Research Paper

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Response of Ocimum tenuiflorum variety CIM-AYU to sulphur fertilization in the semi arid tropical region of Deccan plateau in India

Chemistry

KEYWORDS : CIM-AYU, Sulphur nutrition , Essential oil, Eugenol , Carophyllene, β-elemene

Pandu Sastry	CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre, Boduppal, Hyderabad-500092, Andhra Pradesh, India
Kakaraparthi	CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre, Boduppal, Hyderabad-500092, Andhra Pradesh, India
Dharmendra K. Rajput	CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre, Boduppal, Hyderabad-500092, Andhra Pradesh, India
Niranjan K. Arigarui	CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre, Boduppal, Hyderabad-500092, Andhra Pradesh, India

ABSTRACT

The essential oil obtained from the herb of Ocimum tenuiflorum is one of the industrially important essential oils, these essential oils are composed of aroma compounds such as eugenol, methyl eugenol, citral, linalool, geraniol and thymol. These are used as raw materials for the pharmaceutical, comestics and food industries. These essential oils exhibit biological activities such as antimicrobial, insecticidal, antioxidant and analgesic activities.

Sulphur (S) is required for realisation of optimum yields from aromatic crops cultivated in semi-arid tropical areas in India. A field experiment involving varying levels of sulphur (0-150 kg/ha) in combination with standard levels of other nutrients was conducted on a red sandy loam soil at the research farm of Central Institute of Medicinal and Aromatic Plants (CIMAP), Hyderabad, India to identify the optimum level of sulphur required for higher herb and essential oil yield of Ocimum tenuiflorum variety CIM-AYU.

Sulphur application resulted in increased herb and essential oil yield of Ocimum tenuiflorum variety CIM-AYU due to better growth of plants (plant height, number of leaves /plant and dry weight of plant) and the optimum dose of sulphur required is 50 kg/ha. The chemical composition of the essential oil analysed at different stages indicated that eugenol is the major constituent of the essential oil followed by carophyllene and β-elemene.

1. Introduction

Ocimum belonging to the family lamiaceae is very well distributed in tropical and subtropical Africa, Asia and South America. They occur in various parts of the world and grow from sea-level to an altitude of about 1800 m (Gupta, 1994). The herb of *Ocimum* on steam distillation, yield a bright yellow, volatile oil possessing a pleasant odour. The essential oils are composed of aroma compounds such as eugenol, methyl eugenol, citral, linalool, geraniol and thymol which are required as raw materials for the pharmaceutical, comestics and food industries(Gupta, 1994, Bizzo *et al*., 2009).

Many of these essential oils exhibit biological activities such as antimicrobial (Prasad *et al*., 1986, Nakamura *et al*.,1999), insecticide (De Paula *et al*.,2003, Paula *et al*., 2004), antioxidant (Ganiyu, 2008) and analgesic activities (Franca *et al*.,2008).

Ocimum extracts are used in indigenous Indian systems of medicine as remedies for common colds, headaches, stomach disorders, inflammation, heart disease, various forms of poisoning, malaria and an effective treatment for diabetes by reducing blood glucose levels.

There is a significant growth in the world trade for essential oils and in India essential oils are produced from many species and those of Ocimum are gaining importances due to their multi-utility.

Sulphur plays a pivotal role in various plant growth and development processes being a constituent of sulphur containing amino acids. Sulphur (S) is involved in amino acid and protein synthesis, enzymatic and metabolic activities in plants, which account for approximately 90% of organic S in the plant. Sulphur is required early in the growth of rice plants and non availability during early growth will reduce tiller number and therefore final yield. It is increasingly being recognized as the fourth major plant nutrient after nitrogen, phosphorus and potassium.

Sulfur is deficient in rainfed semi-arid tropical (SAT) Alfisols because of low organic-matter content in soil, coarse texture of the soils, more removal of S than its application, and use of fertilizers without any S content. The native plant-available S [0.15% calcium chloride (CaCl2)– extractable S] in rainfed Alfisols in the SAT regions rarely exceeds 10–20 kg /ha and the soils are mostly categorized as low to medium in S (Takkar,1988).

