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Research Article

EFFECT OF PLANT GROWTH REGULATORS ON GROWTH AND ESSENTIAL OIL CONTENT IN PALMAROSA *(CYMBOPOGON MARTINII)*

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ABSTRACT

Objective: The aim was to examine the responses *viz*., herbage yield, essential oil content and oil composition to varying levels of gibberellic acid (GA_3), kinetin and indole acetic acid (IAA) in Palmarosa (*Cymbopogon martinii*).

Methods: Essential oils were obtained by hydro-distillation using a Clevenger-type apparatus and oils composition was analyzed by gas chromatography-mass spectrometry. Nitrate reductase (NR) activity was evaluated by *in-vivo* assay. Protein content was evaluated by Lowry method. Chlorophyll was estimated by Arnon method.

Results: Plant growth was improved by GA_3 significantly, and the increase was maximum at 100 ppm concentration in plant height, leaf area, tiller number and herbage yield. Chlorophyll content, protein content, NR activity and oil content increased in the plants due to GA_3 treatment compared to untreated plants, and the increase was maximum at 100 ppm concentration. Effect of IAA and kinetin was similar to GA_3 but maximum effect was observed at 50 ppm concentration. Geraniol content of the essential oil of Palmarosa increased due to GA_3 and kinetin treatment while percentage of geranyl acetate decreased.

Conclusion: On a comparative basis, among the three promotional hormones, GA_3 was most effective in stimulating growth, essential oil content, leaf area, tiller number and metabolic parameters (protein content, chlorophyll content and NR activity).

Keywords: *Cymbopogon martinii*, Essential oils, Plant growth regulators, Oil compositon.

INTRODUCTION

Palmarosa (*Cymbopogon martinii* [Roxb.] Wats. Var. motia) is a perennial grass, widely distributed in tropical and subtropical regions. It contains essential oil, whose main components are geraniol and geranyl acetate [1]. The essential oil from *C. martinii* is widely used as a valuable component for perfumes, cosmetic and pharmaceutical products [2,3] and against the action of various bacteria, fungi and microorganisms [4,5]. The essential oil also has some of the important properties such as insect repellent and is also used in aromatherapy [6-9]. Due to these applications, essential oil demand in the domestic and international market has increased, which has stimulated its cultivation.

Many factors including age, seasonal variation, nutrition, temperature and phytohormones have an influence on accumulation and metabolism of secondary metabolites [10]. There are numerous reports in the literature concerning the effects of growth regulators on growth and development of various aromatic plants [10]. Most of these growth substances have exhibited influences by modifying the growth characters. Gibberellins are endogenous plant hormones, which improve the growth of plants. In aromatic plants, where active principle is generally present in the leaves or flowers, gibberellins have been used to improve growth and herb yield of aerial parts of the plants. Gibberellic acid (GA_3) also significantly enhanced the foliage yield of *Cymbopogon jwarancusa* [11]. Auxins and cytokinins constitute other major groups of plant hormones. Auxins are well known for their role in cell elongation and differentiation in the plants. Similarly, role of cytokinins in cell division and differentiation in the plants is well established. Both these hormones have been used for improvement of growth and development of aromatic plants. Indole acetic acid (IAA) also stimulated leaf growth in *C. jwarancusa* [11]. Effect of kinetin on herbage and oil yield has been reported earlier in *Mentha* specie [12,13]. The present study was under-taken to examine the effect of plant growth regulators on the essential oil biosynthesis in Palmarosa and plant growth.

Biosynthesis of terpenoids is dependent on primary metabolism, e.g. photosynthesis and oxidative pathways for carbon and energy supply [10]. Therefore in this study, effect of plant growth regulators on primary metabolic parameters like nitrate reductase (NR) activity, chlorophyll content and protein content was also determined.

METHODS

Chemicals and reagents

Sodium nitrite, potassium dihydrogen phosphate, potassium nitrate, sulfanilamide, N-(1-naphthyl) ethylene dihydrochloride (NED-HCL), anhydrous sodium sulfate, trichloroacetic acid, anthrone, Tween 80, sodium potassium tartrate, cupric sulfate, acetone, sodium sulfate, bovine serum albumin etc., were purchased from Hi-media.

Plant material

Seedlings of Palmarosa (*C. martinii* [Roxb. Wats. var. motia Burk] cv. Trishna) were purchased from Central Institute of Medicinal and Aromatic Plants, Lucknow, India and cultivated in pots at herbal garden of Integral University, Kursi road, Lucknow, India.

