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Effect of Different Fertilizers on Crop Growth, Oil yield and Chemical Composition of Lemongrass (*Cymbopogon citrates* (DC.) Stapf)

U.G.A.T. Premathilake[1], D.L. Wathugala[2*] and R.M. Dharmadasa[3]

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ABSTRACT

Purpose: *Lemongrass (Cymbopogan citrates (DC) Stapf.) is ranked among the top ten oil bearing crops in the world and the essential oils of lemongrass are widely used in pharmaceutical, cosmetics, food and flavor, agriculture industries and possess an array of medicinal uses. Aromatic grasses respond well to application of manures and fertilizers. However, farmers do not use fertilizer. Therefore, it is very important to find out the possibility to increase oil yield and improve the quality of essential oil by applying inorganic and organic fertilizer. Therefore, a study was conducted to investigate the effect of fertilizers on crop growth, bio mass production, oil yield and chemical composition of Lemongrass.*

Research Method: *Three different fertilizer mixtures (F1:compost only, F2: inorganic fertilizer (urea, triple super phosphate and muriate of potash at the rate of 278, 296 and 175kg/ha respectively) and F3: inorganic fertilizer + Compost) were used with (F0) control (without fertilizer). Randomized Complete Block Design (RCBD) was used as the experimental design. Number of leaves, tillers and plant height (cm) were recorded. Oil yield and chemical composition of five months old plants were evaluated. Essential oil was extracted by hydro distillation and chemical composition of essential oil was analyzed by Gas Chromatography and Mass Spectrometry.*

Findings: *Highest herbage yield (15.94 t ha-), dry matter yield (4.82 t ha-) and oil yield (fresh wgt. basis) (59.16 kg ha-) were recorded in the treatment of F3 (Compost 106.5g: Urea 2.96g: TSP 3.15g: MOP 1.86g per plant). Citral, β- Citral, Geraniol, α Pinene, cis – Verbenol were major chemical compounds identified in oil.*

Research Limitations: *The study focused on whether there were benefits of using organic and inorganic fertilizers for lemongrass cultivation. However, the research was done only for a limited number of fertilizer mixtures. Further research with different fertilizer mixtures will offer most suitable mixture of fertilizers.*

Originality/Value: *Present study revealed that, application of inorganic fertilizer along with compost result best growth performances and highest oil yield with high quantities of economically important major chemical constituents in lemongrass.*

Keywords: *Chemical composition, Compost, Cymbopogon citrates (DC) Stapf, Essential oil, Fertilizer mixtures*

INTRODUCTION

The essential oils are natural products obtained from plants. These are formed by heterogeneous and complex volatile mixtures of chemical compounds, with predominance of terpene associated to aldehyde, alcohols and ketone which are deposited in several structures of the plant (Linares *et al*., 2005). *Cymbopogan citrates* (DC) Stapf (Lemongrass), belongs to family Poaceae and has acquired a great interest

[1] *Department of Export Agriculture, Uva Wellassa University of Sri Lanka*
[2*] *Department of Crop Science, University of Ruhuna, Sri Lanka*
wgdlakmini@crop.ruh.ac.lk
ORCID https://orcid.org/0000-0003-1276-8060
[3] *Industrial Technology Institute, Sri Lanka*

due to its commercially valuable essential oils and it is ranked among the top ten oil bearing crops in the world (Ravinder *et al.*, 2010). Essential oils of lemongrass are widely used in pharmaceutical, cosmetics, food and flavor, agriculture industries and possess an array of medicinal uses.

Lemongrass grows in dense clumps that can grow up to 1.8 m in height and about 1.2 m in width, with short rhizome. Its straps like leaves are 1.3-2.5 cm wide, 0.9 m long, and have gracefully drooping tips (Ravinder *et al.,* 2010). The plant possesses strong lemony odor due to its high content of the aldehyde citral, which has two geometric isomers, geranial (citral A) and neral (citral B) (Shahi *et al*., 2005). It contains 1-2 % of essential oil on dry basis (Carlson *et al*., (2001) and the chemical composition of essential oil is varying widely upon genetic diversity, habitat and agronomic practices (Paviani *et al*., 2006).

Most of the poor soil types in Sri Lanka are suitable for cultivation of Lemongrass but higher yield can be obtained from soils with high humus. It can be successfully grown in almost all climatic zones in Sri Lanka but tropical hot areas with high sun shine are most suitable. In addition, the crop can be grown even in the pathana lands, marginal tea lands, severely eroded lands and lands which are not suitable to grow other crops. In general, aromatic grasses respond well to application of manures and fertilizers. But normally, farmers do not use fertilizer. Therefore, it is very important to find out the possibility to increase oil yield and improve the quality of essential oil by applying inorganic and organic fertilizer.

