INFLUENCE OF WATER STRESS AND PLANT AGE ON THE YIELD AND CHEMICAL COMPOSITION OF ESSENTIAL OIL FROM *Cymbopogon winterianus* JOWITT**

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ABSTRACT

Citronella oil, which is also known as Java citronella oil, is typically obtained from the leaves of *Cymbopogon winterianus* Jowitt and has been widely used as an antiseptic, fragrance flavor and many other applications. However, systematic studies on the factors affecting the yield and composition of citronella oil are limited. Hence, this study was carried out to investigate the influence of water stress (low to high) and plant age (0.5 to 5 years) on the yield and composition of citronella oil. Citronella leaves were dried at room temperature (26-30 °C) with a relative humidity of 62-74% for 3 days before the citronella oil was extracted using a steam distillation unit. Introducing a high water stress condition to the plants increased the yield from 0.8% (control) to 1.4% (high water stress) dry weight. The geraniol content slightly increased from 15% to 17%, whereas the citronellal content considerably increased from 31% to 44% when the plants were subjected to high water stress in comparison to the control. The oil yield increased with plant age before it reached its plateau at 3.5% dry weight at age 4 years. However, the composition of citronella oil did not significantly vary with plant age.

Keywords: citronella, citronellal, plant age, water stress, yield

INTRODUCTION

In 2011, the worldwide market of essential oil was approximately US$ 24 billion and still increasing at about 10% per year, making essential oils a very promising market as these are currently used in flavor, fragrance and pharmaceutical industries (Chen & Viljoen 2010; Govindasamy *et al.* 2013; Bezzera *et al.* 2019). Citronella oil is among the most utilized essential oil apart from orange and eucalyptus oil. It is typically obtained from the leaves of *Cymbopogon winterianus* Jowitt which is a native plant in the tropical and semitropical countries of Asia, such as Indonesia and India (Manurung *et al.* 2015).

Citronella oil typically consists of 32-55% citronellal and 10-12% geraniol as the major components as well as 11-15% citronellol, 3-8% geraniol acetate, 2-4% citronellal acetate and other terpene and terpenoid compounds (Timung *et al.* 2016). Citronella oil which is also known as Java citronella has been widely used for perfumery, body care products and pharmaceutical products due to antifungal and antimicrobial property (Wei & Wee 2013). The global demand of citronella oil is around 2000-2500 tonnes/year but only 50-60% of this demand is available in the market. China as the major producing country of citronella oil supplies approximately 500-600 tonnes/year. Indonesia as the third largest producer can only provide 200-250 tonnes/year (Rizal *et al.* 2009).

The limited supply of citronella oil in the market is mainly due to its low yield and high production cost. Steam distillation is the traditional process to obtain citronella oil from the leaves. This method is also extensively used in the aromatic industry due to its cheaper

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operating cost when compared with the supercritical fluid extraction (Malauluan & Malauluan 2015). However, the yield of citronella oil obtained through steam distillation of the citronella leaves is only about 1.5% to 3% of dry weight (Hanaa *et al.* 2012; Kakaraparthi *et al.* 2014). In addition, low amount of citronellal and geraniol content in the citronella oil also limits the supply of high-quality citronella oil to be used for fragrance and therapeutic applications.

Systematic studies on the factors affecting the yield and composition of citronella oil particularly drought stress and plant age are scarce. Previous studies have shown that drought stress affect water potentials in the root zone and consequently affects the growth and metabolism of essential oil baring plants (Caparros *et al.* 2019). In another study, harvesting time of 10 to 90 days have influenced the yield and composition of citronella oil (Kakaraparthi *et al.* 2014). An increase in age of plant resulted to an increase in limonene, citronellol and geraniol content of the essential oil. This study attempted to investigate the influence of water stress and plant age on the yield and composition of citronella oil as these two factors proved to be very important in determining the yield and composition of citronella oil (Kakaraparthi *et al.* 2014; Rocha *et al.* 2014).

MATERIALS AND METHODS

Sample Source

Citronella plants (*Cymbopogon winterianus* Jowitt) were obtained from Kebun Percobaan Manoko (KPM), West Java which is the center of citronella plantation in Indonesia. The plantation is located at an altitude of 1200 m over the sea level with a geographical bearing of 6°48'23"S, 107°36'49"E. The region has a tropical with an average temperature of 21 °C and a relative humidity of 82%. Its mean annual rainfall of this region is generally in the range of 1,500-2,250 mm per year.

