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Influence of different levels of gypsum on growth, herb and essential oil yields of lemongrass.

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Abstract:

The essential oil obtained from lemongrass (*Cymbopogon flexuosus*) is an industrially important essential oil being used widely for the isolation of citral which can be converted into ionones. Improving the economic yield of the aromatic grass lemongrass is part of the rural development mandate of Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP). Salt affected soils occupy wide regions scattered all over the world and India has considerable salt-affected soils. The experiment was conducted on a red sandy loam soil at the research farm of CSIR-CIMAP, Hyderabad, India with five levels of gypsum as treatments(0-4 tons/ha).The soils of the experimental site are on the leeward side of an industrial area and the ground water has become saline due to industrial effluents. A field experiment was initiated to study the influence of gypsum in soil remediation and its influence on the growth and herb yield of lemongrass. Due to application of four tons of gypsum /ha a progressive decrease in the soil pH was observed and soil pH also decreased with advancement in time and it decreased from 7.73 to 7.40 at 120 days after planting due to gypsum application. EC increased progressively in all the treatments with time. The increase was less due to gypsum treatments. Similar trend was noticed in case of bicarbonate content of the soil and carbonates were absent in the soil. Gypsum application resulted in increased herb and essential oil yield of lemongrass 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 gypsum required is four tons/ha.

Key words: lemongrass herb, essential oil , gypsum Citral, ,ionones

1. Introduction

The steam volatile essential oils extracted from the leaves of aromatic grasses belonging to Cymbopogon spp. are used in perfumery, cosmetics, pharmaceuticals, and flavoring industries [10,25]. *Cymbopogon flexuosus* (lemongrass) is a tropical perennial grass which yields an aromatic oil containing 70-90% citral. Lemongrass oil is being used widely for the isolation of citral which can be converted into ionones having the odour of violets. They are used in flavours, cosmetics and perfumes. ß-ionone is used for the commercial synthesis of vitamin A.

The annual world production of lemongrass oil is around 1000 tonnes. In some Far Eastern countries like Java, Japan, China and India the leaves are used for flavouring foods, drinks and tea and for scenting bathwater.

Cymbopogon spp. are known to be very hardy plants that can grow in soil types ranging from rich loam to poor laterite but with a preference to well drained and nutrient rich soils. These plants are also known for their tolerance to soil salinity and alkalinity to a higher level than other crops [12].

Salt affected soils occupy wide regions scattered all over the world (about 954 millions of hectares). India has 6.73 m ha of salt-affected soils, of which 3.77 m ha is sodic soil [23]. Application of gypsum and a mixture of organic matter was found to reduce the adverse soil properties associated with sodic soils [24] and application of farmyard manure mixed with gypsum also significantly reduced both EC and ESP values. Combined application gypsum and FYM improved pH, EC, soluble ions, SAR and ESP of soils and the amendments increased the downward movement of heavy metals and reduced the chemically available heavy metals in the studied soil in some cases application of gypsum increased soil salinity and decreased sodium adsorption ratio (SAR) and pH [21].

The soils of the experimental site are on leeward side of an industrial area and the irrigation water is

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contaminated with industrial effluent water. The water became saline and to combat the adverse affects of saline irrigation water an experiment was initiated to study the influence of gypsum on the growth and herb yield of lemongrass. Though lemongrass is known to respond significantly to fertilizers, not much information is available regarding the cultivation of lemongrass in marginal soils amended with gypsum.

2. Material and Methods

2.1 Experimental site and design of the experiment

The present study was undertaken to evaluate the influence of gypsum on the herb and essential oil yield of lemongrass 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 treatments consisted of five levels of gypsum (0, 1, 2, 3, and 4 tons gypsum / ha) through commercial gypsum. These treatments were laid out in a randomized block design (RBD) with four replications.

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 ploughed twice using a tractor-drawn disc and was harrowed twice. The field was levelled with a tractor drawn leveller after complete removal of stubbles of previous crop for providing fine tilth.

Mature healthy slips of lemongrass variety Krishna were collected from three year old plantation 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. Gypsum as per treatments was incorporated in to the plots 30 days before planting and the plots were irrigated thrice before planting.

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 observations recorded at 30 days interval starting from 30 Days after planting (DAP) . Data were recorded for six morphometric traits viz., plant height , number of leaves /plant , number of tillers /plant plant weight , leaf area, leaf weight and dry weight of plant (g) .