Plant growth regulators treatment

The study was conducted during April-July 2013 in a randomized block design with three replications. One seedling was transplanted in each pot. There were three pots for each treatment in one replication. The effect of plant growth regulators on herbage yield was evaluated by spraying plant foliage with solutions of GA_3, kinetin and IAA each at 25, 50 and 100 ppm. Three foliar sprays of these hormones were given at 15 days intervals starting from 45 days after transplanting. Tween 80 (0.01%) was used as wetting agent. In addition, untreated

plants were sprayed with distilled water to serve as control. The plants were harvested at maximum flowering stage.

Growth analysis

The effect of plant growth regulators on plant height, herb yield (fresh weight of aerial part of the plant), tiller number, leaf area, oil content and oil constituents *viz*., geraniol and geranyl acetate was studied. Fresh leaves were sampled for biochemical analysis.

Determination of chlorophyll content, protein content and NR activity

The chlorophyll pigment was extracted and estimated according to the procedure of Arnon [14]. The total leaf protein was extracted and estimated according to Lowry *et al*. [15].

NR (EC 1.6.6.1) activity (NR activity) was measured by *in-vivo* method according to Hangeman and Huckelsby [16]. The NR activity was expressed as the amount of nitrite formed per gram fresh weight per hour. (μmoles NO^{-2} formed g^{-1} leaf (fr.wt.) h^{-1}).

Determination of essential oil content

The extraction of essential oil was carried out according to Guenther [17] by water distillation procedure with Clevenger's apparatus and is expressed on a fresh weight basis. For this, aerial parts were chopped into pieces, and 100 g samples was taken in duplicate from each sample.

Estimation of oil composition

The gas chromatography-mass spectrometry (GC-MS) analysis of palmarosa oil of performed using a GC-MS (model; QP 2010 Plus, Shimadzu, Tokyo, Japan) equipped with a VF-5 ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 μm film thickness. The column oven temperature was programmed from 50°C (3.0 minutes hold) to 200°C for 4°C per minutes (3.0 minutes hold) to 310°C (3 minutes hold) ionization of the sample components was performed in electron impact mode (EI, 70 eV). The temperature of the injector was fixed to 230°C and one of the detector to 280°C. Helium (99.9995% purity) was the carrier gas fixed with a flow rate of 1.21 ml/minute. The injector volume of each sample was 0.2 μl. MS condition was: Start time: 3 minutes, end time: 61.49 minutes, ACQ mode: Scan, start m/z: 40.00, end m/z: 650. The percentage composition of the major component of the oil is reported as total peak area. The constituents of oils were identified by comparing their retention indices and patterns of mass spectra with reference to Wiley Registry of Mass Spectral Data's, New York (WILEY 8) and NIST11 LIB.

Statistical analysis

The data were statistically analyzed using one-way analysis of variance by Graph Pad Instat plus 3.3 software (Graph Pad Software Inc. CA, USA). Mean values were statistically compared by Tukey multiple comparison test to calculate the significance at ($p<0.05$). Data were presented as mean±standard deviation (n=3).

RESULTS

Effect of GA_3 on growth and oil content

Plant height and leaf area were significantly increased by treatment with GA_3 at all concentrations and the increase was 20.6-54% over the control (Table 1). The GA_3 treatment at 100 ppm increased tiller number, and the increase was 61.5% over the untreated plants. Herbage yield was increased significantly at 50 and 100 ppm concentration, and the increase was 28.6% and 48.6%, respectively over the control.

GA_3 application at 50 and 100 ppm significantly increased chlorophyll content and it increased by 14.5% and 62% respectively, over untreated plants (Table 2). NR activity increased significantly by the application of GA_3 (50 and 100 ppm) and the increase was 33-82.3%. Protein content increased significantly by GA_3 application at 50 and 100 ppm and the increase was in the range of 14.7-41.8% over control.

Oil content increased significantly due to application of GA_3 at 50 and 100 ppm but oil content was not affected due to application of GA_3 at 25 ppm (Table 2). Oil content increased by 51% and by 111% due to application of GA_3 at 50 and 100 ppm, respectively. The amount of geraniol increased in the oil by 7-51.8% following application of GA_3 at 50 and 100 ppm while geranyl acetate decreased.

Effect of IAA on growth and oil content

Plant height, leaf area, tiller number and herbage yield increased significantly due to IAA treatment at 50 ppm concentration and increase in plant height was 17.6%, in leaf area 42.3% and in tiller number 22% over the untreated plants (Table 3). Application of IAA increased herbage yield by 25% over the untreated plants.