In this study, the effect of water stress and plant age on the yield and chemical composition of citronella oil was carried out simultaneously. Growing of the plant and harvesting of the leaves for both experiments were performed at KPM from January to June 2015.

Water Stress Treatment

Matured healthy slips (rooted tiller) of citronella variety "G2" were planted in twelve polybags at KPM for approximately 0.5 year (6 months) under standard conditions. Afterwards, the plants were subjected under different water stress conditions for 45 days particularly moderate and high water stress as well as control (four polybags as replicates per water stress conditions). This was achieved by watering the plants once every three days (600 mL/three days) for moderate water stress. High water stress was achieved by not watering the plants for 45 days whereas the control (unstressed) plants were watered daily (600 mL/day). Watering of the plants was carried out in the morning around 09.00 am.

Plant Age Treatment

Citronella plants aged 0.5 to 5 years (three healthy clumps per plant age) were selected from KPM. These plants have not been subjected to harvest during the past 30-60 days. After 45 days, their leaves were harvested and weighed. The harvested leaves were dried (shade-drying) at room temperature (26 °C to 30 °C) with a relative humidity of 62-74% for 3 days before the citronella oil was extracted using a steam distillation unit. After the extraction, the yield and composition of the citronella oil were determined.

Extraction of Citronella Oil

For the essential oil extraction, approximately 180-610 g of citronella leaves were subjected to a 1-L capacity of steam distillation unit. The distillation was carried out at 97 °C and 0.9 atm for 4 h. The cooling water temperature in the condenser was set at 27 °C with a flow rate of 10 mL/s. The distilled sample containing the water and citronella oil was separated using a separatory funnel. The citronella oil was weighed to calculate the yield and analyzed using GC-MS to determine its composition.

Data Analysis

The oil yield is defined as the amount of distilled citronella oil obtained from a certain amount of citronella leaves (eq. 1).

$$\text{Yied (\% dry weight)} = \frac{\text{amount of distilled citronella oil (g)}}{\text{amount of citronella leaves (g)}} \times 100 \quad (1)$$

The productivity of citronella oil is defined as the product of oil yield multiplied by the mass of citronella leaves divided by the plantation area and the harvesting period (eq. 2).

$$\text{Productivity}\left(\frac{\text{kg}}{\text{ha.year}}\right) = \frac{\text{yield (\% dry weight) x mass of citronella leaves (kg)}}{\text{plantation area (ha) x harvesting period (year)}} \quad (2)$$

The distance between plants is assumed at 1 x 1 m and that the citronella leaves can be harvested at the age of 0.5 year and after 1 year the leaves can be harvested every 0.25 year thereafter (Sukamto *et al.* 2011).

Analytical Methods

The total moisture content of the samples was determined by drying the fresh leaves in the oven at 80 °C until constant weight. The composition of citronella oil was analyzed by gas chromatography-mass spectrometry (GC-MS) using a Shimadzu GCMS-QP2010 Plus device. A capillary column of Zebron ZB5MS (30 m length x 0.32 mm internal diameter and 0.25 μm film thickness) was used. Helium was used as the gas carrier at 86.1 kPa. The oven temperature was initially set at 90 °C for 4.5 min. Then, the temperature was increased to 150 °C at a rate of 7 °C/min. The temperature was then increased at 10 °C/min up to 170 °C and held for 8 min at this temperature. The injector and detector temperature were set at 250 °C.

RESULTS AND DISCUSSION

Effect of Water Stress on the Weight and Moisture Content of Citronella Leaf

The effect of moderate and high-water stress on the citronella plants were investigated by adjusting the amount of water input given to the plants. After 45 days of treatment, the leaves were harvested and shade-dried at room temperature of 26 °C to 30°C. The total weight of the harvested leaves and the moisture content were determined (Fig. 1; Fig. 2). After 45 days of treatment, the fresh weight of citronella leaves per clump that were subjected to high water stress was almost three times lower than those of the control plants. The remaining moisture content only slightly decreased for citronella plants subjected to moderate water stress, whereas for the plants subjected to high water stress, the moisture content considerably decreased by 20.4% (Fig. 2).