The present study was undertaken to evaluate the influence of elemental sulphur on the herb and essential oil yield of *Ocimum tenuiflorum* variety CIM-AYU.

2. Material and Methods

2.1. Experimental site and design of the experiment

The present study was undertaken to evaluate the influence of elemental sulphur on the herb and essential oil yield of *Ocimum tenuiflorum* variety CIM-AYU during 2012. The experiment was laid out at the research farm of Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Boduppal, Hyderabad, Andhra Pradesh, India.

The experimental site is located at an altitude of 542 m above mean sea level with a geographical bearing of 78°8' longitude and 17°32' latitude. The climate of the region is semi-arid tropical with hot summers and mild winters. The mean annual rainfall of this region is generally 750 mm and accounts for approximately 42% of annual potential evapo-transpiration (1754 mm).

The soil of the experimental field is a red sandy loam (alficusto chrept) with pH 8.27 (1.25 soils to solution ratio), EC– 1.21 ds / m, organic C -0.583%, available nitrogen (215.40 Kg/ha), available P- 10.30 kg/ha and exchangeable K-103.08 kg/ha.

Before planting, the experimental field was plowed twice using a tractor-drawn disc and was harrowed twice. The field was leveled with a tractor drawn leveler after complete removal of stubbles of previous crop for providing fine tilth for the crop.

2.2. Nursery

Nursery beds of size 1x1x1 m were prepared and mixed with well prepared manure. Seeds of the *Ocimum tenuiflorum* variety CIM-AYU were broadcasted in the beds and covered with dry grass. The beds were irrigated twice a day till the seeds germinated. After the seeds germinated the grass was removed and the beds irrigated once a day for twenty days. Fully grown seedlings of size 4-6" were planted in field as per the lay out plan. The crop was planted during First week of January, 2012. Uniform doses of P and K was applied during ploughing. Nitrogen was applied in four splits. Sulphur as per treatments was band placed 15 days after planting. The crop was kept weed free and regularly irrigated

The experimental treatments consisted of seven levels of Sulphur (0, 25, 50, 75 and 100 kg S /ha) through elemental sulphur (85% S). These treatments were laid out in a randomized block design (RBD) with four replications. The crop was managed as per standard practices under irrigated conditions.

First harvest of the crop was taken in the first week of June and thereafter herb was harvested for the second time in the first week of September. During the experimental period three harvests were taken but data pertaining to the first and second harvest are presented in this paper.

2.3. Observation on morphometric traits

After transplanting, the crop was harvested at 150 days after transplanting (first harvest) and at 240 days after planting (second harvest) for recording data on herb and oil yield /ha. During the first harvest period (0-150 days after transplanting) and second harvest period (150-240 days) five randomly selected plants were harvested from each treatment plot in all the replications at monthly intervals. During the first harvest period from January to June observations were recorded five times at 30, 60, 90,120 and 150 days after transplanting (DAP) for the morphometric traits viz., plant height (cm), number of leaves /plant, fresh weight of leaf / plant (g) , leaf area (cm^2) and dry weight of the plant (g). During the second harvest period (June-September) observations were taken for the same morphometric traits for three times at monthly intervals.

Growth indices were computed from the data recorded on leaf area and dry weight /plant. Oil yield /plant was calculated from the data recoded on oil content and fresh weight /plant.

2.4. Essential oil extraction

The aerial parts of citronella were collected from five random plants in each plot at monthly intervals. For the extraction of essential oils, freshly collected herbage was subjected to hydrodistillation using a Clevenger-type apparatus for 3.5 h. The essential oils obtained were collected and dried over anhydrous sodium sulphate and stored at 4 °C until the GC analysis was carried out. Essential oil content and quality were analyzed at monthly interval. During two harvest periods observations on essential oil content were recorded eight times at monthly intervals in all the treatments replicated four times. The samples were also subjected to GC analysis.

