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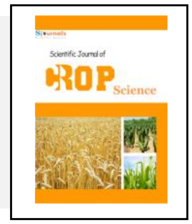

Original article

Response of Java citronella (*Cymbopogon winterianus jowitt*) to sulphur fertilization in the semi arid tropical region in India

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A B S T R A C T

The essential oil obtained from the herb of Citronella is an industrially important essential oil and a known plant-based insect repellent and bio-pesticide. Improving the economic yield of the aromatic grass citronella is part of the rural development mandate of the institute CSIR-Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP) and this study is a part of the activity. 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 (CSIR-CIMAP), Hyderabad, India to identify the optimum level of sulphur required for higher herb and essential oil yield of citronella. Sulphur application resulted in increased herb and essential oil yield of citronella due to better growth of plants (plant height, number of leaves /plant , number of tillers / clump and weight of plant / clump) and the optimum dose of sulphur required is 75 kg/ha.

1. Introduction

Citronella oil obtained from Cymbopogon winterianus Jowitt (Java citronella) is one of the industrially important essential oils used in perfumery, soaps, detergents, industrial polishes, and is a known plant based insect repellent (Guenther,1950).

Essential oils, commonly used as fragrances and flavouring agents, are recommended as mosquito repellents and can be applied to humans like conventional mosquito repellents with little or no harmful effects (Mahidol, 2004). Use of natural substances for mosquito repellence is strongly recommended. Citronella oil is reported to be a mosquito repellent.

In India Cymbopogon winterianus Jowitt (Java citronella) is cultivated mainly in the lower hills and the Southern State of Andhra Pradesh also makes a significant contribution to the total citronella oil production in the country. CSIR-CIMAP introduced this crop to the hill tribal farmers in the Visakhapatnam district of Andhra Pradesh, India to improve the economy of the ethnic tribal people as part of the rural development programmes of the institute.

Sulphur (S) is involved in the synthesis of amino acids, proteins, fatty acids , increases protein quality (Havlin,et al.,1999), increases nitrogenase activity in soils , nitrogen fixation in sulphur deficient soil (Kandpal and Chandel,1993) and plays an important role in the synthesis of vitamins and chlorophyll (Jez,2008). S is accumulated in plants in low concentrations (Scherer et al., 2008; Abdallah et al., 2010) and the deficiency of S is emerging in areas under oilseeds and pulses due to higher removal of S b y crops (Singh and Kumar, 2009). Essentiality of sulphur for soybean (Bhuiyan et al., 1998; Dubey and Billore 1995; Fontanive et al., 1996; Srivastava et al.,2000), oilseed crops (Das and Das, 1994) and peas (Kedar and Rajendra, 2003) are very well documented.

S 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 ($CaCl_2$) – 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; Morris, 1987).

There is a gradual increase in pH from 6.9 to 8.1 in experimental site over a period of thirty years. The EC of ground water also increased and this experiment was initiated to study the influence of sulphur as a soil amendment and also to study its role in balanced nutrition. This experiment is a part of the agrotechnology development for improving the herb and essential oil yield of citronella in rural areas inhabited by the tribal farmers.

2. Materials 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 citronella during 2011-12. 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 leveller after complete removal of stubbles of previous crop for providing fine tilth for the crop.

Mature healthy slips of Citronella variety Bio-13 collected from three year old plants were planted in rows following a row spacing of 60 cm between rows and 60 cm between plants in 4.8m x 6.0 m plots. The crop was planted during First week of January, 2011. A fertilizer dose of 100:40:40 Kg/ha of N: P: K was applied to the crop. Uniform doses of P and K were applied during ploughing. Nitrogen was applied in four splits. Sulphur as per treatments was band placed 15 days after planting.

The experimental treatments consisted of seven levels of Sulphur (0, 25, 50, 75, 100, 125 and 150 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. The plots were kept weed free.

First harvest of the crop was taken in May, 2011 and thereafter herb was harvested once in every 100 days. During the experimental period four harvests were taken.

2.2. Observation on morphometric traits

Five randomly selected plats were harvested from each treatment plot in all the replications and the harvested herb was collected. Data were recorded for six morphometric traits viz., plant height , plant weight , leaf area, number of leaves /plant , number of tillers /plant and dry weight of plant [g] .