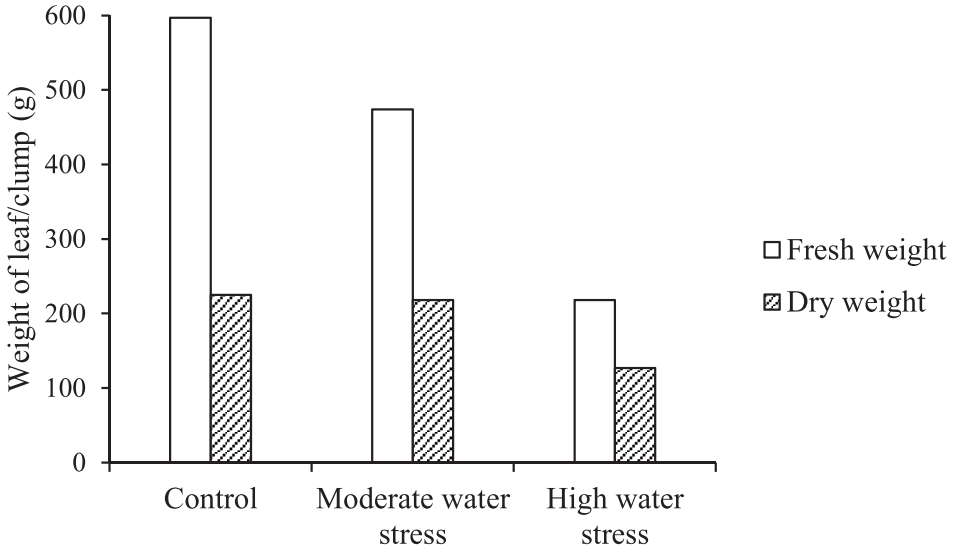

Figure 1 Total weight (fresh and dry basis) of citronella leaf after 45 days of water stress treatment (plant age of approximately 0.5 year)

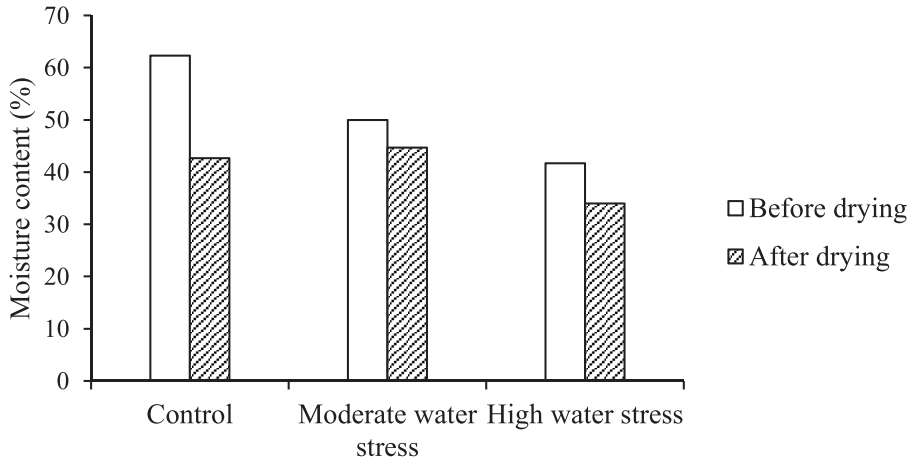

Figure 2 Moisture content of citronella leaf after 45 days of water stress treatment (plant age of approximately 0.5 year)

The dry weight of citronella leaves subjected to moderate water stress only slightly decreased by 3.1% over the control. Whereas, that of the leaves subjected to high water stress, significantly decreased by 43.6% as compared to the control. These results confirmed the previous research which explained that citronella plants assumed an adaptive strategy by sacrificing growth for long term survival under water stress conditions (Timung *et al.* 2016).

