Enhancement of Drought Tolerance in Patchouli (*Pogostemon cablin*, Benth) by Modifying Micro Climate and Frequency of Fertilization

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**ABSTRACT**

Drought stress is one of abiotic stresses and has caused a significant deterioration in growth and yield of patchouli. This research was conducted in Reuleut Timu Village, Muara Batu District, Aceh Utara Regency, from July to December 2016, using split plot design in a Randomized Block Design with three replications. The main plot was mulch treatments (without mulch, rice straw mulch and black silver plastic mulch). The subplots were treated with different frequency of fertilizer application (once, twice and three times). The results revealed that the application of rice straw mulch has reduced the effects of drought stress, suppressed the soil temperature fluctuations, maintained soil moisture, which resulted in an increase of patchouli growth. Different frequency of fertilizer application did improve the oil yield, proline accumulation and drought resistance.

**Key words:** Drought stress, Mulch, Proline, Soil moisture content, Soil temperature.

**INTRODUCTION**

Patchouli (*Pogostemon cablin*, Benth) is a species of a plant from family Lamiaceae and it is commercially important for its essential oil known as patchouli oil. Indonesia is the major producer and exporter of this oil in the world market with a contribution about 90%. This plant grows well at altitudes up to 1,200 m above sea level (ASL), humidity 70-90%, rainfall evenly throughout the year between 1500-3000 mm/year, temperatures 24-28°C. For good growth, sapphires require pH 5.5 - 7 on alluvial, latosol and regosol soils (Swamy *et al.*, 2010; Putri *et al.*, 2017).

Aceh Utara is one of the regencies in the Province of Aceh producing patchouli oil in Indonesia which has rainfall of 1470 mm/year. This amount of rainfall is below the average water needs by patchouli plants. Patchouli cultivation on dry land with low rainfall has the potential to experience drought stress. Plants that grow in conditions of lack of water resulted in stunting and low yield. In conditions with drought stress the plant will maintain its survival by making adjustments both physiologically and morphologically (Basu *et al.*, 2016).

Drought stress is a major cause of the decline in growth and crop yields throughout the world. Lack of water followed by large evaporation of water becomes an obstacle in crop cultivation. Mulch has the role of reducing excessive evaporation of ground water thereby helping to retain soil moisture (Ramakrishna *et al.*, 2006), suppressing soil temperature fluctuations, increasing infiltration rates, reducing run off (Scott, 2007), maintaining soil nutrient content (Uwah and Iwo, 2011).

Plants must have enough water and nutrients which is applied in proper time. The adequacy of those will stimulate plants to flourish and grow better, produce more leaves and produce higher yield. Lack of water and improper amounts of nutrients will reduce the rate of growth and crop production (Elewa *et al.*, 2017). The application of fertilizers in the right dose of patchouli is expected to stimulate vegetative growth and increase the process of plant assimilation and oil production (Mahtouz and Sharaf-Eldin, 2007).

Drought stress and low nutrient content for plants can be solved by applying mulching technology and proper time of fertilizer application. This application can increase plant growth which creates drought resistance in plants. This study aimed at investigating the effect of mulching and different frequency of fertilizer application on patchouli in an effort to reduce the effects of drought stress.
MATERIALS AND METHODS
The study was conducted in Reuleut Timu Village, Muara Batu District, Aceh Utara Regency at a height of ±8 m above sea level (asl) from July-December 2016. The materials used were patchouli seeds variety Tapaktuan, cow manure, urea fertilizer, phosphate fertilizer, potassium fertilizer and pesticides. The tools used are Traceable® digital soil thermometer (range between-50°C-300°C), tensiometer, oven and digital scales.

This research was conducted using split plot design divided by two factors. The first factor was mulch (M) as the main plot, consisting of M₀ (without mulch), M₁ (rice straw mulch), M₂ (silver black plastic mulch) and the second factor was different frequency of fertilizer application, consisting of C₁ (at once, all doses at 0 days after planting), C₂ (twice, ½ dose at 0 day after planting + ½ dose at 90 days after planting) and C₃ (three times, 1/3 dose at 0 day after planting + 1/3 dose at 60 days after planting + 1/3 dose at 120 days after planting). Each treatment unit was repeated three times. The observational data were analyzed with ANOVA and further tested with LSD at probability level 5%.