2.3. Essential oil extraction

The aerial parts of citronella were collected from five random plants in each plot. For the extraction of essential oils, freshly collected herbage was subjected to hydro-distillation using a Clevenger-type apparatus for 3.5 h. The essential oils obtained collected were dried over anhydrous sodium sulphate and stored at 4 °C until the GC analysis was carried out. The crop was harvested four times and the oil content and oil quality were observed in all the harvests in all the treatments.

2.4. 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.

2.5. 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 /clump, leaf area and number of tillers /clump

Comparison of means of morphological characters like plant height, number of leaves /clump, Leaf area (cm2) and number of tillers /clump in citronella as influenced by different doses of sulphur application are presented in Table 1. The results indicated that application of sulphur significantly influenced all the evaluated traits except plant height at third harvest only.

The herb was harvested at five months after planting for the first time and thereafter three harvests were taken at 100 days interval. In total there were four harvests. The crop responded to application of sulphur in all the harvests and the response was noticed only up to 75 kg/ ha dose and differences noticed beyond 75 kg/ ha dose were not significant.

Application of sulphur up to 75 kg per hectare produced significantly taller plants with higher number of leaves and tillers /clump. The average leaf area was also significantly high.

The highest values for plant height (86.60, 99.60, 113.90 and 89.30cm), number of leaves /clump (116.50, 226.75, 459.25, and 350.00) and number of tillers /clump (74.50, 98.85, 160.75 and 108.50) were noticed due to

75 kg sulphur application/ha. Among the harvests highest values were noticed in third harvest followed by fourth, second and first harvests for the characters.

Application of sulphur from 0 to 75 kg /ha in the form of elemental S resulted in significantly higher plant height at all the harvests over control (65.70, 76.70, 101.70 and 77.80 cm). Observations recorded on number of leaves/clump, number of tillers/clump and average leaf area (Table 1) also indicated the same trend.

The increase in number of leaves / clump due 75 kg sulphur application at first , second, third and fourth harvests was 18.6, 41.9, 80.3, and 89.7 per cent , respectively compared to control. Similarly increase was noticed in case of number of tillers per clump also (37.9, 16.8, 34.2 and 10.9 % at first, second, third and fourth harvests, respectively).

The increase in plant height, leaf area, number of leaves and tillers /clump 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 tillers. Similar finding was also reported by (Mengel , 1987).

The above results are in conformity with the results of Joshi and Billore (1998) who reported a gradual increase in the yield attributes of soybean with increasing levels of sulphur.

Chaubey et al., (2000) also reported similar type of results in groundnut. Application of sulphur increased the height of plants in rice (Rahman et al., 2008; Singh et al., 2011) and produced highest LAI at anthesis in rice (Sahaa et al., 2007).

3.2. Effect of different levels of sulphur on fresh weight of herb/clump, oil content, oil yield /clump and oil yield /ha

The data on the yield contributing characters like fresh weight of clump, oil content , oil yield /clump and oil yield /ha as influenced by different doses of sulphur application are presented in Table 2. The results indicated that application of sulphur significantly influenced all the evaluated traits.

Fresh weight of clump, oil yield /clump and oil yield /ha increased significantly up to 75 kg /ha dose only. The highest values for fresh weight of herb/clump (342.50, 364.00, 433.09 and 384.25 g/clump), oil yield per clump (4.49, 4.15,5.54 and 5.19 g/clump) and oil yield /ha (53.84, 49.80, 66.52 and 62.25 kg/ha) were noticed due to 75 kg sulphur application/ha. Among the Harvests, highest values were noticed in third harvest followed by forth, second and first harvests for the characters studied.

The increase in fresh weight of herb/clump due 75 kg sulphur application compared to control was 65.1, 40.3, 51.5, and 40.5% respectively. Similar improvements were also noticed in case of oil yield per clump (76.1, 33.4, 55.2, 37.7%) and oil yield /ha (75.8, 33.3, 55.1, 37.4%).

The above results are in conformity with the results of Joshi and Billore (1998) who reported a gradual increase in the yield attributes of soybean with increasing levels of sulphur. Chaubey et al., (2000) observed that yield attributes in groundnut were significantly influenced by the application of sulphur. Sulphur treatments brought about significant effect in the herb and oil yields in citronella. Similar yield improvements were noticed in rice (Bharathi and Poongothai, 2008).