2.3 Observations on soil chemical properties

To study the influence of gypsum on the soil properties over a period of 120 days after planting, soil samples were collected from a depth of 15 cm from ground level at randomly selected spots about 15-20 cm away from the main stem of the plant at 15 days interval starting from 15 DAP. Samples were collected at five randomly spots in each plot and observations on pH, Electrical conductivity (EC) , Carbonate and bicarbonate content were recorded immediately as per standard laboratory procedures. The data were subjected to statistical analysis.

2.4 Essential oil extraction

The aerial parts of lemongrass 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. The data pertaining to first two harvests is presented in this paper.

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 (C_6-C_{19}). 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.

2.6 Statistical analysis

Analysis of variance was performed to determine the effect of different levels of gypsum on morphological characterstics , herb yield and essential oil yield of lemongrass using statistical software IRRISTAT (IRRI, Manila, Philippines). Means were compared using least significant differences (LSDs) at 5% probability levels.

3.1 Effect of different levels of gypsum on soil pH, EC and bicarbonate content of the soil

Observations were recorded on soil pH, EC and bicarbonate content of the soil at 15 days interval. The data pertaining to soil pH, EC and bicarbonate content of the soil are presented in Table 1. The results indicated that application of gypsum significantly reduced soil pH. The differences observed were not significant at 30, 60, 75 and 120 DAP. There is progressive decrease in the soil pH with advancement in time and it decreased from 7.73 to 7.40 at 120 days after planting due to application of 4 tons of gypsum /ha.

Application of gypsum reduced the EC of the soil at all stages of observation compared to control. But EC increased progressively in all the treatments with time. The increase was less in case of gypsum treatments. Similar trend was noticed in case of bicarbonate content of the soil also. The tests conducted for carbonates were negative.

3 Results and Discussion

Table 1: Influence of different levels of gypsum on the soil pH , EC (ds/m) and bicarbonates(m.e./l) at different stages.

Gypsum t/ha	**pH** Days after planting							
	15	30	45	60	75	90	105	120
0	8.15	8.03	7.80	7.83	7.84	7.93	7.85	7.88
1	7.98	8.00	7.74	7.72	7.88	7.93	7.85	7.33
2	8.18	7.94	7.98	7.73	7.76	7.63	7.98	7.50
3	8.18	7.80	7.72	7.60	7.67	7.58	7.75	7.50
4	7.73	7.86	7.61	7.70	7.73	7.45	7.13	7.40
C.D.(P=.05)	0.31	NS	0.29	NS	NS	0.41	0.34	NS
C.V.%	3.13	3.86	3.01	4.36	4.43	4.21	3.46	3.60
Gypsum t/ha	**E.C. (ds /m)** Days after planting							
	15	30	45	60	75	90	105	120
0	0.37	0.42	0.70	0.70	0.87	0.57	0.74	0.83
1	0.25	0.29	0.49	0.57	0.62	0.53	0.65	0.80
2	0.23	0.26	0.46	0.49	0.54	0.53	0.63	0.76
3	0.20	0.25	0.40	0.38	0.47	0.51	0.60	0.64
4	0.20	0.23	0.33	0.35	0.37	0.44	0.56	0.54
C.D.(P=.05)	0.02	0.02	0.03	0.03	0.05	0.04	0.03	0.05
C.V.%	7.76	6.61	5.63	4.43	6.46	6.39	3.81	5.13
Gypsum t/ha	**Bicarbonate content (m.e./l)** Days after planting							
	15	30	45	60	75	90	105	120
0	1.02	0.63	2.01	1.77	0.62	1.39	1.60	1.40
1	0.62	0.78	2.21	2.92	0.62	0.82	1.19	0.47
2	0.57	0.72	2.16	2.58	0.59	0.86	1.37	0.57
3	0.60	0.57	2.28	2.33	0.72	0.83	1.24	0.45
4	0.56	0.67	1.94	2.55	0.60	0.73	1.24	0.50
C.D.(P=.05)	0.04	0.04	0.15	0.10	0.05	0.06	0.19	0.04
C.V.%	4.92	5.02	5.74	3.35	6.89	4.92	13.05	6.16

