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Determination of Optimum Spacing and Harvesting Age of Citron Scented Gum (*Eucalyptus citriodora H.*) for Maximum Biomass and Essential Oil Yield at Wondo Genet, South Ethiopia

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Abstract

The experiment was conducted at Wondo Genet south Ethiopia for three years. The study was aimed for determination of optimum plant spacing and harvesting age on *E. citriodora*. The treatment consists of three levels of harvesting ages and four levels of plant spacing. The character that manifested for data collection is: plant height, leaf weight/plant stem weight/plant leaf to stem ratio, leaf and stem yield/ha, essential oil content and essential oil yield/ha were collected. The whole plant had been cut using sharp saw at 20 cm height above the ground and essential oil was extracted from the leaf part. The results of the study revealed that harvesting age had very highly significant effect on plant height, fresh leaf to stem ratio, essential oil content and essential oil yield/hectare. Spacing had a very high significant effect on fresh leaf yield/hectare and fresh stem/hectare. The interaction effect of spacing and harvesting age had significant effect on essential oil content. The highest leaf weight/pant was obtained with spacing of 1.5 m and 2 m between plants and raw respectively. Maximum fresh leaf yield/hectare was obtained with narrower spacing (1.5 m between plant and row) and the highest essential oil yield/hectare was obtained when the plant harvested at 10 months after transplanting but it was not significantly different with harvesting age at 9 months after transplanting. Based on the overall result use of plant spacing 1.5 × 2 m and 9 to 10 MATS were recommended for citron scented for Wondo Genet and its vicinity.

Keywords: Citron scented gum · Harvesting age · Planting space · Leaf and essential oil

Introduction

Citron scented gum (*Eucalyptus citriodora* Hook) is belongs to the genus *Eucalyptus* and family *Myrtaceae* [1]. It is commonly known as '*Lemon-Scented Eucalyptus*' or '*Lemon-Scented Gum* and called '*Shitobahirzaf*' in Amharic [2]. The lemon-scented leaves originated from Australia [3]. It is extensively planted and coppiced for the extraction of essential oil that is rich in citronellal and used in perfumery and as flavoring agent [3]. It is a multipurpose crop used for; timber, charcoal, firewood, grown as ornamental but commercially mainly cultivated for its essential oil distilled from the leaves [1]. Its oil is widely used in a number of perfumery formulations, toiletries and as disinfectants. The leaves reported to possess antiseptic properties and are used in the treatment of various skin diseases.

Oil of *E. citriodora* reported to possess antibacterial, antifungal, acaricidal and insect repellent activities [4-6]. The oil is also observed to be phytotoxic and has potential to be used as herbicide [7,8]. In traditional medicine, essential oil is used for treatment of joint pains [9]. Due to its aforementioned numerous advantages, *E. citriodora* is one of the most commonly grown species across the world [10].

In Ethiopia many different Eucalyptus species were introduced to during

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the reign of emperor Menilek II in 1895 to supply fuel wood and construction wood [11]. Its cultivation gradually spread throughout the country with effort of different organizations such as academic, research and development institutions including Alemaya College of Agriculture, Institute of Agricultural Research and Chilalo Agricultural Development Unit [12].

Currently different eucalyptus species were being grown for its multipurpose advantage to Ethiopian public and Wondo Genet Agricultural Research Center was adapted and registered one variety of Citron scented gum for its aroma of essential oil at national level. Leaf and essential oil yield are highly influenced by; location, season, nature of soil, age of plant and planting population density [13]. Even though different Eucalyptus tree species had been grown in Ethiopia for over a century and people developed traditional knowledge for eucalyptus management, still there is knowledge and information gap to obtain optimum essential oil yield of Citron scented gum [14]. Despite different Eucalyptus species reach for harvest after 5-8 years after planting for its wood product, the determination of proper spacing and harvesting age of the leaves is highly required for higher essential oil yield of *E. citriodora*. Because of seldom research activities under taken on this plant in Ethiopian condition for essential oil, this experiment was aimed to determine optimum plant spacing and harvesting age of citron scented gum.