Materials were taken from patchouli shoots with a length of 15-20 cm which is grown in a polybag containing a mixture of soil and manure. Seedlings that have buds and leaves are planted 60 cm x 40 cm apart in a plot of 2.5 m x 2.5 m. Each hole is planted with one patchouli seedling. Rice straw mulch is applied after planting, while black silver plastic mulch is applied before planting. Watering is done until 30 days after planting, after that the plants would be obtaining water only from rainfall. Weed control was done conventionally, while plant pest was controlled using chemicals.

Soil temperature was observed every 15 days, when the plants were 30-180 days old at 7.30 am, 1.30 pm and 5.30 pm. Soil moisture content was observed every 7 days at 7.30 am and 5.30 pm. Plant growth was observed at 60, 120 and 180 days after planting, included plant height, number of branches, number of leaves, root length, leaf area, root dry weight, plant dry weight, leaf dry weight. Observation of oil production was carried out by distillation of dried patchouli leaves after harvest, while the analysis of proline content in leaf shoots was carried out using method by (Bates et al., 1973) at 180 days after planting.

RESULTS AND DISCUSSION
The results of ANOVA revealed that the application of mulch had a significant effect on soil temperature, soil moisture content, number of branches, number of leaves, leaf area and root dry weight. Frequency of fertilizer application also significantly affected the plant dry weight, oil production and proline content. There was no interaction between application of mulch and frequency of fertilizer application at all observed parameters (Table 1 and Fig 1).

The unmulched soils (M₀) possessed higher soil temperatures in the afternoon and evening than mulched soils (M₁ and M₂), while they demonstrated lower temperatures in the morning. There were significant differences between mulched and unmulched soils at various depths (Fig 1). The high soil temperature in untreated soils is caused by solar radiation that is easily absorbed by the soil, resulted in rapid increase of soil temperature. Awal and Sultana (2011) stated that the soil temperature in soil without mulch application increased rapidly resulting from solar radiation enters the soil surface easily. Mulching could have benefits on soils by inhibiting the increase in soil temperature, maintains higher soil moisture content and has an albedo effect (Buerkert et al., 2000; Awal and Sultana, 2011). The application of mulch modifies microclimates, thereby creating an energy balance in the soil and producing plants that are healthy, strong and resistant to plant disease pets (Bhardwaj, 2013).

Rice straw mulch has the ability absorbing water, facilitating infiltration, preventing evaporation and maintaining water moisture which resulted in lower soil temperature. The application of natural mulch with rice straw, which contains higher specific heat than plastic synthetic materials or soil without mulch, demonstrated better inhibition of soil temperature. Mulch application in the morning and evening had a good effect on moisture content of soils. Rice straw mulch had better ability in maintaining higher moisture content, followed by silver black plastic mulch and without mulch. Moisture content in the soils was lower in the afternoon. Mulching resulted in lower soil moisture fluctuations (1.62% by rice straw mulch, 3.29% by silver black plastic mulch and 14.31% in unmulched soils) (Fig 1). The application of organic mulches have good benefits in soils by maintaining higher soil moisture content compared to soils without mulches in cauliflower plantation (Kumar et al., 2019). Mulching affects the vegetative growth of patchouli plants better. This is indicated by the number of leaves and leaf area treated with rice straw mulch and root dry weight treated with black silver plastic mulch (Table 1). The higher growth of vegetative parts in plants was influenced by the ability of plants to absorb the nutrients, resulted from high humidity due to mulching. This high humidity attributed to high absorption of nutrients in soil which resulted in better plant growth compared to plants grown in unmulched soils (Marwain and Ray, 2019).