When IAA (50 and 100 ppm) was applied, chlorophyll content increased significantly, and the increase was 25% and 13% over untreated plants. NR activity increased significantly by the application of IAA at 50 and 100 ppm and the increase was 110% and 59% respectively, over untreated plants. Protein content increased significantly by the application of IAA at 50 ppm concentration, and the increase was 34% over control. The effect of 100 ppm IAA on protein content was not significant.

Oil content increased significantly by IAA at 50 ppm and the increase was 60% over untreated plants (Table 4). Oil content was not affected significantly due to application of IAA at 25 and 100 ppm concentration. Geraniol content increased by IAA application at 50 ppm and the increase was 39.3% over control. Geranyl acetate content decreased at 50 and 100 ppm IAA and the decrease was 61% and 27.8%, respectively over untreated plants.

Effect of kinetin on growth and oil content

Plant height, increased by kinetin application at 50 and 100 ppm concentration and the increase was 18.5% and 5%, respectively (Table 5). Leaf area and tiller number increased by kinetin at 50 ppm and the increase was 46.3% and 57% respectively, over the untreated plants. Herbage yield increased by kinetin at 50 and 100 ppm and the increase was 42.8% and 4.8%, respectively over the control.

When kinetin (50 ppm) was applied, chlorophyll content increased significantly, and the increase was 26.1% over untreated plants. NR activity increased significantly by the application of kinetin at 50 and 100 ppm and the increase was 79.6% and 38.9% respectively, over the untreated plants. Protein content increased significantly by the application of kinetin at 50 ppm concentration, and the increase was 56.2% over the control. Effect of 100 ppm kinetin on protein content was not significant.

The oil content increased significantly by kinetin at 50 ppm and the increase was 72.7% over the untreated plants (Table 6). Oil content was not affected significantly due to application of kinetin at 25 and 100 ppm concentration. Geraniol content increased by 11.1% and 20.6% due to 100 ppm and 50 ppm kinetin, respectively. Geranyl acetate decreased, and the decrease was in the range of 11.1-33.3% over untreated plants.

DISCUSSION

Among all three growth regulators tested on *C. martinii*, GA_3 was most effective in improving growth and essential oil. Increase in growth, oil content due to the application of growth regulators is also reported earlier. Increase in terpenoid biosynthesis through mevalonate isoprenoid pathway has been reported in *Mentha* species [12,18]. In *Rosa damascena,* kinetin application increased oil content and citronellol+geranyl acetate level in the oil [19]. In the Washington strain of *Artemisia annua*, both GA_3 and kinetin enhanced oil content by 42%. The quality parameter (Artemisia ketone) of the oil has also been increased [20]. IAA (100 ppm) decreased the carvone content in dill essential oil, but increased percentage of dihydrocarvone. The maximum contents of limonene, pinene and dipentene in the oil were obtained from plants treated with IAA [10]. Similarly, GA_3 also increased oil yield and quality in *Ocimum sanctum* [21]. Increase in chlorophyll content, NR activity and protein content by the application of plant hormones is similar to report on *Mentha arvensis* [14]. Increase in NR activity may be due to increasing in availability of NADH or due to synthesis/activation

Table 1: Plant height, area of the leaves, tiller no and herb yield of *C. martinii* as affected by foliar spray of GA_3

Treatment	Plant height (cm)	Leaf area (cm^2)	Tiller no/plant	Herbage yield (g/plant)
Untreated	124.55±6.47	31.91±1.89	26.00±2.88	350±10.00
GA_3 (25 ppm)	150.21±0.76***	40.56±1.03**	27.00±3.60	386.67±15.27
GA_3 (50 ppm)	165.66±2.60***	49.98±3.55***	32.00±0.57	450±20***
GA_3 (100 ppm)	191.67±0.52***	58.09±2.06***	42.00±2.00***	520±26.45***

*p<0.05, **p<0.01, ***p<0.001, GA_3: Gibberellic acid, *C. martinii: Cymbopogon martinii*

Table 2: Effect of GA_3 on chlorophyll content, protein content, NR, geraniol and geranyl acetate percentage and oil biosynthesis of *C. martinii*

Treatment	Chlorophyll content (mg/g fr wt.)	Protein content (mg/g fr wt.)	NR activity (µmoles NO_2^- formed/g fr wt.) hr^{-1}	Geraniol (%)	Geranyl acetate (%)	Oil content (ml/100 g.fr wt.)
Untreated	3.38±0.05	3.40±0.04	7.02±0.45	56	34	0.90±0.10
GA_3 (25 ppm)	3.48±0.03	3.66±0.05	6.96±0.61	47	14	0.73±0.03
GA_3 (50 ppm)	3.87±0.05***	3.90±0.40*	9.34±0.53**	60	27	1.36±0.05***
GA_3 (100 ppm)	5.48±0.16***	4.82±0.06***	12.80±0.36***	85	11	1.90±0.10***