Excess of cations such as sodium and anions like carbonate, bicarbonate and chloride present in irrigation water, increase soil pH, EC and Exchangeable Sodium Percentage (ESP) affect the growth and yield of the crop [18]. Application of gypsum and a mixture of organic matter reduced the adverse soil properties associated with sodic soils [24]. Similarly, application of farmyard manure mixed with gypsum was found to significantly decrease both EC and ESP values [20], these amendments increase the downward movement of heavy metals and reduce the chemically available heavy metals in the soil. It was also reported that application of gypsum increased soil salinity and decreased sodium adsorption ratio (SAR) and pH [21] , use of gypsum in the irrigation water improved soil physical and chemical properties and should be considered as an alternative in the process of reclamation of saline-sodic and sodic soils [19]. Desulfurized gypsum was found to be an effective amendment for saline-sodic soil in northeast China[3], incorporating gypsum full depth (20 cm) compared to superficial mixing was superior compared with the other placement methods for the improvement of soil properties [15,16]. In this experiment also application of gypsum decreased the soil pH, EC and bicarbonate contents of the soil.

3.2. Effect of different levels of gypsum on plant height (cm), number of tillers /clump and number of leaves /clump

Comparison of means of morphological characters like plant height, number of leaves /clump, and number of tillers /clump in lemongrass as influenced by different doses of gypsum application are presented in Table 2. The results indicated that application of four tons of gypsum/ha significantly increased all the evaluated traits at all the stages of observation starting from 30 DAP up to 210 DAP. A progressive increase was noticed in plant height, number of tiller and number of leaves /clump with passage of time from 30 to 210DAP.

Plant height increased from 64.36 cm in control to 71.65 cm at 30 DAP, from 95.75 to 129.75 cm at 120 DAP and from 149.75 cm to 169.50 cm at 210 DAP due to application of four tons of gypsum/ha. Number of tillers /clump increased from 14.50 to 23.25 at 30 DAP, from 23.50 to 36.00 at 120 DAP and from 18.75 to 33.00 at 210 DAP due to application of four tons of gypsum compared to control. Number of leaves /clump also increased significantly due to gypsum application and highest numbers of leaves were observed due to four ton gypsum treatment compared to all other treatments.

3.3. Effect of different levels of gypsum on leaf area (cm ^{2}), fresh weight of leaves /clump(g) and dry weight of plant (g)

The crop was harvested twice during the experimental duration for the extraction of essential oil at 120 (first harvest) and 210 DAP (second harvest). During first harvest period observations on leaf

area, fresh and dry weights were recorded at 30, 60, 90, and 120 DAP and during second harvest period observations were recorded at 150, 180 and 210 DAP. Leaf area, fresh weight of leaves and dry weight of plants increased significantly due to gypsum application (Table 3). Progressive increase in leaf area, fresh and dry weights was noticed in all the treatments from 30 to 120 DAP during first harvest period and from 150 to 210 DAP during second harvest period. The differences due to treatments were significant and highest values were noticed due to application of four tons of gypsum /ha at all observation stages.

Application of gypsum to reduce salinity hazards is well understood [2,5,9] and it was reported to increase nitrogen, potassium and phosphorous absorption in roots, stimulate photosynthesis, increase the plant size and improve fruit quality in various vegetables like tomato and sugar beet etc., [7,8]. Application of CaSO4 in saline conditions increased fruit yield and improved quality of strawberry [14]. In this study also application of gypsum increased the morphological traits of lemongrass.

3.4. Effect of different levels of gypsum on oil content (% , herb yield (kg/ha) and oil yield (kg / ha)

Data pertaining to the oil content (%), herb yield (kg/ha) and oil yield (kg/ha) in lemongrass variety Krishna as influence of different levels of gypsum is presented in Table 4. The differences observed in the oil content at first and second harvest were not significant due to treatments. Herb yield increased significantly due to gypsum application. During the first harvest the increase in herb yield was from 2670 in control treatment to 4620 kg/ha due to application of four tons of gypsum/ha. Similarly, a significant improvement was noticed in the herb yield during the second harvest also (5552.50 to 8845 kg/ha). This was reflected in the total herb yield /ha (Table 4). Oil yield /ha which is a product of oil content and herb yield followed the same course as that of herb yield/ha. Oil yield was significantly high due to four ton gypsum

application during first harvest (41.30 kg/ha) and second harvest also (78.06 kg /ha) compared to treatments that did not receive gypsum application (22.54 and 48.47 kg/ ha in the first and second harvests, respectively).