Rice straw mulch significantly affected vegetative growth of patchouli plants because it facilitated infiltration, better aeration (Kusumastuti, 2013), increased nutrient availability through weathering (Lu et al., 2009; Kumar et al., 2019), maintaining more stable soil temperatures with high minimum soil temperatures and lower maximum soil temperatures in wheat and mustard (Chen et al., 2007; Awal and Sultana, 2011), inhibiting evaporation in wheat plants (Balwinder et al., 2011), maintaining soil moisture in cereal, groundnut, wheat and potato plantations (Buerkert et al., 2000; Ghosh et al., 2006; Ramakrishna et al., 2006; Chakraborty et al., 2008; Hamdani, 2009). The increasing of plant growth was influenced by micro climate modification. Research of Abhiyakti et al. (2016) revealed that the
Table 1: Results of parameters observed.

| Parameters                  | Mulch (M) | Frequency of fertilizer application (C) |
|-----------------------------|-----------|----------------------------------------|
|                             | 60 DAP    | 120 DAP                                |
|                             | 180 DAP   | 60 DAP                                |
|                             | 120 DAP   | 180 DAP                                |
| Plant height                | 0.17 ns   | 1.55 ns                                |
|                             | 3.44 ns   | 2.67 ns                                |
|                             | 1.39 ns   | 1.81 ns                                |
| Number of branches          | 7.35 *    | 6.29 ns                                |
|                             | 1.16 ns   | 0.63 ns                                |
|                             | 0.12 ns   | 1.16 ns                                |
| Number of leaves            | 14.09 *   | 4.46 ns                                |
|                             | 2.13 ns   | 2.58 ns                                |
|                             | 0.74 ns   | 2.46 ns                                |
| Root length                 | 3.63 ns   | 5.14 ns                                |
|                             | 2.96 ns   | 1.83 ns                                |
|                             | 0.37 ns   | 2.95 ns                                |
| Leaf area                   | 10.98 *   | 67.22 **                               |
|                             | 64.02 **  | 0.07 ns                                |
|                             | 0.74 ns   | 0.50 ns                                |
| Root dry weight             | 9.69 *    | 3.03 ns                                |
|                             | 0.40 ns   | 0.03 ns                                |
|                             | 0.96 ns   | 2.86 ns                                |
| Plant dry weight            | 2.20 ns   | 4.08 ns                                |
|                             | 0.17 ns   | 0.18 ns                                |
|                             | 1.60 ns   | 4.54 *                                 |
| Dry weight of leaves        | 0.35 ns   | 4.79 ns                                |
|                             | 3.19 ns   | 0.68 ns                                |
|                             | 2.79 ns   | 2.53 ns                                |
| Oil production              | - - -     | 4.85 ns                                |
|                             | - - -     | - -                                    |
|                             | - - -     | 6.88 *                                 |
| Proline accumulation        | - - -     | - -                                    |
|                             | - - -     | - -                                    |
|                             | - - -     | - -                                    |

ns = non significant, * = significant ** = significant.

Fig 1: Soil temperature (°C) and soil moisture (%) with mulch treatment.

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Application of mulch provided benefits to the modified microclimate which resulted in increasing of tomato growth. The use of silver black plastic mulch produced a greater oil of 5.53 grams and the accumulation of proline of 17.36 µ mol/g, but it did not show any significant difference between the treatments (Table 2). This is happened due to the plants did not experience severe drought stress so that the accumulation of proline at each treatment was similar. Proline accumulation in coriander plants will increase if the plants experience a drastic drought stress (Farahani et al., 2008). At optimum water conditions, proline accumulation was still relatively similar, but in soils experiencing drought stress, the accumulation of proline could be greater in *corn and Indigofera zollingeriana* plants (Etendi, 2009; Lestari et al., 2019). Mafakheri et al. (2010) stated that proline is an organic compound that accumulates a lot in plants when experiencing drought stress. The accumulation of these proline compounds can reduce the negative effects of drought stress in quinoa plants in Egypt (Elewa et al., 2017).