Even though *Cymbopogon citrates* (DC.) Stapf has an array of usages, export potential and suitable soil and climatic conditions to cultivate in Sri Lanka, it is not extensively grown due to several reasons including unawareness of the community, absence of proper protocol for commercial cultivation, and poor attention of authorized organizations. The objective of present study was to find out the most suitable fertilizer on higher crop growth, biomass production, oil yield and quality of Lemongrass.

MATERIALS AND METHODS

Field experiment was conducted at the experimental field of the Uva Wellassa University, Badulla during the period March, 2015 to June 2016. Badulla belongs to up country intermediate zone (670 m). All weather parameters (total rainfall, average annual temperature and relative humidity) of the area during the study period were recorded. Soil pH and Electrical Conductivity (EC) values of the soil were measured using pH and EC meters (Thermo Electron Corporation, Orion 3 star). Soil nitrogen content was tested by using Kjeldhal method) (McGill and Figueiredo, 1993) and soil organic matter content was analyzed by Wet oxidation method) (Schumacher, 2002). Soil type of the experimental site was Red Yellow Podsolic according to FAO classification (www.cea.lk/, 2014).

Field establishment and maintenance

The selected site was cleared and ploughed up to 30 cm depth and harrowed to obtain fine tilth to facilitate proper aeration, root penetration and to increase the water holding capacity. The size of a raised bed was 1.8 x 3 m and 1.2 m width drains were prepared between two beds. Suckers were used as planting material. Well grown mother bushes were selected from mother stock and 15 cm height slips were planted at 60 × 60 cm spacing with one slip per hole. (www.exportagridept.gov.lk, 2010). Planted slips were irrigated daily during first two weeks and then at two day intervals during dry weather. Weeding was carried out manually. One month after field establishment, all plants were cut at same height prior to data collection.

Three different mixtures of organic and inorganic fertilizers (Table 01) along with the control were used as treatments. Fertilizer was applied during field establishment. The different fertilizer combinations used in the experiment were as follows.

Table 01: Different combinations of organic and inorganic fertilizer added per plant

Treatment	Compost (g/Plant)	Urea (g/Plant)	TSP (g/Plant)	MOP (g/Plant)
F_0	-	-	-	-
F_1	213	-	-	-
F_2	-	5.93	6.31	3.73
F_3	106.5	2.96	3.15	1.86

1. (F0) Only soil without fertilizer - Control treatment
2. (F1) Compost only- 1000 kg/ha (recommendation of the Department of Export Agriculture)
3. (F2) Inorganic fertilizer only- Urea, TSP and MOP into 278, 296 and 175kg/ha respectively (recommendation of the Department of Export Agriculture)
4. (F3) Inorganic fertilizer + Compost- 1:1 ratio of both compost and inorganic fertilizer (recommendations of the Department of Export Agriculture)

The composition of compost used in the experiment is shown in table 2.

Randomized Complete Block Design (RCBD) with 3 replicates was used as the experimental design. Each block consisted 27 plants and border plants were not taken for data analysis to remove border effect. As growth parameters, number of tillers per plant, number of fresh leaves per plant and plant height (cm) were taken every 30 days.

Plants were harvested five months (www.exportagridept.gov.lk, 2010) after planting. Harvesting was done by sickle and leaves were cut 15 cm above the ground level. After harvesting, fresh weight (g) and dry weight (g) of the leaves were measured.

Fresh weight of herbage was recorded to calculate leaf yield, from net area of each plot and converted into hectare.

Extraction of Essential oil

Essential oil of lemongrass was extracted by hydro distillation. Fresh leaves were harvested and dried in shade in room temperature for 24 hours. Leaves were cut into small pieces (1 cm x 1 cm) and 200 g of each sample was put into 2 L round bottom flask and covered with distilled water. Distillation process was carried out for 2.5 hrs. The distillate was collected into a separating funnel to separate oil from water and oil was dried over anhydrous sodium sulfate to remove existing moisture from the oil.

Yield of essential oil was calculated on fresh weight basis and dry weight basis as proposed by Ranitha *et al.,* (2014).