2.5. GC analysis

GC analysis was carried out using Varian CP-3800 with Galaxie chromatography data system fitted with flame ionization detector (FID) and an electronic integrator. Separation of the compounds was achieved employing a Varian CP-Sil 5CB capillary column (ID: 50 m X 0.25 mm; film thickness 0.25 µm) with 5% dimethyl polysiloxane. Nitrogen was the carrier gas at 0.5 ml/min constant flow rate. The column temperature program was: 120°C (2 min) to 240°C (6 min) at 8°C/min ramp rate. The injector and detector temperature were 250°C and 300°C respectively. Samples (0.2 µL) were injected with a 20:80:20 split ratio. Retention indices were generated with a standard solution of *n*-alkanes (C6-C19). Peak areas and retention times were measured by an electronic integrator. The relative amounts of individual compounds were computed from GC peak areas without FID response factor correction.

Oil samples were collected from all the treatments replication wise but data pertaining to the chemical composition of oil pooled over replications was presented in this paper since no significant differences were observed due to treatments and the data is inconsequential with respect to treatments.

2.6. Statistical analysis

Analysis of variance was performed to determine the effect of varieties, sowing dates, and interaction [varieties x sowing dates] on root yield and quality parameters using statistical software IRRISTAT [IRRI, Manila, Philippines]. Means were compared using least significant differences [LSDs] at 5% probability levels.

3. Results and discussion

3.1. Effect of different levels of sulphur on plant height, number of leaves /plant and fresh weight of leaf/plant

Comparison of means of morphological characters like plant height, number of leaves /plant and fresh weight of leaf/plant in Ocimum tenuiflorum variety CIM-AYU (CIM-AYU) as influenced by different doses of sulphur application is presented in Table 1. The results indicated that application of sulphur significantly influenced all the evaluated traits.

Observations on morphological parameters were recorded at thirty days interval starting from 30 days after planting (DAP). In total during the first harvest period observations were recorded at five stages (30, 60, 90,120 and 150 DAP). The crop was harvested to 30 cm above ground level at 150 DAP and then it was allowed to grow. Observations were again taken at 30 days interval after the first harvest i.e., chronologically on 180, 210 and 240 DAP.

Application of sulphur up to 50 kg per hectare produced significantly taller plants with higher number of leaves and higher leaf weight /plant.

In case of plant height, a significant decrease in the plant height was noticed due to application of sulphur beyond 50 kg/ha. The decrease was significant at all stages except 30 and 60 DAP. In case of number of leaves /plant a significant improvement was noticed up to 50 kg sulphur/ha, thereafter there is a decrease in the number of leaves per plant. The decrease was significant at 150,180 and 240 DAP only. The differences noticed were not significant at 210 DAP.

Fresh weight of leaves/plant also showed significant improvement up to 50 kg sulphur level only, thereafter the weight of leaves decreased significantly at all stages.

The highest values for plant height (46.13, 49.92, 50.75, 55.00 and 73.00 cm) number of leaves /plant (301.7, 376.9, 496.2, 956.7 and 1617.3) and fresh weight of leaf /plant (107.13, 136.75, 189.75, 414.00 and 915.75) were noticed due to 50 kg sulphur application/ha at 30, 60, 90,120 and 150 DAP. Similar trend was noticed at 180,210 and 240 DAP during the second harvest period.

The increase in plant height (27.5 and 67.0 %) , number of leaves / plant (24.3 and 74.8%) and fresh weight of leaves (20.5 and 22.3%) due 50 kg sulphur application at 150 and 240 DAP) were significant compared to control.

The increase in plant height, number of leaves/plant and weight of leaf /plant might be due to the function of sulphur which plays a pivotal role in various plant growth and development processes since sulphur is a constituent of amino acids. With increased supply of sulphur, the process of tissue differentiation and development might have increased, resulting in increase in number and size of leaves and branches. The above results are in conformity with the results reported in soybean (Joshi and Billore, 1998) and groundnut (Chaubey *et al*., 2000). Application of sulphur increased the height of plants at all the growth stages in rice (Rahman et al. 2008) and produced highest LAI at anthesis in rice (Sahaa et al., 2007).

3.2. Effect of different levels of sulphur on dry weight of herb/plant and leaf area (cm^2/leaf)

The data on the yield contributing characters like dry weight of herb /plant and average leaf area /leaf as influenced by different doses of sulphur application are presented in Table 2. The results indicated that application of sulphur significantly influenced both the evaluated traits.