3.3. Effect of different levels of sulphur on the chemical constituents in the essential oil

The 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 (Table 3). GC analyses of the oil samples indicated that Citronellal (25.71-30.98%), geraniol (20.37-25.65%) and Citronellol (8.53-13.88%) are the major components and Citronellyl acetate (1.90-2.24%), geranyl acetate (3.13-4.88%) and elemol (1.36-2.25%) are minor coponets of the essential oil. Similarly, it was observed that oil content and quality in aromatic crops was not influenced by nutrients in lemongrass (Singh et al., 1996), Ocimum basilicum (Gulati et al., 1978) and geranium (Ram et al., 2006). In contrast, it was also observed that content of principal constituents of basil oil (methylchavicol and linalool) were higher under integrated nutrient management especially when vermicompost was applied in combination with NPK (Anwar et al., 2005).

The essential oil samples obtained in individual harvests were pooled and the samples were analysed for chemical constituents. Each observation is an average of three replicates. Comparison of the active substances in the essential oil obtained at four different harvests is presented in Table 4.

Table 1
Plant height, no. of leaves/clump, leaf area (cm2**)** and No. of tillers/clump in citronella as influenced by different levels of sulphur.

Sulphur	Plant height, cm				No of leaves/clump				Leaf area, cm2				No. of tillers/clump			
kg/ha	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
0	65.70	76.70	101.70	77.80	98.25	159.75	254.75	184.50	24.75	26.00	66.50	63.00	54.00	83.25	119.75	93.25
25	69.60	78.70	102.70	79.70	97.00	165.25	320.00	199.50	27.00	27.50	73.00	67.75	57.25	86.00	134.50	89.25
50	74.60	81.70	102.70	83.30	105.50	173.00	349.25	214.50	25.75	29.25	80.50	77.00	62.75	90.50	133.00	98.25
75	86.60	99.60	113.00	89.30	116.50	226.75	459.25	350.00	30.00	38.25	107.00	100.25	74.50	98.85	160.75	108.50
100	86.00	88.30	107.00	85.70	120.50	194.75	405.75	312.25	29.25	32.75	90.50	87.25	73.25	93.25	145.00	104.50
125	82.60	82.30	110.30	87.00	118.25	184.50	374.75	285.50	28.75	30.25	86.25	84.75	71.00	87.00	130.00	87.25
150	81.33	81.30	104.30	85.16	118.75	173.75	230.25	238.25	27.00	30.25	78.50	82.75	62.00	91.00	129.00	91.25
'F'-test	*	*	NS	*	*	*	*	*	*	*	*	*	*	*	*	*
CD(P=.05)	9.20	2.28	21.42	2.50	6.40	5.58	9.08	9.26	3.75	5.71	5.67	7.57	5.04	7.52	6.76	7.18
CV%	7.80	1.89	13.62	2.01	3.90	2.06	1.64	2.62	9.19	12.56	4.68	6.23	5.23	5.49	3.35	5.22

*Significant at 5% level of probability NS: Not significant. I, II, III & IV– First, second, third and fourth harvests, respectively.

Table 2
Fresh weight of herb/clump, Oil content (%,w/w), Oil yield (g/clump) and Oil yield (kg/ha) in citronella as influenced by different levels of sulphur.

	Fresh weight of herb/clump				Oil content (%,w/w)				Oil yield (g/clump)				Oil yield, kg/ha				
Sulfur kg/ha	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	Total
0	207.50	259.38	285.88	273.50	1.23	1.20	1.25	1.38	2.55	3.11	3.57	3.77	30.63	37.35	42.88	45.29	156.15
25	231.75	262.75	312.50	277.00	1.29	1.09	1.28	1.33	2.99	2.86	4.00	3.68	35.87	34.37	48.00	44.21	162.45
50	282.63	291.50	346.25	305.00	1.10	1.19	1.30	1.38	2.56	3.11	4.11	4.21	30.71	37.34	49.34	50.51	167.89
75	342.50	364.00	433.09	384.25	1.31	1.14	1.28	1.35	4.49	4.15	5.54	5.19	53.84	49.80	66.52	62.25	232.41
100	319.86	325.75	371.75	337.00	1.19	1.25	1.25	1.33	3.81	4.07	4.65	4.48	45.68	48.86	55.76	53.79	204.09
125	281.00	283.00	363.25	312.00	1.28	1.03	1.33	1.33	3.60	2.91	4.83	4.15	43.16	34.98	57.97	49.80	185.91
150	259.75	289.50	360.00	316.00	1.28	1.18	1.25	1.28	3.32	3.42	4.50	4.04	39.90	40.99	54.00	48.54	183.43
'F'-test	*	*	*	*	NS	NS	NS	NS	*	*	*	*	*	*	*	*	*
CD(P=.05)	9.49	11.97	7.03	8.05	0.35	0.22	0.18	0.20	0.91	0.47	0.66	0.34	10.92	5.64	7.92	4.08	6.56
CV%	2.34	3.02	14.65	2.43	19.02	12.76	9.43	9.84	15.60	9.38	10.37	5.83	6.35	3.82	2.01	3.14	2.42