Analysis of the chemical composition of essential oil

Chemical composition of essential oil was analyzed by Gas chromatography and Mass spectroscopy (GC-MS). A Thermo-scientific TRACE 1300 GC-MS was used and RTX WAX was used as capillary column. The operating conditions were: Injection Mode: Split (1:50). GC-MS analysis was done at 1700 eV. Helium was used as the carrier gas. Oven temperature program: 60˚C (0.00 min.), 60˚C to 240˚C (@ 5˚C/min.), 240˚C (10.00 min.) Quad temperature was 250˚C. MS Source temperature was 250˚C. Scan parameters: 50-450 (amu). Library Search: NIST.

Table 02: Chemical composition of compost used in the field experiment

Ingredients	Cow dung, Poultry litter, Green manure, Plant ash, Dolamite
Nitrogen content	1.2%
Phosphorus content	0.9%
Potassium content	1.3%

(Soil testing laboratory, Department of Agriculture, Gannoruwa, Peradeniya)

Statistical analysis

Data were statistically analyzed using Analysis of variance (ANOVA) and means were compared using Tukey test. Statistical analysis was performed with Minitab 17 software.

RESULTS AND DISCUSSION

The total rainfall received during the experimental period, ranges between 500-700 mm whereas during the 2 months of the second inter-monsoon season the district received as much as 500-750 mm. The average annual temperature of the district varied between 20° to 25 °C. Relative humidity was 70% . Soil pH of the experimental field was 5.4 and Electrical Conductivity (EC) was 1.42 dS/m. According to the analysis, soil nitrogen content was 1.22 g/kg (Kjeldhal method). Soil organic matter content was 1.02% (Wet oxidation method).

Effect of different fertilizer mixtures on crop performances

Plants exhibited higher crop performances with fertilizer application compared to the control treatment. When comparing the different fertilizer mixtures, the highest leaf growth was observed in F3 fertilizer mixture which was the combination of inorganic fertilizer and compost in 1: 1 ratio (Table 03). This is an agreement with Gajbhiye *et al.,* (2013) who reported that the number of leaves per clump of lemongrass was significantly higher in plants applied inorganic fertilizer and farm yard manure together.

Number of tillers per plant was progressively increasing with the advance in age of the plants in each treatment during the study period. The highest rate of tiller growth was observed during the period of three to five months after planting. There was no any significant difference among the fertilizer mixtures during three, four and five month after establishment. But F3 fertilizer mixture gave a significantly ($P \leq 0.05$) higher mean number of tillers per plant (59.77) at six month after planting (Table 04).

Similar results were observed by Gajbhiye *et al.,* (2013) who investigated that significantly lower number of tillers per clump was observed in plants without application of fertilizer. Moreover, Singh (1998) reported the favorable effect on number of tillers per clump at fertilizer level 100 kg N ha.

Plant height was increasing at an increasing rate up to four months and thereafter, minor increment was observed during the study period. According to the results, plant height was not significantly ($P \leq 0.05$) affected by the application of different fertilizer mixtures (Table 05). Complementary to the present results, Gajbhiye *et al.,* (2013) observed significantly lower plant height in control treatment. Singh *et al.,* (1998) revealed that positive improvement in plant height under increased N application and they further explained that it might be due to nitrogen which is one of the key nutrients required for plant growth.

Effect of different fertilizer mixtures on leaf yield

Leaves are the economically important part of *Cymbopogon citrates* which are commonly used for extraction of essential oil.

Table 03: Effect of different fertilizer mixtures on mean number of leaves per plant

Fertilizer mixture	Duration (Months after establishment)					
	1	2	3	4	5	6
F0	4.55^{a}	13.22^{c}	39^{b}	139.89^{b}	187^{b}	228.89^{c}
F1	5.55^{a}	15.78^{b}	62^{a}	183.44^{a}	240.33^{a}	267.11^{b}
F2	5.22^{a}	15.88^{b}	61.33^{a}	183.88^{a}	247.11^{a}	277.22^{ab}
F3	5.55^{a}	17.67^{a}	64.44^{a}	187.11^{a}	242.44^{a}	286.66^{a}
P Value	0.148	0.000	0.000	0.000	0.000	0.000

Table 04: Effect of different fertilizer mixtures on mean number of tillers per plant

Fertilizer mixture	Duration (Months)					
	1	2	3	4	5	6
F0	1.2[b]	3.22[a]	7.78[b]	27[b]	35[b]	42.22[c]
F1	1.78[ab]	3.56[a]	11.33[a]	35.44[a]	45.44[a]	55.11[b]
F2	1.89[a]	3.67[a]	11.22[a]	34.33[a]	46.66[a]	56.33[b]
F3	1.78[ab]	3.67[a]	11.33[a]	35.78[a]	48.33[a]	59.77[a]
P Value	0.003	0.309	0.000	0.000	0.000	0.000