Leaf area and dry weight of herb increased significantly up to 50 kg /ha dose and thereafter application of sulphur resulted in decrease in these parameters. The decrease in dry weight was significant at 90 DAP only during first harvest period whereas the decrease in dry weight was significant at 180 and 210 DAP during second harvest period.

3.3. Effect of different levels of sulphur on the growth indices CGR and NAR

From the observations made on leaf area/m^2 and dry weight of plant/m^2 growth indices NAR and CGR were computed (Table 3). The data did not follow any particular pattern and indicative of any trends. On an average over the observations with increase in the dosage of sulphur applied to the soil the NAR decreased initially but with further increase in dose NAR showed higher values during first harvest period. During second harvest period NAR decreased continuously with increase in the dose of sulphur applied with higher values noticed in control treatment.

CGR showed an increase - decrease pattern with increase in the dose of sulphur applied during both the harvests on an average.

3.4. Effect of different levels of sulphur on the oil content and oil yield /plant

Oil content varied from 0.43 to 0.53 % due to the treatments in the first 150 DAT. It varied from 0.32 to 0.47 % during the period from 150 to 240 DAT. During the first harvest period significant differences in oil content were noticed at 90 and 120 DAT only. At both the stages significantly lowest oil content was noticed due to application of 75 kg sulphur / ha (120 DAT) and 100 kg Sulphur /ha (90DAT).

On an average over treatments, the oil content varied from 0.45 to 0.46 % during the first harvest period and from 0.34 to 0.45 during the second harvest period. The oil content was the lowest at 180 DAT or 30 days after first harvest only. The oil content remained almost constant during the rest of the experimental period ranging between 0.43 to 0.47 %.

Similarly, it was reported that inorganic fertilizer levels did not influence the oil content in other aromatic crops like patchouli and rosemary (Singh et al., 2007; Singh and Guleria, 2013)

Oil yield /plant was significantly high due application of 50 kg sulphur /ha and additional application of sulphur beyond 50 kg dose resulted in decrease in oil yield /plant. This is a reflection of dry weight of plant which was significantly high at 50 kg sulphur dose. The oil yield /plant varied from 0.098 to 0.128 g/plant at 30 DAT and from 0.39 to 0.49g /plant at 150 DAT. It ranged from 0.16 to 0.19 at 180 DAT to 0.26 to 0.40 g/plant at 240 DAT. The oil yield /plant was high during the January to June period because of increase in day and night temperatures and availability of moisture in the soil due to assured irrigation. It started declining in the second harvest period with the onset of monsoon. and intermittent rains with reducing day and night temperatures.

In contrast , in Pelargonium sps. it was observed that during summer harvests (April to June) the crop yields lower biomass and essential oil. Rainy/monsoon (August and September) and autumn (October and November) season months were characterized by high rainfall, cloudy days and short photoperiods. These favourable environmental conditions encouraged crop growth and produced highest biomass yields, essential oil yields and maximum concentration of essential oil in rose-scented geranium plants (Rajeswara Rao et al. 1996).

3.5. Effect of different levels of sulphur on the chemical composition of the essential oil

Essential oil samples obtained in all the treatments in the first harvest were subjected to GC analysis and the data was statistically analysed. The differences noticed in the chemical constituents in the essential oil were not significant due to sulphur treatments. Hence the data averaged over different treatments at different stages is presented in Table 4.

GC analyses of the oil samples indicated that eugenol is the major constituent in the essential oil (64.6 to 76.75 % during the first 150 DAT and 63.85 to 69.49 % during 210-240 DAT), followed by carophyllene (10.80 to 16.70% and 10.71 to 16.50 %) and β- elemene (9.03 to 13.03% and 8.45 to 9.43%). Eugenol showed a decrease- increase pattern during the first harvest period and a steady decrease pattern during the second harvest period.

Carophyllene increased up to 120 DAT and later decreased during the first 150 DAT during the later harvest it showed a steady increase. In terms of number of days after transplanting or number of days after harvesting carophyllene increased only up to 90 days and later decreased . Similar pattern was noticed in case of β- elemene also.