*Significant at the 5% level. NS- Not significant; I,II,III & IV – First, second, third and fourth harvests , respectively.

Table 3
Chemical constituents in the essential oil (%) of citronella in the first harvest as influenced by different levels of sulphur.

Sulphur kg/ha	Chemical constituents in the essential oil, %					
	Citronellal	Citronellol	Geraniol	Citronellyl acetate	Geranyl acetate	Elemol
0	26.46	11.78	21.88	2.11	4.11	1.86
25	29.44	10.04	23.23	1.90	3.13	2.25
50	26.68	11.59	20.37	2.01	4.01	1.36
75	28.78	8.53	23.72	2.24	4.88	1.84
100	30.98	11.96	25.65	2.13	3.72	2.10
125	26.56	13.88	24.23	2.13	4.13	1.77
150	25.71	10.06	24.57	2.22	4.28	1.47
'F'-test	NS	NS	NS	NS	NS	NS
CD(P=.05)	6.23	6.21	5.13	0.50	1.40	1.43
CV%	15.09	37.58	14.76	15.90	23.44	53.39

NS: Not significant; I,II,III & IV – First, second, third and fourth harvests , respectively.

Table 4
Chemical constituents in the essential oil (%) of citronella at different stages of harvest.

Chemical constituents (%)	Stage of harvest			
	I	II	III	IV
Citronellal	27.92*	18.75	24.64	33.13
Citronellol	12.43	9.46	8.88	12.61
Geraniol	22.74	27.23	21.68	30.66
Citronellyl acetate	1.97	5.29	2.32	2.28
Geranyl acetate	2.32	3.17	4.34	2.84
Elemol	2.75	1.60	2.02	0.75

*Each observation is an average of three replicates; I,II,III & IV – First, second, third and fourth harvests , respectively.

Oil composition was found to vary with date of harvesting. The data indicated that essential oil obtained from February harvest had higher concentration of citronellal (33.13 %), citronellol (12.61%),and geraniol(30.66%) followed by May harvest (citronellal 27.92% and citronellol 12.43%). Minimum concentrations of these constituents were observed in the essential oil obtained from November followed by August harvest. The exception was geraniol content which was high in the essential oil obtained from August harvest (27.23%) followed by May (22.74 %) and November (21.68 %) harvests. In Pelargonium sps. it was observed that during summer harvests (April to June) the crop yields lower biomass and essential oil. Evaluation of terpenoid compositions showed minimum concentrations (% of essential oil) of linalool, geraniol and its esters and maximum concentrations of citronellol and its esters during summer months. The percentages of geraniol and its esters were highest during cool winter season months of December and January followed by rainy and autumn season months (Rajeswara Rao et al. 1996).

Rainy/monsoon (August and September) and autumn (October and November) season months were characterised 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.

In contrast, in the essential oil obtained from Pelargonium grown in high altitude areas it was observed that geraniol content in the oil decreases with increase in the temperature and citronellol increase with increase in temperature. High temperature during day and absence of rainfall resulted in lower geraniol and higher citronellol in the same crop at high altitudes. Quality of the essential oil is dependent on weather parameters, latitude longitude and altitude.

4. Conclusions

The findings from this study show that application of 75 kg of elemental sulphur /ha has resulted in significant improvent in the morphological characters of the citronella and rcaused an increase in the herb and oil yield /ha.

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