Table 05: Effect of different fertilizer mixtures on mean plant height (cm)

Fertilizer mixture	Duration (Months)					
	1	2	3	4	5	6
F0	31.88[a]	56.33[a]	80.11[a]	99.22[a]	104.26[a]	108.44[a]
F1	33[a]	57.44[a]	81.55[a]	99.78[a]	104.44[a]	112.33[a]
F2	32.33[a]	56[a]	81.55[a]	101.66[a]	105.88[a]	110.66[a]
F3	32.44[a]	56[a]	82.22[a]	101.66[a]	106.33[a]	110.33[a]
P Value	0.561	0.788	0.299	0.217	0.783	0.084

In Lemongrass cultivation, fresh herbage yield and dry yield are considered as main oil yield contributing characters (Gajbhiye *et al.,* 2013). Significantly, ($P \leq 0.05$) lower herbage yield (9.7 t/ha^{-}) and dry matter yield (2.8 t/ha^{-}) was recorded in control treatment. As shown in Figure 01, there was no any significant ($P \leq 0.05$) difference in leaf yield among the three different fertilizer mixtures but highest herbage yield (15.94 t ha^{-}) and dry matter yield (4.82 t ha^{-}) were produced in F3 fertilizer mixture (Inorganic fertilizer and compost 1: 1 ratio). Similarly, Gajbhiye *et al.,* (2013) reported that fertilizer application significantly increased the herbage yield compared with control. Similar observations were made by Silva *et al.,* (2003) who reported that the application of organic and inorganic fertilizers produced highest dry matter content of lemongrass. Chandra *et al.,* (1991) observed that the dry matter accumulation increased significantly with increase in nitrogen levels. Similar results have also been reported by Wong and Ho (1991) on amending soils with composts. NPK fertilizers are more efficient than the organic manures in supplying N, P and K in the short run, while the compost had an advantage in supplying other macro and micro nutrient elements not contained in NPK fertilizer in the long term as well as in slow releasing nature. Rotkittikhun *et al.,* (2007) showed the suitability of application of high proportions of compost with lower doses of inorganic fertilizer for higher biomass production of field crops such as Java citronella and sunflower.

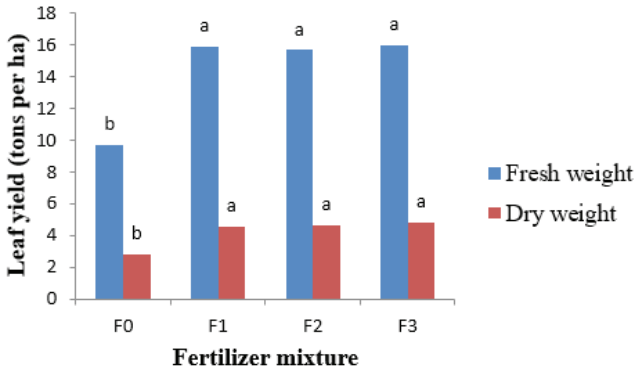

Figure 01: Effect of different fertilizer mixtures on leaf yield (tons per ha)

Effect of different fertilizer mixtures on essential oil yield

Oil yield is the main consideration for economic value of lemongrass. The oil yield of *Cymbopogon citrates* (DC.) Stapf was significantly ((P≤0.05) influenced by fertilizer application. (Figure 02)

Oil yield (Fresh wgt. basis) (32.1 kg ha^{-}) of the control was significantly (P≤0.05) lower when comparing plants which applied any type of fertilizer. There was no any significant difference among oil contents which are obtained with the different fertilizer mixtures, but the highest oil yield (Fresh wgt. basis) (59.16 kgha^{-}) was recorded in F3 fertilizer mixture which was the combination of inorganic fertilizer and compost in 1: 1 ratio. Results of the current study are in agreement with Linares *et al*.(2005) who observed that oil content of *Cymbopogon citrates* (DC.) Stapf positively responded to the application of fertilizers compared to the control. Based on the results of the fertilizer trial conducted by Gajbhiye *et al.,* (2013) concluded that application of 5 t FYM ha^{-1} (Farm Yard Manure) and 60:30:30 kg NPK ha^{-1} was found superior in oil content, whereas, 5 t FYM ha^{-1} and 90:45:45 kg NPK ha^{-1} was found superior in oil yield of lemongrass. Singh *et al*., 2002 observed that application of N at 100 kg ha^{-1} increased significantly oil yield as compared with that control (no fertilizer). Increase in the oil yield by nitrogen levels was due to increase in herbage yield. But Tajidin *et al.,* (2012) reported that nitrogen deficiency affected increases in the citral content of lemongrass. He postulated that it was primarily due to the increase in leaf age caused by nitrogen deficiency. But in contrast, Linares *et al.,* (2005) reported that, differences in yield and quality were not found when the crop was fertilized or when it was not.