Effect of Water Stress on the Yield and Composition of Citronella Oil

Citronella oil was extracted from the air-dried leaves using a steam distillation unit. After extraction, the yield and productivity of the citronella oil were determined (Table 1). The yield varied from 0.8% to 1.4% dry weight which is equivalent to an increase of 75%. The essential oil obtained from citronella belongs to the terpenes, particularly monoterpenes. The biosynthesis pathway of citronella oil includes several stages to produce geranyl pyrophosphate (GPP) which is later transformed into geraniol which is then converted into citronellol via enol formation, while oxidation converts citronellol to form citronellal. Water stress increased the enzymatic activity of geraniol dehydrogenase (GeDH) in producing geraniol and citronellal (Timung *et al.* 2016). Hence, the increased enzymatic activity of GeDH resulted in a higher amount of citronella oil synthesized by the plants when subjected under high water stress (Table 1).

Table 1 Experimental yield of and estimated productivity of Citronella oil after 45 days of treatment (plant age of approximately 0.5 year)

Treatment	Yield of citronella oil (% dry weight)	Productivity of citronella oil (kg/ha/year)
Control	0.8 ± 0.19	45 ± 11
Moderate water stress	0.9 ± 0.10	49 ± 5
High water stress	1.4 ± 0.18	45 ± 6

The yield of citronella oil obtained here is considerably lower than in other studies which ranged from 1.5% to 3% dry weight (Hanaa *et al.* 2012; Kakaraprthi *et al.* 2014) which may be contributed by the relatively high moisture content of the leaves used in this study (34-45%) for extraction of citronella oil. Previous studies have reported that reducing the moisture content of the leaves up to 10% of its weight may soften the plant cell membrane which lead to higher oil release and consequently improved oil yield (Moncada *et al.* 2014; Timung *et al.* 2016).

The composition of citronella oil obtained from the water stressed conditions was analyzed with GC-MS (Table 2). The major compounds are citronellal (27-44%), citronellol (21-22%) and geraniol (15-17%). These geraniol and citronellal contents are well within the range obtained in other studies; 12-27% for geraniol and 27-45% for citronellal (Kakaraparthi *et al.* 2014). Other components include limonene (4%), caryophyllene (3-4%), δ-cadinene (2-4%), germacrene-D (1-2%) as well as other terpenes. When the plants were subjected to high water

stress, the geraniol content increased up to 17% whereas the citronellal content considerably increased up to 41%.

The total citronellal and geraniol content of the citronella oil increased with increasing water stress from 75.7% (unstressed) to 81.5% (high water stress). High water stress treatment increased the citronellal content up to 43.7% which lies at the high end of the reported citronellal content of 32-45%. A high citronellal content is an indication of a high quality and price of citronella oil (Beneti *et al.* 2011; Timung *et al.* 2016; Gavahian *et al.* 2018). Such condition was achieved by introducing high water stress to citronella plants. Hence, the introduction of water stress to citronella plants increased both the oil yield and the total content of citronellal and geraniol in the citronella oil.

Effect of Water Stress on the Estimated Productivity of Citronella Oil

The productivity of citronella oil is estimated from the oil yield and mass of the leaves per citronella plant (Table 1). It is assumed that there are 10,000 Citronella plants in one-hectare land (1 x 1 m distance between plants) and that the citronella leaves can be harvested at the age of 0.5 year and can be harvested twice in the first year of plantation (Sukamto *et al.* 2011). Based on the 0.5-year old citronella plants used in this study, the productivity of citronella oil for the control is 45 ± 11 kg/ha/year. When subjected to moderate water stress conditions, the productivity slightly increased to 49 ± 5 kg/ha/year. At higher water stress conditions, the productivity decreased to 45±6 kg/ha/year. Although the oil yield increased when subjected to water stress conditions, the productivity did not significantly increase due to overall decrease in the total weight of citronella leaves after 45 days of treatment (Table 1).

Effect of Plant Age on the Morphological Characters of Citronella Leaf

Citronella plants at the age of 0.5 to 5 years were grown at KPM under standard conditions (watered on a daily basis) for 30-60 days before the leaves were harvested. The total weight and number of leaves per plant as well as the length and width of the leaves were measured (Table 3). The plant age influenced the total weight and number of leaves per plant as well as the length and width of the leaves. At the age of 4 years, citronella plants had 453 ± 6.7 leaves per plant which is 72% more than the plant at the age of 0.5 year. The total weight of the leaf also greatly increased by 116% as compared with that of the plant at the age of 0.5 year. The length of each leaf also increased considerably by 47% in comparison the 0.5-year old plant. The weight of the individual citronella leaf was in the range of 0.8-1.3 g and did not vary with the plant age.