Materials and Methods

The experiment was conducted at Wondo Genet Agricultural Research Center for three years starting from 2017 up to 2019. This trial was consisted three levels of harvesting ages (8, 9 and 10 months after transplanting) and four levels of plant spacing (1.5 m × 1.5 m, 1.5 m × 2 m, and 2 m × 2.5 m between plant and row, respectively. Treatments arranged in factorial Randomized Complete Block Design (RCBD) with three replications. Twelve treatments combined and each treatment combination was assigned randomly within the block. A single plot area was 80 m² having 8 m length and 10 m width. A spacing of 1 m and 2 m was maintained between plants and rows respectively.

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Seedlings were raised in the nursery bed for three months using plastic pots and transplanted on the main field at the onset of rainy season. Hoeing and weeding was done as required. Supplemental irrigation was applied during the dry seasons when rain was not enough as well as where there was no rain at all. The whole plant had been cut using sharp saw at 20 cm height above the ground during harvesting. Harvesting was done for three consecutive years. The harvested herb was distilled for the production of essential oil using hydro distillation in a Clevenger type apparatus according to Gunter [15].

Data collection

Both agronomic and chemical traits data were collected for this study. The character that manifested for data collection are: plant height, leaf weight/plant (kg), stem weight/plant (kg), leaf to stem ratio, leaf yield/ha (kg), stem yield/ha (kg), essential oil content (%) and essential oil yield/ha were collected.

Data analysis

The collected data were subjected to analysis of variance (ANOVA) using SAS version 9.3 [16] statistical software packages. Means were separated using the Least Significant Difference (LSD) procedure at the 5% and 1% level of significant.

Results and Discussion

Plant height

Plant height was very highly significantly (p<0.01) influenced by age, plant population density and harvesting Cycle (Table 1). As plant population density, harvesting age and harvesting cycle increased plant height was increased (Table 2). Maximum plant height was obtained when plants planted with spacing narrower (1.5 m \times 1.5 m) and lower plant height was obtained from wider spacing (2 m \times 2.5 m) between plant and row respectively. This is due to plant grown on narrower spacing was highly competed each other for the sunlight. This result was in line with the result reported by SAS Institute [17] on haricot bean that Plants with a dense population were grown rapidly higher than small population per a given cultivation land area.

Fresh leaf weight kg/plant

The result of this study indicated, fresh leaf weight/plant was not significantly affected by plant spacing and harvesting age (p>0.05). Even though Fresh leaf weight/plant was not significantly affected by plant population density and harvesting age, it was significantly influenced by harvesting cycle (Table 1). Fresh leaf weight per plant was increased from harvesting cycle one (1.76 kg) to harvesting cycle three (4.12 kg) (Table 2). This is may be due to plants perennial character that plant coppiced and produce a number of branches that bear tremendous leaves than the plant preexisting harvest. Maximum fresh leaf weight/plant (2.79 kg) was recorded with the spacing of 1.5 m and 2 m between plant and row respectively harvested at the age of 10 month after transplanting and minimum fresh leaf weight/plant (2.42 kg) was obtained from the wider spacing (2 m and 2.5 m) between plant and row respectively harvested at age of 8 month after transplanting (Table 2). This result revealed that *E. citriodora* require specific plant and row spacing

for optimum leaf weight/plant that not too narrow and wider. As harvesting cycle increased fresh leaf weight/plant also highly increased (from 1.23 kg to 4.72 kg). From this experimental observation optimum spacing for Citron scented gum for essential oil production is $1.5 \text{ m} \times 2 \text{ m}$ between plant and row respectively. Maximum leaf weight/plant was obtained when harvested at age of 10 month after transplanting. This is due to root of a plant well organized absolved enough water and nutrient from a deep soil. Welu, Gebremedhin [18] also reported similar result that older *E. citriodora* was high yielder than the younger one.

