higher essential oil content in Sage (Sharafzadeh & Zare, 2011). Farooqi *et al.* (1996) have studied the effect of GA_3 and kinetin in *Artemisia annua* (Washington strain) and found that essential oil content of the herbs can increase up to 42%. The results were in support with Zlatev *et al.* (1978) who in their studies on *Mentha citrata*, a plant from the Lamiaceae family, exposed that the application of gibberellic acid (GA_3) @ 50ppm had also resulted increased essential oil content. Bhagya *et al* (2015) informed regarding the mechanisms of growth regulators in general and their metabolic actions are initiated through increased activity of enzymes that synthesize essential oils. Although very few researches were done on accumulation and biosynthesis of patchoulol and other sesquiterpenes in patchouli leaves, Henderson *et al* (1970) declared that the sesquiterpenes were accumulated in peltate glandular trichomes as well as in internal glands (oil bearing glands) in leaves of plants within Lamiaceae family.

Conclusion

The results from the present experiment showed that the maximum dry matter content in patchouli leaves was obtained in plants treated with GA_3 @ 200 ppm which was at par with GA_3 @ 100 and 150 ppm. Maximum amount of essential oil in leaves was also found in the treatment GA_3 @ 150 ppm which was at par with 100 and 200 ppm. Hence, it can be concluded that the foliar spray with GA_3 @ 150 ppm is beneficial for obtaining greater essential oil content in patchouli leaves but, in respect of dry matter accumulation combined with essential oil content, treatments with 100 and 200 ppm are also found statistically equally effective.

Figure 1. Extraction of essential oil of patchouli; (a) Fresh sample of patchouli leaves. (b) Shade dried patchouli leaves. (c) Extraction of essential oil of patchouli using Soxhlet apparatus. (d) Essential oil of patchouli.

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