Table 2 Composition of citronella oil after 45 days of water stress treatment

Component	Content (%)			Ref.[b]
	Control	Moderate water stress	High water stress	
Limonene	3.88	3.71	3.58	2.2-3.91
Linalool	0.67	0.63	0.77	0.76-0.8
Citronellal	31.08	40.12	43.74	27.0-45.0
Isopulegol	0.44	0.43	0.58	0.3-3.67
Neral	0.25	0.24	n.d.[a]	0.31
Citronellol	21.82	20.97	20.88	5.4-6.9
Nerol	0.33	0.31	0.34	0.6-0.8
Geraniol	14.50	14.61	16.88	12-26.7
Geranial	0.18	0.21	n.d.[a]	0.82-1.0
Citronellyl acetate	n.d.[a]	0.23	0.23	2.21-5.3
Eugenol	0.66	0.49	0.29	0.9-1.06
Geranyl acetate	0.35	0.38	0.40	9-14.8
ß-Elemene	0.52	0.68	0.59	0.7-1.0
Caryophyllene	3.36	3.77	3.32	0.21-0.28
α –Humelene	0.47	0.52	0.43	-
Germacrene-D	1.48	1.37	1.26	0.4-0.7
γ-Murrolene	n.d.[a]	0.78	0.76	0.2-0.79
γ –Cadinene	n.d.[a]	0.91	1.28	0.57-1.0
δ-Cadinene	3.74	3.23	1.50	1-2.32
Elemol	0.78	1.16	0.2	6.19-8.3

Notes: [a]n.d. = not detected based on GC-MS analysis.
[b]Kakaraparthi *et al.* 2014; Simic *et al.* 2015.

Table 3 Morphological characters of harvested citronella leaves after 30-60 days grown under standard conditions at KPM

Age (year)	Total weight of leaf per clump (g)	Total number of leaf per clump	Length (cm)	Width (cm)
0.5	205.1 ± 1.4	264 ± 9.4	55.6 ± 2.0	1.4 ± 0.1
1	357.6 ± 9.2	286 ± 6.8	62.0 ± 1.0	1.9 ± 0.1
2	407.8 ± 7.4	321 ± 4.0	67.9 ± 0.4	1.7 ± 0.1
3	411.3 ± 14.4	409 ± 2.0	74.2 ± 0.8	2.4 ± 0.1
4	443.7 ± 13.3	453 ± 6.7	81.7 ± 1.5	2.2 ± 0.1
5	418.7 ± 6.4	437 ± 7.8	70.0 ± 1.3	2.2 ± 0.1

Effect of Plant Age on the Yield, Composition and Estimated Productivity of Citronella Oil

The oil yield lies in the range of 2.7-3.5% dry weight (Table 4). The yield increased with plant age before it slightly decreased after the age of 4 years. Most probably, periodic harvesting of the leaves may trigger the self-defensive mechanism within the plant by producing more essential oil which reached its plateau at the age of 4 years. Citronella oil which primarily consist of terpenes may become toxic when accumulated at very large amounts particularly at the age of 4 years. Hence, the biosynthesis of citronella oil within the plant was reduced with increasing age. The results are in accordance with the results obtained in another study that the yield of essential oil from *Cymbopogon citratus* increased from 2.0% to 2.5% as the plant age increases from 3 months to 12 months (Rocha *et al.* 2014).

Previous studies have reported that the quantity and quality of essential oil from *Cymbopogon* spp. are highly dependent on plant age because the yield and composition of the essential oils are very much connected with the developmental stage of the whole plant, organs and cells (Rocha *et al.* 2014; Verma *et al.* 2015; Majewska *et al.* 2019). Similar findings are also observed in this study that the yield and composition of the extracted citronella oil varies with plant age. The composition of citronella oil obtained from different plant age was analyzed with GC-MS and the major compositions are shown in Fig. 3. The major compounds of citronella oil are citronellal (38-46%), citronellol (16-21%) and geraniol (13-21%). Other components include caryophyllene (3-5%), limonene (3-4%), δ-Cadinene (2-5%), γ-Cadinene (1-3%) as well as other terpenes (data not shown).