Fresh leaf yield/hectare/harvest

The result indicated that harvesting age had no significant influence but plant population density highly significant effect (P<0.01) on fresh leaf yield ha-1 (Table 1). Even though harvesting age had no significant effect on fresh leaf yield, it was increased as harvesting age increased (Table 2). Maximum leaf yield ha-1 of single harvest was obtained at age of 10 month after transplanting (7.37 tones) and minimum was obtained when harvested at age of 8 months after transplanting (6.55 tones) (Table 2). This experiment was revealed that E. citriodora fresh leaf yield ha-1 was increased as plant population density becoming increased. Maximum leaf yield ha-1(7.41 tons) was obtained at narrower (1.5 m × 1.5 m) spacing but not significantly different with the spacing of 1.5 m × 2 m between plant and row respectively. This is may be due to maximum number of plant/hectares. Minimum Fresh leaf vield ha⁻¹ (5.04 tons) was obtained from wider spacing (2 m × 2.5 m) or lower population density. Ramamurthy et al. [19] reported oppose this result that narrower spacing was lower herbage biomass this is may be due to further late harvesting age decreases leaf yield since E. citriodora when plant grow further it highly compete for water and nutrient. Harvesting cycle also highly influenced leaf yield at (P<0.01) and as harvesting cycle increased leaf yield increased from 4.86 tons to 10.60 tons. This experiment was confirmed that harvesting age and plant spacing highly affect leaf of citron scented gum yield per hectare as reported by Ramamurthy et al. [19].

Fresh stem weigh/plant

Fresh stem weight significantly influenced by plant population density (planting space), harvesting age and cycle (Table 1). Stem weight/plant was increased as harvesting age increased from 8 to 10 month after transplanting. This is due to the eucalyptus perennial tree is a plant that rapidly grow or increase its height and size after transplanting. Low fresh stem weight/plant (2.23 kg) was obtained when the plant was harvested at 8 months after transplanting and maximum weight (2.63 kg) was obtained at 10-month age after transplanting. Even though wider spacing had no significant difference, maximum weight was recorded with the spacing of 1.5 m and 2 m between plant and row respectively. Akhtar, Javaid et al. [20] reported similar finding on plant spacing that wider spacing the lower number of trees decrease stem or wood yield per hectare.

Fresh leaf to stem ratio

Fresh leaf to stem ratio significantly affected (P<0.01) by harvesting age and cycle (Table 1). Fresh leaf to stem ratio was decreased as harvesting age increased and maximum Fresh leaf stem was recorded at the spacing of 1.5 m

Table 1. Analysis of variance for tested traits of Citron scented gum to determine optimum plant.

sv	DF	PH	PBN	SBN	FLWP	FSWP	FLSR	FLYHH	FSYHH	EOC	EOYHH
REP	2	0.034	32.7	1612.009	0.47	0.44	0.03	2774907	968146	0.02	161.89
HA	2	1.84	64	3811.74	1.87	1.46	0.33	6157093	6941882	0.32	5295.6
SP	3	0.26	140.63	3883.25	0.81	0.87	0.15	36148298	27208204	0.19	1808.27
HC	2	8.31	483.76	55620	63.68	142.82	4.41	3.61E+08	9.24E+08	2.57	123724.6
HA × SP	6	0.82	102.38	589.14	0.52	0.62	0.03	4565249	6864679	0.29	1414.05
Error	92	21.63	53.54	1270.04	0.415	0.41	0.049	3382937	3140780	0.062	704.63
CV (%)		9.5	19.46	24.55	24.91	26.52	16.95	26.44	27.66	21.41	31.23
Mean		2.31	37.6	145,15	2.59	2.42	1.31	6955,297	6406,768	1.16	84,99

Where: SV (Source of Variance), DF (Degree of Freedom), PH (Plant Height), PBN (Primary Branch Number/Plant), SBN (Secondary Branch Number/Plant). FLWP (Fresh Leaf Weight/Plant), Fresh Stem Weight/Plant, FLSR (Fresh Leaf to Stem Ratio) FLYHH (Fresh Leaf Yield/Hectare Per Harvest), FSYHH (Fresh Leaf Yield/Hectare Per Harvest), EOC (Essential Oil Content), and EOYHH (Essential Oil Yield/ Harvest/Hectare)

Table 2. Mean value of traits tested to determine effect of plant population density and harvesting age of Citron scented gum for optimum essential oil yield.