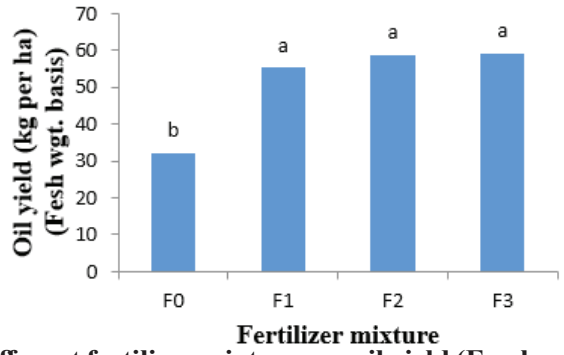

Figure 02: **Effect of different fertilizer mixtures on oil yield (Fresh wgt. basis)**

Table 06: **Effect of different fertilizer levels on essential oil composition of *Cymbopogon citrates* (DC) Stapf**

Compound	Fertilizer mixture							
	F0		F1		F2		F3	
	RT	%	RT	%	RT	%	RT	%
Citral	24.90	33.19	20.55	35.89	20.53	33.29	20.54	35.97
β- Citral	19.17	22.71	19.16	26.47	19.15	24.74	19.17	26.50
Citral diethyl acetal	18.82	21.87	18.82	21.78	18.81	23.59	18.82	19.58
α Pinene			5.61	0.94	5.58	0.33	5.60	2.00
cis - Verbenol							19.16	26.30
Epoxy- linalooloxide	25.70	0.58	25.71	0.46	25.70	0.24	25.68	0.56
Geraniol							23.55	`1.16
(R) – lavanduly acetate							21.33	1.13

(RT: Retention Time)

Effect of different fertilizer mixtures on chemical composition

Quality of the essential oil mainly influenced by its chemical constituents (Linares *et al*., 2005).Chemical composition of essential oil of *Cymbopogon citrates* (DC.) Stapf analyzed by Gas chromatography and Mass spectrometry (GC-MS) is as follows (Table 06).

The highest Citral percentage (35.97) was observed in essential oil extracted from plants which was treated with F3 fertilizer mixture (combination of inorganic fertilizer and compost in 1: 1 ratio).The lowest Citral percentage (33.19) was recorded in essential oil sample respected for control treatment which plants without any fertilizer application. Since the demand for the lemongrass oil is determined by Citral content of the oil, of *Cymbopogon citrates*, oil with higher Citral is key factor of lemongrass essential oil (Tajidin *et al*., 2012). Interestingly, Citral has been identified as a compound which is mainly responsible for antimicrobial properties of lemon grass oil (Paranagama *et al*., 2002; Palhano *et al*., 2003). According to the US National Toxicology Program (NTP) the Citral did not cause cancer in male or female rats receiving 4000 ppm citral in the feed for 2 years. The acceptable daily intake of Citral is 5 mg Citral/ kg body weight and it was given, to generally recognize as safe status in the United States. Moreover, it is one of the most common used flavor compounds in the world and it is used in different concentrations (National Toxicology Program, 2003).

With regard to the β- Citral, the highest percentage (26.50) was found in essential oil extracted plants which applied F3 fertilizer mixture. α Pinene was present in oil samples which was extracted from plants applied any type of fertilizer mixture but the compound was not recorded in control sample. Geraniol,cis – Verbenol and (R) – lavanduly acetate were only identified in essential oil sample extracted from plants which applied combination of inorganic fertilizer and compost in 1: 1 ratio. Linares *et al.,* (2005) reported that the application of any type of fertilizers increases the quality of essential oil of lemongrass.

CONCLUSION

Current study revealed that there was a significant effect of fertilizer application on crop growth, leaf harvest, yield and the chemical composition of essential oil of *Cymbopogon citrates* (DC.) Stapf. Combination of inorganic fertilizer and compost in 1: 1 ratio (Compost 106.5g: Urea 2.96g: TSP 3.15g: MOP 1.86g per plant) was the most promising fertilizer mixture for best crop performances, oil yield and quality of lemongrass.

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