*p<0.05, **p<0.01, ***p<0.001, GA_3: Gibberellic acid, NR: Nitrate reductase, *C. martinii: Cymbopogon martinii*

Table 3: Analysis of growth and development (plant height, area of the leaves, tiller no and herb yield) of *C. martinii* in pot by foliar spraying method of IAA

Treatment	Plant height (cm)	Leaf area (cm^2)	Tiller no/plant	Herbage yield (g/plant)
Untreated	107.33±7.33	32.77±1.59	34.66±0.57	337.33±6.42
IAA (25 ppm)	100.33±1.20	29.47±1.5	31.00±1.00	310±10
IAA (50 ppm)	126.44±1.38***	46.61±1.3***	42.33±4.61*	421.67±18.93***
IAA (100 ppm)	110.66±2.09	34.50±0.85	33.00±3.00	340.67±5.13

*p<0.05, **p<0.01, ***p<0.001, IAA: Indole acetic acid, *C. martinii: Cymbopogon martinii*

Table 4: Effect of IAA on chlorophyll content, protein content, NR, geraniol, and geranyl percentage and oil biosynthesis of *C. martinii*

Treatment	Chlorophyll content (mg/g fr wt.)	Protein content (mg/g fr wt.)	NR activity (µmoles NO_2^- formed/g fr.wt.) hr^{-1}	Geraniol content (%)	Geranyl acetate content (%)	Oil content (ml/100 g fr. wt.)
Untreated	3.63±0.21	2.91±0.07	5.18±0.25	56	36	1.06±0.10
IAA (25 ppm)	3.32±0.11	2.46±0.43	4.99±0.11	58	34	0.76±0.02*
IAA (50 ppm)	4.54±0.12***	3.90±0.04**	10.88±0.49***	78	14	1.60±0.11**
IAA (100 ppm)	3.16±0.13*	2.20±0.11*	8.23±0.27***	47	26	1.27±0.04

*p<0.05, **p<0.01, ***p<0.001, IAA: Indole acetic acid, NR: Nitrate reductase, *C. martinii: Cymbopogon martinii*

Table 5: Effect of kinetin on plant height, area of the leaves, tiller no and herb yield of *C. martinii* after

Treatment	Plant height (cm)	Leaf area (cm^2)	Tiller no/plant	Herbage yield (g/plant)
Untreated	113.00±2.33	28.26±1.00	26.33±2.88	312.33±2.51
Kinetin (25 ppm)	105.99±2.08	23.56±1.02	23.33±1.15	250.33±6.4***
Kinetin (50 ppm)	133.88±2.91***	41.34±3.94***	41.33±1.15***	446.00±4.00***
Kinetin (100 ppm)	118.78±0.38	33.44±2.25	28.66±1.15	327.33±2.51**

*p<0.05, **p<0.01, ***p<0.001, *C. martinii: Cymbopogon martinii*

Table 6: Effect of kinetin on chlorophyll content, protein content, NR, oil content and its major constituents in intact plant of *C. martinii*

Treatment	Chlorophyll content (mg/g fr.wt.)	Protein content (mg/g fr.wt.)	NR activity (µmoles NO_2^- formed/g fr. wt.) hr^{-1}	Geraniol content (%)	Geranyl acetate (%)	Oil content (ml/100 g fr.wt.)
Untreated	3.29±0.05	3.13±0.11	5.94±0.11	63	18	0.77±0.04
Kinetin (25 ppm)	2.82±0.18	2.78±0.03*	5.07±0.81	60	15	0.76±0.05
Kinetin (50 ppm)	4.15±0.17***	4.89±010***	10.67±0.76***	76	12	1.33±0.05***
Kinetin (100 ppm)	3.52±0.08	3.20±0.05	8.22±0.12**	70	16	1.06±0.11**

*p<0.05, **p<0.01, ***p<0.001, NR: Nitrate reductase, *C. martinii: Cymbopogon martinii*

of the enzyme due to hormones treatment. Ananda *et al*. [22] has also reported hormonal regulation of NR gene expression in *Hordeum vulgare*.

It seems that the application of GA_3, IAA and kinetin on growth characters, NR activity and protein content was favorable for an increase in oil accumulation.

CONCLUSION

On a comparative basis, among the three promotional hormones, GA_3 was found to be the most effective hormone in stimulating growth, essential oil content, leaf area and tiller number as well as biochemical parameters such as protein content, chlorophyll content and NR activity.

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