Harvesting age	PH (m) Mean	PBN Mean	SBN Mean	FLWP (kg) Mean	FSWP (kg) Mean	EI CD	FLYH (kg) Mean	FSYH (kg) Mean	EOC (%) Mean	EOYHH (kg) Mean
						FLSR				
						Mean				
8MAT	2.07	36.27	134.79	2.4	2.23	1.37	6545.4	5957.7	1.06	71.31
9MAT	2.34	37.58	145.3	2.52	2.38	1.36	6948	6427.4	1.24	89.23
10MAT	2.51	38.94	155.37	2.84	2.63	1.2	7372.5	6835.2	1.19	94.43
LSD (0.05)	0.1	3.43	16.68	0.3	0.3	0.1	861.01	829.62	0.12	12.43
				:	Spacing					
1.5 m × 1.5 m	2.38	39.19	153.81	2.47	2.44	1.24	8143.7	7413.6	1.04	86.51
1.5 m × 2 m	2.35	38.91	150.44	2.79	2.56	1.33	7471	6794.4	1.17	93.69
2 m × 2 m	2.34	38.04	148.93	2.68	2.51	1.25	6768.9	6375.5	1.21	85.84
2 m × 2.5 m	2.16	34.25	127.43	2.42	2.16	1.24	5437.6	5043.6	1.23	73.93
LSD (0.05)	0.26	3.96	19.26	0.35	0.35	0.12	994.21	957.97	0.13	14.35
				Harv	esting cycle					
HC1	2.06	33.88	111.15	1.76	1.23	1.5	4863.9	3390	0.97	47.53
HC2	2	41.21	136.12	1.88	1.3	1.51	5403.7	3574.8	1.05	54.89
HC3	2.86	37.7	188.19	4.12	4.72	0.9	10598.3	12255.5	1.47	152.55
LSD (0.05)	0.1	3.43	16.68	0.3	0.3	0.1	861.01	829.62	0.12	12.43

Where: HC1 (Harvesting Cycle One) HC2 (Harvesting Cycle Two) HC3 (Harvesting Cycle Three) HC1 (Harvesting Cycle One) PH (Plant Height), PBN (Primary Branch Number/ Plant), SBN (Secondary Branch Number/Plant). FLWP (Fresh Leaf Weight/Plant), Fresh Stem Weight/Plant, FLSR (Fresh Leaf To Stem Ratio) FLYHH (Fresh Leaf Yield/Hectare Per Harvest), FSYHH (Fresh Leaf Yield/Hectare Per Harvest), EOC (Essential Oil Content), and EOYHH (Essential Oil Yield/Hectare/Harvest).

Table 3. Mean value of the interaction effect of plant spacing and harvesting age on tested traits to determine optimum population density and harvesting age of citron scented gum appointment.

Level of HA	Level of SP	PH	PBN	SBN	FLWP	FSWP	FLSR	FLYH	FSYH	EOC	EOYHA
8MAT	1.5 × 1.5 m	2.11	34.3	138.39	2.24	2.14	1.36	7309.2	6063.86	0.87	65.72
8MAT	1.5 × 2 m	2.22	42.02	148.05	2.84	2.7	1.41	7888.39	7462.47	0.97	82.69
8MAT	2 × 2 m	2.05	37.91	134.39	2.31	2.18	1.3	5794.88	5434.24	1.2	70.22
8MAT	2 × 2.5 m	1.88	30.86	118.34	2.22	1.91	1.4	5189.27	4870.17	1.18	66.62
9MAT	1.5 × 1.5 m	2.42	42.89	160.24	2.56	2.6	1.29	8472.35	7999.8	0.93	80.06
9MAT	1.5 × 2 m	2.35	36.41	143.32	2.55	2.29	1.33	7090.54	6128.44	1.41	107.07
9MAT	2 × 2 m	2.47	36.98	157.78	2.85	2.72	1.29	7502.25	7136.16	1.23	99.08
9MAT	2 × 2.5 m	2.13	34.04	119.85	2.12	1.93	1.53	4726.87	4445.27	1.39	70.72
10MAT	1.5 × 1.5 m	2.62	40.38	162.81	2.61	2.59	1.08	8649.66	8177.12	1.33	113.73
10MAT	1.5 × 2 m	2.45	38.29	155.42	2.97	2.69	1.26	7434.02	6792.33	1.13	91.31
10MAT	2 × 2 m	2.52	39.22	159.16	2.88	2.63	1.17	7009.47	6555.96	1.19	88.23
10MAT	2 × 2.5 m	2.48	37.86	144.09	2.92	2.62	1.27	6396.66	5815.4	1.12	84.46