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Table 1

Influence of different levels of sulphur on the morphological parameters plant height (cm), number of leaves / plant and weight of leaf (g/plant) in *Ocimum tenuiflorum* variety CIM-AYU

Sulphur, kg/ha	Plant height, cm							
	First harvest period					Second harvest period		
	Days after transplanting							
	30	60	90	120	150	180	210	240
0	35.12	42.71	47.11	48.45	57.25	41.25	58.25	50.75
25	42.51	47.58	49.94	53.31	68.91	47.25	68.50	80.00
50	46.13	49.92	50.75	55.00	73.00	50.50	70.00	83.50
75	43.26	46.88	48.09	51.63	65.75	44.25	68.25	75.75
100	44.38	46.74	47.00	49.16	64.75	40.50	65.50	72.00
LSD at 5%	4.037	3.802	2.260	3.651	2.739	1.286	2.675	3.468

Sulphur, kg/ha	Number of leaves / plant							
	Days after transplanting							
	First harvest period					Second harvest period		
	30	60	90	120	150	180	210	240
0	215.6	325.3	421.2	448.7	1301.2	209.2	366.0	380.8
25	291.8	374.1	487.9	930.8	1539.9	258.1	445.3	632.5
50	301.7	376.9	496.2	956.7	1617.3	306.7	460.3	665.6
75	295.9	373.2	490.8	942.6	1461.1	291.7	420.3	621.1
100	298.1	378.9	487.2	924.9	1304.4	276.0	391.8	570.4
LSD at 5%	16.83	38.026	22.85	134.001	143.48	17.08	NS	18.47
Sulphur, kg/ha	Fresh weight of leaf, g/plant							
	Days after transplanting							
	First harvest period					Second harvest period		
	30	60	90	120	150	180	210	240
0	77.69	96.50	155.25	318.75	759.25	151.26	313.51	690.75
25	95.19	123.50	177.25	408.25	887.50	182.99	627.54	763.25
50	107.13	136.75	189.75	414.00	915.75	194.53	653.75	844.50
75	100.63	112.75	165.50	385.75	845.00	170.03	622.75	719.75
100	95.88	106.50	159.75	346.25	823.25	166.25	599.50	712.00
LSD at 5%)	3.843	3.320	4.12	6.023	14.05	6.006	NS	9.81

NS : Not significant

Table 2
Influence of different levels of sulphur on dry weight of plant (g/plant), leaf area (cm^2) and NAR (g/m^2/day) in *Ocimum tenuiflorum* **variety CIM-AYU**

Sulphur, kg/ha	Dry weight of plant, g/ plant							
	First harvest period					Second harvest period		
	Days after transplanting							
	30	60	90	120	150	180	210	240
0	23.00	37.00	36.25	42.50	94.75	41.75	59.25	67.25
25	27.75	41.25	43.25	47.50	114.25	48.25	63.25	79.50
50	30.50	45.75	48.25	51.50	112.50	50.75	67.25	80.25
75	30.50	42.00	44.75	48.00	109.25	49.50	64.25	77.00
100	28.50	42.50	47.00	50.00	110.25	47.50	63.75	82.25
LSD at 5%	3.63	3.29	2.61	3.512	6.49	1.66	2.30	2.25
Sulphur, kg/ha	Leaf area, cm^2/leaf							
	Days after transplanting							
	First harvest period					Second harvest period		
	30	60	90	120	150	180	210	240
0	22.75	40.00	46.75	56.25	57.50	28.00	45.50	80.75
25	34.75	41.50	53.00	61.75	74.50	36.25	57.25	85.25
50	37.50	42.25	55.75	65.75	79.00	41.25	59.50	87.00
75	32.50	41.25	53.25	60.75	76.50	37.50	57.75	85.50

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100	30.25	39.75	51.25	64.00	75.75	41.00	54.50	85.25
LSD at 5%	2.23	2.16	2.66	3.00	3.53	2.33	2.42	2.52