Gypsum and pyrites treated plots increased the grain yields of rice and wheat over untreated plots.The yields from gypsum-treated plots were significantly higher than those from pyrites-treated plots, and the differences in the yield of wheat due to treatments were more pronounced than those in the case of rice [26]. Better yields of wheat and rice crops were obtained with the application of amendments like gypsum or sulfuric acid. However, Kallar grass yield was somewhat suppressed with these amendments [27]

In this experiment also improvement in the soil chemical properties due to gypsum application resulted in significant improvement in the growth of lemongrass which in turn was reflected in higher herb and oil yields obtained /ha.

3.5. Effect of different levels of gypsum on different levels of gypsum on the chemical constituents of the essential oil (%) at different harvests in lemongrass variety Krishna

The data pertaining to the chemical composition of the essential oils collected from different treatments at both harvests (pooled samples of the replicates) is presented in Table 5. Citral is the major constituent of the essential oil and it varied from 70.31 to 73.58 % at 120 DAP and from 71.23 to 73.41 % at 210 DAP. Geraniol varied from 0.85 to 3.15% in the first harvest and it varied from 1.04 to 3.69% in the second harvest. The variability noticed due to different treatments in case of geranyl acetate was 0.06 to 0.48 at 120 DAP and from 0.04 to 0.24% at 210 DAP. Gypsum did not show marked influence on the quality of oil.

Table 2: Influence of different levels of gypsum on the plant height (cm), number of tillers /clump and number of leaves/clump in lemongrass variety Krishna.

Treatment, Gypsum t/ha	Plant height, cm Days after planting						
	30	60	90	120	150	180	210
0	64.36	84.76	91.50	95.75	30.50	64.75	149.75
1	64.58	95.46	91.75	97.50	34.00	67.00	152.00
2	68.80	94.20	98.25	94.75	35.00	71.50	159.25
3	67.97	96.11	98.50	107.50	35.50	78.50	161.75
4	71.65	98.26	107.00	129.75	74.75	148.58	169.50
C.D.(P=.05)	2.92	2.52	4.45	4.12	1.79	3.85	4.27
C.V.%	3.43	2.13	3.34	3.38	3.39	3.60	2.14
Treatment, Gypsum t/ha	No of tillers /clump Days after planting						
	30	60	90	120	150	180	210
0	14.50	15.75	20.25	23.50	9.26	16.25	18.75
1	17.00	18.00	22.75	27.25	11.25	21.00	24.50
2	16.25	19.75	22.75	29.25	13.25	25.25	25.00
3	18.00	21.50	23.50	32.00	14.25	26.25	25.00
4	19.50	23.25	24.00	36.00	17.75	30.75	33.00
C.D.(P=.05)	2.72	2.40	1.73	2.04	1.17	2.55	2.89
C.V.%	9.53	11.01	7.09	5.46	7.05	8.23	9.33
Treatment, Gypsum t/ha	No of leaves/clump Days after planting						
	30	60	90	120	150	180	210
0	44.75	50.25	61.05	71.00	30.73	66.00	89.50
1	44.50	52.25	65.00	72.25	48.23	93.00	91.75
2	54.50	53.00	63.50	82.25	50.01	96.75	90.75
3	54.25	55.00	69.25	84.75	55.00	107.50	94.25
4	57.00	61.00	72.50	92.25	64.00	121.50	96.75
C.D.(P=.05)	3.64	1.79	3.77	5.17	3.28	2.95	3.64
C.V.%	5.64	2.61	4.52	5.10	5.24	2.42	3.12

Table 3: Influence of different levels of gypsum on the leaf area (cm^2), fresh weight of leaf / clump (g) and dry weight of plant (g) in lemongrass variety Krishna.

