INTRODUCTION

Clove (Syzygium aromaticum L.), which produce essential oil for pice or traditional medicine, is one of the most important cash crops in Indonesia (Baietto, 2014; Danthu et al., 2020; Nurdjannah & Bermawie, 2012; World Bank Group, 2017). The major component of the essential oil is eugenol, which is mainly contained in bud and leaves (Bhuiyan, 2012; Bhuiyan, Begum, Nandi, & Akter, 2010; Gaylor et al., 2014; Kamatou, Vermaak, & Viljen, 2012; Kaur, Kaushal, & Rani, 2019; Pino, Marbot, Agüero, & Fuentes, 2001.). Leaf letter collection is the most important and frequent harvesting method (Alighiri, Eden, Cahyono, & Supardi, 2018; Gaylor et al., 2014).

Recently, clove plantation was mostly established in monoculture systems for production efficiency (Arimalala et al., 2019; Danthu et al., 2014; Martin, 1991). The frequent litter collection associated with intensive soil surface disturbances in a whole area of the crop field, however, is causing deterioration plant species diversity of ground vegetation. Therefore, mixed culture by intercropping of clove with other crop species is suggested as an alternative method which would reduce the impact of frequent litter collection on biodiversity of ground vegetation for sustainable management of clove production.

Though the mixed culture is expected to have ecological advantages in terms of biodiversity conservation, at the same time, it will also bring inter-species competition of resources such as light or soil water (Jensen, 1993; Jose, Gillespie, & Pallardy, 2004; Leštianska, Fleischer Jr, Fleischer, Merganičová, & Střelcová, 2020; Liu, Kuchma, & Krutovsky, 2018; Prasad et al., 2010; Pretzsch, 2014). Thus, it is quite important to know the

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ABSTRACT

The growth responses of clove seedling to light and soil water regimes were studied by a field experiment with shading and irrigation treatments to clarify their growth traits at the early stage of plantation establishment. Eighteen-month-old clove seedlings were subjected to twelve treatments, that is, 3 shading treatments (0%, 60% and 80% shading) × 4 watering treatments (1.0, 0.75, 0.5 and 0.25 l/m²/day), for ca. 6 months. Increment ratio of seedling height (IH), number of newly created buds (NB) during the experimental period and dry mass per plant at the end of the experiment (leaves: LM, stem and branch: SM, root: RM and total plant: TM) were compared among the treatment. The growth of clove seedlings (height < 150 cm) was generally more susceptible to water stress than to low light availability. The growth retardation by water shortage was observed in all the examined parameters except for SM. The effect of the shading treatment appeared to be limited; however, LM and NB showed significantly lower values under the heavy shade (80% shading). We concluded that dense planting of clove seedling with other competitive crops should be avoided to ensure the fast growth of clove seedlings at the establishment stage.

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responses of clove plants to the limited light and soil water availabilities in order to grow them successfully under the mixed culture system. In particular, the early stage of the establishment after planting of juvenile plants is critical for clove planted in the mixed culture because they may suffer from severer competition compared to that at the later stages when the plant grow taller than other (mainly annual) crop species. However, the growth responses of clove to light and soil water regimes at the juvenile stage have not been well documented.

In this study, we aimed to explore the growth responses of juvenile plants of clove to light and soil water regimes. We conducted a field experiment with shading and irrigation treatments, and discussed the growth traits of clove at the early stage of their establishment.

MATERIALS AND METHODS

Study Site and Experimental Design

The experiment was conducted in a private nursery located in Kesamben Distric, Blitar Regency, East Java, Indonesia (190 m asl). The site is situated under the tropical monsoon climate with a dry season from May to October and a rainy season from November to April. Annual average temperature and annual rainfall are 25.7°C and 2060 mm, respectively.

Thirty-six plots with each size of 2.5 m × 2 m were constructed. Twenty clove seedlings (18 months old, ca. 120 cm in average height) were planted in each plot on February 7, 2017. After one year planting (July 4, 2018), three levels of shading treatments using by shading net [0% (S0), 60% (S60) and 80% (S100)] and four levels of watering treatments [1.0 l/m²/day (W100), 0.75 l/m²/day (W75), 0.5 l/m²/day (W50) and 0.25 l/m²/day (W25)] were applied in combination with three replication based on split-plot design. The experiment was carried out for 6 months until January 2, 2019.

Measurements

During the experiment (starting from January 2, 2018), the height of the seedlings was measured every month except on October. Number of buds were also counted at the beginning and the end of the treatments to determine the number of newly created buds. At the end of the experiment, all the seedlings were harvested to determine the dry weight of leaves, stems and branches, and roots. Dry weight of each organ was measured separately after the respected plant organs were dried up using dry oven at the constant temperature of 72°C for 48 h.