Sulphur, kg/ha	NAR,							
	Days after transplanting							
	First harvest period					Second harvest period		
	30-60	60-90	90-120	120-150	Average	180-210	210-240	Average
0	0.0237	0.0003	0.0024	0.0227	0.0123	0.0392	0.0050	0.0221
25	0.0133	0.0016	0.0013	0.0153	0.0079	0.0227	0.0072	0.0150
50	0.0132	0.0020	0.0015	0.0146	0.0078	0.0200	0.0048	0.0124
75	0.0149	0.0026	0.0012	0.0177	0.0091	0.0229	0.0043	0.0136
100	0.0117	0.0006	0.0031	0.0236	0.0098	0.0232	0.0046	0.0139
LSD at 5%	0.0023	0.0007	0.0007	0.0035		0.0059	0.0010	

Table 3
Influence of different levels of sulphur on CGR (g/m2/day), oil content (%) and oil yield/plant (g) in *Ocimum tenuiflorum* variety CIM-AYU

Sulphur, kg/ha	CGR							
	First harvest period					Second harvest period		
	Days after transplanting							
	30-60	60-90	90-120	120-150	Average	180-210	210-240	Average
0	0.0161	0.0004	0.0046	0.0542	0.0188	0.0211	0.0085	0.0148
25	0.0145	0.0028	0.0039	0.0748	0.0240	0.0196	0.0207	0.0202
50	0.0146	0.0035	0.0047	0.0673	0.0225	0.0210	0.0170	0.0190
75	0.0129	0.0042	0.0030	0.0671	0.0218	0.0177	0.0125	0.0151
100	0.0096	0.0009	0.0057	0.0545	0.0177	0.0154	0.0150	0.0152
LSD at 5%	0.0022	0.0007	0.0013	0.0108		0.0032	0.0024	

Sulphur, kg/ha	Oil content							
	Days after transplanting							
	First harvest period				Second harvest period			
	30	60	90	120	150	180	210	240
0	0.43	0.46	0.48	0.48	0.44	0.36	0.43	0.43
25	0.44	0.45	0.53	0.50	0.47	0.35	0.46	0.45
50	0.46	0.46	0.48	0.47	0.46	0.34	0.47	0.47
75	0.46	0.48	0.48	0.44	0.48	0.35	0.46	0.46
100	0.45	0.46	0.43	0.48	0.46	0.32	0.44	0.44
LSD at 5%	0.03	0.04	0.04	0.05	0.04	0.03	0.03	0.02

Sulphur, kg/ha	Oil yield							
	Days after transplanting							
	First harvest period				Second harvest period			
	30	60	90	120	150	180	210	240
0	0.0989	0.1588	0.1749	0.2055	0.3997	0.1635	0.2532	0.2680
25	0.1273	0.1726	0.2219	0.2460	0.5414	0.1827	0.2783	0.3799
50	0.1595	0.2295	0.2352	0.2521	0.5477	0.1964	0.3342	0.4010

75	0.1389	0.2028	0.2267	0.1885	0.5387	0.1667	0.2853	0.3795
100	0.1283	0.1958	0.2238	0.2400	0.4936	0.1520	0.2930	0.3629
LSD at 5%	0.0147	0.0244	0.0243	0.0391	0.0354	0.0374	0.0337	0.0363

Table 4
Influence of different levels of sulphur on the chemical composition (%) of the essential oil at different stages in *Ocimum tenuiflorum* **variety CIM-AYU**

Chemical constituents	Composition , Per cent							
	First harvest period					Second harvest period		
	Days after transplanting							
	30	60	90	120	150	180	210	240
Limonene	0.43	0.82	0.23	0.33	0.22	0.41	0.81	0.20
Linalool	0.07	0.50	0.24	0.24	0.17	0.07	0.59	0.17
Methyl Chavicol	0.18	0.40	0.03	0.05	0.04	0.43	0.56	0.03
Geraniol	0.16	0.22	0.09	0.17	0.07	0.42	0.22	0.14
Eugenol	69.62	64.60	65.81	69.10	76.75	69.49	63.85	66.20
Methyl Eugenol	2.51	5.38	0.70	0.05	0.06	3.21	5.53	1.22
β- Elemene	9.33	9.32	9.03	13.03	8.40	8.71	9.43	8.45
Caryophyllene	11.85	12.28	16.70	10.80	7.81	10.71	12.75	16.50

- Data pooled over treatments.

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