Table 4 Experimental yield and estimated productivity of citronella oil after 30-60 days grown under standard conditions at KPM

Plant age (year)	Yield of citronella oil (% dry weight)	Productivity of citronella oil (kg/ha/year)
0.5	2.7 ± 0.12	139 ± 7
1	2.8 ± 0.03	362 ± 5
2	3.1 ± 0.13	464 ± 20
3	3.7 ± 0.10	533 ± 28
4	3.8 ± 0.24	629 ± 42
5	3.5 ± 0.04	528 ± 10

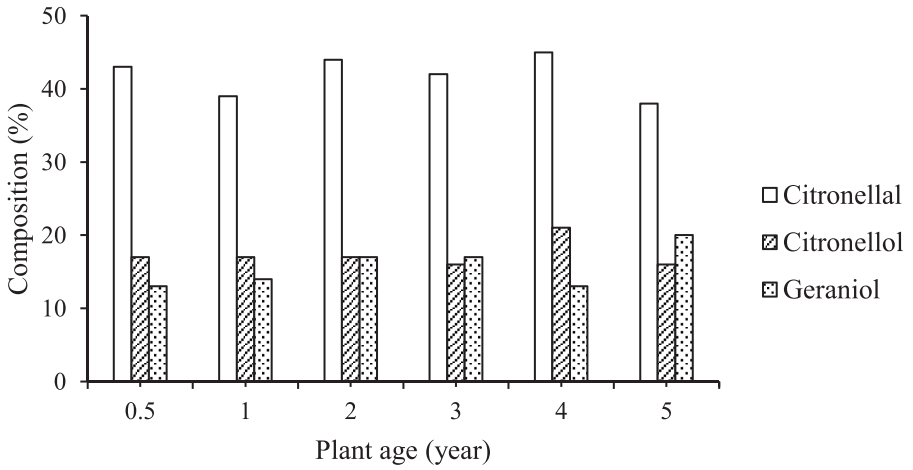

Figure 3 Composition of citronellal, citronellol and geraniol in citronella oil as a function of plant age

The essential oil extracted from the plant age of 4 years had the highest amount of citronellal and citronellol and the lowest amount of geraniol (Fig. 3). In contrast, the essential oil extracted from the plant age of 5 years had the lowest amount of citronellal and citronellol and the highest amount of geraniol. The total amount of citronellal, citronellol and geraniol was the highest for the essential oil extracted from the plant age of 4 years (79%) and decreased to 74% when the plant age was 5 years. This finding supported the results of previous studies that the chemical composition of essential oil was highly influenced by plant age (Rocha *et al.* 2014; Verma *et al.* 2015; Majewska *et al.* 2019) and harvesting time (Kakaraparthi *et al.* 2014; Costa *et al.* 2016).

The productivity of citronella oil was estimated from the oil yield and mass of the leaves per citronella plant (Table 4), assuming that there are 10,000 Citronella plants in one-hectare land (at 1 x 1 m spacing distance between plants) and that the Citronella leaves can be harvested at half a year old (age 0.5 year) and at every 0.25 year thereafter (Sukamto *et al.* 2011). The productivity increased from 139 ± 7 kg/ha/year to 629 ± 42 kg/ha/year as the plant age increased from 0.5 to 4 years. As the plant age increased, the leaf area and total number of leaves per plant increased and consequently more cells were able to synthesize citronella oil (Kakaraparthi *et al.* 2014). At age of 5, the oil yield decreased and consequently the productivity also decreased to 528 ± 10 kg/ha/ year. Assuming an oil yield of 0.8-1.2% (fresh weight basis), the estimated productivity of citronella in year 4 ranged from 422-633 kg/ha/year. In this study, the estimated productivity of citronella oil obtained at plant age of 4 years was 629 kg/ha/year (Table 4).

CONCLUSION

Water stress condition has positively affected the yield and composition of citronella oil. Introducing a high-water stress condition to the plants increased the yield from 0.8% dry weight (control) to 1.4% dry weight (high water stress). The geraniol content slightly increased from 15% to 17% whereas the citronellal content considerably increased from 31% to 44% when the plants were subjected to high water stress. The oil yield also increased with plant age before it reached its plateau at 3.5% dry weight at age 4 years. However, the composition of citronella oil did not vary with plant age. Moreover, it was estimated that 629 kg/ha/year of citronella oil can be obtained from citronella plants with a productive age of 4 years.

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