The results illustrated that the application of fertilizer twice (C2) exhibited higher oil production, while increased dry weight of plants and higher accumulation of proline content were demonstrated by plants which were applied fertilizer for three times (C3) (Table 2). The application of fertilizers at 90 days after planting has provided adequate nutrition for patchouli plants by applying fertilizer in appropriate time, which is increasing production of patchouli oil. The balance of nutrients is needed to support the growth and production of patchouli (Singh and Guleria, 2012; Syakir and Gusmani, 2012). Fertilization with the proper dose and in the right time is an effort to support patchouli plants to grow better and produce high number of leaves which resulted in high yield of patchouli oil (Wahyuni et al., 2011).

The highest accumulation of proline was exhibited by plants with the highest frequency of fertilizer application (three times every two months/C3) which attributed to the reduction of dose at each step of application. This has affected the balance and amount of nutrients absorbed by the plants during its growth. Atanasova (2008) revealed that the increase of proline could be an indicator of unbalanced nutrients in plants. One time fertilizer application has reduced plant stress, which is shown by lower accumulation of proline (12.90 µ mol g). It was significantly different from the result of plants which have been applied fertilizer for three time. Jawad et al. (2015) found that the addition of nutrients to wheat plants with drought stress could reduce plant stress and reduce the accumulation of proline in plants. Surprisingly, Maheswari et al. (2008) confirmed the dissimilar
Table 2: The results of growth and yield of patchouli treated with mulches and fertilizer.

| Parameters observed | NB at 60 DAP | NL at 60 DAP | LA at 60 DAP | LA at 120 DAP | LA at 180 DAP | RDW at 60 DAP | PDW at 180 DAP | OP | Proline |
|---------------------|--------------|--------------|--------------|--------------|--------------|--------------|---------------|-----|---------|
| Mulch (M) | | | | | | | | | |
| M_1 | 8.77 a | 64.00 b | 46.06 b | 34.71 b | 40.39 b | 0.79 b | 43.21 a | 5.24 a | 14.36 a |
| M_2 | 8.37 a | 78.77 a | 62.62 a | 57.62 a | 60.07 a | 0.53 b | 40.08 a | 4.46 a | 12.70 a |
| M_3 | 6.44 b | 64.93 b | 58.29 a | 56.23 a | 57.27 a | 1.68 a | 35.03 a | 5.53 a | 17.36 a |
| LSD 0.05 | * | * | * | * | * | * | ns | ns | ns |
| Frequency of fertilizer application (C) | | | | | | | | | |
| C_1 | 8.55 a | 60.96 a | 54.67 a | 51.23 a | 52.95 a | 0.93 a | 29.74 b | 3.99 b | 12.70 b |
| C_2 | 7.74 a | 77.33 a | 56.10 a | 46.03 a | 51.06 a | 1.00 a | 36.82 ab | 6.03 a | 13.25 b |
| C_3 | 7.29 a | 69.29 a | 56.11 a | 51.30 a | 53.71 a | 1.07 a | 51.77 a | 5.21 ab | 18.61 a |
| LSD 0.05 | ns | ns | ns | ns | ns | ns | * | * | * |

Note: NB = Number of branches, NL = Number of leaves, LA = Leaf Area, RDW = Root Dry Weight at, PDW = Plant Dry Weight, OP = Oil production.

The means followed by the same letters in the same columns did not significantly differ as determined by LSD at probability level 5%.

result in rice plants. They investigated that the increase of nutrients (nitrogen) in rice contributed to the increase of proline content. It was due to nitrogen is a constituent of proline, an amino acid. The increase of proline and amino acid content was resulted from higher N nutrient uptakes by plants, especially by plants grown in rainfed land such as olive plants in Himachal Pradesh India (Kumar and Sharma, 2016).

CONCLUSION

The application of rice straw mulch has reduced the effects of drought stress by suppressing the fluctuations in soil temperature and maintaining soil moisture content and also increasing the patchouli growth. The application of fertilizer twice to the plants significantly increased the oil production compared to plants with fertilizer applied once, while application of fertilizer for three times contributed to the increase of plant dry weight and proline accumulation. The application of fertilizer once could reduce the impact of drought stress which is characterized by low accumulation of proline on patchouli leaves.

Compliance with ethical standards

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The authors declare that they have no conflict of interest.

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