Where: MAT (Month After Transplanting) HA (Harvesting Age), SP (Spacing), PH (Plant Height), PBN (Primary Branch Number/Plant), SBN (Secondary Branch Number/Plant). FLWP (Fresh Leaf Weight/Plant), Fresh Stem Weight/Plant, FLSR (Fresh Leaf To Stem Ratio) FLYHH (Fresh Leaf Yield/Hectare Per Harvest), FSYHH (Fresh Leaf Yield/Hectare Per Harvest), FOC (Essential Oil Content), and EOYHH (Essential Oil Yield/ Harvest)

and 2 m between plant and row respectively. Lower fresh leaf to stem ratio was obtained at early stage and late harvesting age. Maximum fresh leaf to ratio (1.33) was obtained at 9 months after transplanting. This result revealed that too narrower space and too wider is not proper for *E. citriodora* leaf production for essential oil. Since *E. citriodora* essential is extracted from leaves, leaf to stem ratio is very important to get optimum essential oil yield. Similar result reported by Zigene, Zewdinesh Damtew et al. [21] on rosemary that as harvesting age increased leaf to stem ratio was increased. This result indicated that stem growth change was proportionally higher than leaf growth. Based on this finding, leaf to stem ratio can be used as harvesting age indicator for citron scented gum produced for essential oil extraction.

Essential oil content

Result indicated essential oil content of Citron scented gum was high influenced by harvesting age and harvesting cycle as well as by the interaction of harvesting age and plant population density (Table 1). Maximum essential oil content (1.41%) was obtained when harvested at 9-month age after transplanting having the spacing 1.5 meter and 2 meters between plant and row respectively (Table 3). As plant spacing becoming wider essential oil content becoming higher this is due to wider space receive high sun light than narrower spaces which highly influence essential oil content as reported by Rezai, Sedigheh, et al. [22] that essential oil content decreased with increasing shade levels the vice versa was reported by Saleh, M [23] that essential oil content was increased when plants grown under full sunlight.

Essential oil yield/hectare/harvest

The essential oil yield/hectare/harvest had significantly (p<0.01) influenced by plant population density, harvesting age and harvesting cycle (Table 1). The essential oil yield was increased as harvesting age increased from 71.31 kg to 94.43 kg. This is due to plant leaf yield increased as age of a plant increased and also essential oil content is low at early stage. Maximum essential oil yield ha⁻¹(94.43 kg) was obtained at late harvest (10 MAT) and minimum (71.31 kg) at early harvesting age (8 MAT). Plant spacing also the other source of variation for E. citriodora essential oil yield that maximum yield was obtained with the spacing 1.5×2 m this due to maximum essential oil content was obtained using this spacing. Harvesting cycle had also very significant difference on essential oil vield (Table 1). As harvesting cycle increased from one to three essential oil yield/ hectare/harvest increased from 47.53 kg to 152.55 kg, this is due to fresh leaf yield and essential oil content was increased as harvesting cycle increased that directly associated with essential oil yield of the plant. Inline result was reported by Kassahun, Beemnet Mengesha [24] on Japanese mint and pepper mint.

Conclusion and Recommendations

E. citriodorais grown for it multi-purpose advantage and its growing is depend on our purpose and it require 5-8 years to reach first harvest for its wood advantages but it can be harvested less than one year for its essential oil. Since leaf is the main economical part of E. citriodora for essential oil extraction, can be harvested at any time of age but optimum essential oil yield obtained at 9 to 10 month after transplanting. The highest essential oil yield/hectare/harvest was obtained when the plant harvested at 10 months after transplanting (94.43 kg ha/harvest). Maximum essential oil content was obtained at the age of 9 month after transplanting with spacing of 1.5×2 m between plant and row respectively. In this experiment 1.5 × 2m spacing can be recommended as optimal spacing for essential oil content and essential oil yield/hectare. Our experiment had the limitation of quality parameters in which essential oil of Citron scented gum can be affected by population density and harvesting age. Therefore, we recommend that this experiment should be repeated with testing the effect of spacing and harvesting age on the essential quality by determination of essential oil composition using Gas Chromatography Mass-Spectrometer (GC-MS).

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