Treatment, Gypsum t/ha	Leaf area (cm^2) Days after planting						
	30	60	90	120	150	180	210
0	31.00	34.75	33.75	34.25	22.50	37.50	42.50
1	32.50	34.25	36.00	37.25	23.25	39.00	43.00
2	35.95	36.50	41.50	43.50	23.75	46.25	48.62
3	38.87	39.25	45.00	47.25	27.75	47.50	51.50
4	39.57	41.50	47.50	60.00	25.25	47.00	52.00
C.D.(P=.05)	2.15	1.49	1.96	2.19	2.47	2.84	4.05
C.V.%	4.80	3.14	3.74	4.00	8.01	5.19	6.76
Treatment, Gypsum t/ha	Fresh weight of leaf /clump(g) Days after planting						
	30	60	90	120	150	180	210
0	217.00	253.00	282.00	290.00	258.00	677.00	686.00
1	296.00	295.00	345.00	416.00	403.00	793.00	691.00
2	297.00	350.00	351.04	422.00	442.00	871.00	713.00
3	302.00	368.00	370.47	484.48	494.00	946.00	779.00
4	325.00	368.00	415.93	633.00	510.00	968.00	799.02
C.D.(P=.05)	15.85	9.36	18.26	13.27	28.80	26.82	26.28
C.V.%	17.51	8.94	16.70	9.38	21.70	10.01	11.37
Treatment, Gypsum t/ha	Dry weight of plant (g) Days after planting						
	30	60	90	120	150	180	210
0	59.00	89.00	102.00	117.00	82.00	173.00	190.00
1	64.00	105.08	107.00	136.00	110.00	176.00	216.00
2	84.00	109.43	122.00	145.00	139.00	178.10	274.00
3	88.00	112.21	126.00	150.00	147.00	195.69	281.00
4	91.00	131.25	149.00	154.00	152.93	200.09	284.00
C.D.(P=.05)	9.66	15.94	7.52	7.52	7.85	12.51	18.00
C.V.%	39.73	45.18	17.12	19.96	19.74	21.59	22.94

Table 4: Influence of different levels of gypsum on the oil content(%), herb yield (kg/ha) and oil yield (kg/ha) in lemongrass variety Krishna.

Treatment, Gypsum t/ha	Oil content %			Herb yield , kg/ha			Oil yield , kg/ha		
	I	II	Average	I	II	Total	I	II	Total
0	0.88	0.85	0.92	2670.00	5552.50	8295.00	22.54	48.47	73.00
1	0.89	0.82	0.85	3360.00	6305.00	9740.00	28.75	57.77	83.27
2	0.82	0.89	0.86	3735.00	8120.00	12380.00	32.42	72.44	103.12
3	0.93	0.88	0.91	4130.00	8505.00	12960.00	33.97	75.03	109.00
4	0.88	0.89	0.94	4620.00	8845.00	13140.00	41.30	78.06	119.61
C.D.(P=.05)	NS	NS	NS	325.878	430.738	1074.063	2.274	3.794	5.472
C.V.%	39.01	38.02	34.72	6.984	4.579	7.541	5.676	4.537	4.450

I &II first and second harvests (120 and 210 days after planting, respectively)

Table 5: Influence of different levels of gypsum on the chemical constituents of the essential oil (%) at different harvests in lemongrass variety Krishna

Gypsum t/ha	Chemical constituents of the oil, %									
	First harvest					Second harvest				
	Citral- 1 geranial	Citral-2 niral	Total citral	Geraniol	Geranyl Acetate	Citral- 1	Citral-2	Total citral	Geraniol	Geranyl Acetate
0	34.90	37.98	72.88	0.85	0.06	29.54	43.87	73.41	1.69	0.05
1	26.95	43.36	70.31	2.90	0.29	29.23	43.61	72.84	1.13	0.07
2	25.50	45.21	70.71	3.15	0.48	34.72	38.07	72.79	1.15	0.04
3	27.56	45.76	73.32	2.44	0.32	31.89	41.83	72.72	2.13	0.24
4	29.88	43.70	73.58	2.39	0.38	32.74	38.49	71.23	1.04	0.12

I &II first and second harvests (120 and 210 days after planting, respectively)

The first harvest was taken during last week of April and the second harvest was taken during last week of July. The differences noticed in the chemical constituents of the oil during the two harvest were marginal.

Adler and his co-workers [1989] reported that the form of inorganic N (NH4-N versusNO3-N) did not alter the relative quantity in the essential oil of both monoterpenes and phenyl propanoids in greenhouse-grown, potted basil plants. Whereas, application of a mixture of inorganic and organic N affected the chemical composition of essential oil in basil plants, a decrease in linalool and increase in methyl chavicol concentrations was noticed [13]. In this experiment gypsum application did not alter the quality of oil in lemongrass.

4. Conclusions

In a field experiment conducted on a red sandy loam soil at the research farm of CSIR-CIMAP, Hyderabad, India with five levels of gypsum as treatments (0-4 tons/ha) to study the influence of gypsum on soil remediation and its influence on the growth and herb yield of lemongrass, it was observed that gypsum application resulted in increased herb and essential oil yield of lemongrass due to better growth of plants (plant height, number of leaves /plant , number of tillers / clump and weight of plant / clump). The optimum dose of gypsum required is four tons/ha and this has to be incorporated in to the soil at least 30 days before planting and irrigated thrice before planting of the seedlings.

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