Data Analyses

To compare the height of seedlings that had different initial sizes, the increment ratio of seedling height ($IH$, m/m) was calculated by dividing the final size (January 2, 2018) by the initial value at the beginning of the treatments (July 4, 2018). Number of the newly created buds per seedling ($NB$) were also calculated as the differentials of the final and initial numbers of the buds.

All the parameter, that is, the increment ratio of seedling height ($IH$), the number of the newly created buds ($NB$), the leaf dry mass per plants ($LM$), the stem and branch dry mass per plant ($SM$), the roots dry mass per plant ($RM$), and the total dry mass per plant ($TM$) were compared by Two-way ANOVA ($p<0.05$). The treatment means were compared by using the Tukey’s $\omega$-procedure.

RESULTS AND DISCUSSION

Height Growth

Seeding height in treatments of W50 and W25 tended to have slower growth compared to the adequately watered treatments (W100 and W75) throughout the experimental period. The variation of seedling height in each measurement was quite high (Fig. 1). Since the initial height was different among the treatments, growth in height was determined by the comparison of increment ratio of seedling height ($IH$). The watering treatments had significant effects on $IH$ ($p<0.001$). While in the shading treatments and their interaction, the effects were not significant ($p>0.05$) (Table 1). The treatment W100 showed the highest value of $IH$, followed by W75 which has significantly higher values than W50 and W25 (Fig. 2A).

Number of Buds

Number of the newly developed buds ($NB$) was affected by both watering and the shading treatments as well as their interaction ($p<0.001$) (Table 1). $NB$ demonstrated similar results as in $IH$, in which W100 showed the highest value followed by W75 (Fig. 3A). For the shading treatments, S80 had showed the lowest $NB$, while there was no significant difference between S0 and S60 (Fig. 3B). The effects of the watering treatments were obvious under no shading (S0), in that significantly higher NB were detected under W100 and W75 than those under W50 and W25 (Fig. 4). $NB$ in the severest
watering treatment (W4) between full light (S0) and moderate shade (S60) was significantly different, but the difference was negligible (3.1 and 4.0 for S0 and S60, respectively) (Fig. 4).

**Dry Mass**

The total plant dry mass (TM) was affected by both the watering and the shading treatments ($p<0.001$), but not by their interaction (Table 1). The effect was more apparent at the watering treatments. TM showed a decreasing trend along the decrease of the watering rates, with significantly higher in W100 and W75 than in W50 and W25 (Fig. 5A). In contrast, the shading treatments did show a simple decrease. S60 had significantly lower TM than the other two treatments, while S0 and S80 had insignificant difference in TM values (Fig. 5B).

Table 1. Summary of Two-way ANOVA conducted for the watering and the shading treatments on growth parameters of clove seedlings

| Variables                      | Increment ratio of seedling height ($IH$) | Number of the newly created buds ($NB$) | Leaf dry mass ($LM$) | Root dry mass ($RM$) | Stem and branch dry mass ($SM$) | Total dry mass ($TM$) |
|--------------------------------|------------------------------------------|-----------------------------------------|----------------------|----------------------|-------------------------------|----------------------|
| Watering                      | 34.6***                                  | 127.6***                                | 3.78*                | 44.10***             | 2.77                          | 16.4***               |
| Shading                       | 2.06                                     | 108.4***                                | 6.60**               | 28.4***              | 5.06*                         | 15.3***               |
| Watering x Shading            | 2.20                                     | 7.34***                                 | 0.88                 | 4.87**               | 0.44                          | 0.75                 |

Remarks: Figures denote F-value; *, ** and *** indicate the significance level ($p<0.05$, $p<0.01$ and $p<0.001$, respectively).

![Fig. 1](image1.png)

Fig. 1. Changes of seedling height during the experimental periods. Vertical bars indicate standard errors.
Fig. 2. The increment ratio of seedling height ($IH$) under the different watering and shading treatments. A) comparison by the watering treatment, B) comparison by the Shading treatment. Different letters indicate significant differences between treatments ($p<0.05$).

Fig. 3. Number of the newly created buds ($NB$) under the different watering and shading treatments. A) comparison by the watering treatment, B) comparison by the shading treatment. Different letters indicate significant differences between treatments ($p<0.05$).
Fig. 4. Comparison of number of the newly created buds (NB) under the 12 treatments with different watering and shading levels. Different letters indicate significant differences ($p < 0.05$) between treatments according to Tukey's test. Lowercase and uppercase letters denote the comparison among the shading and watering treatments, respectively.

Fig. 5. Total dry mass per plant ($TM$) under the different watering and shading treatments. A) comparison by the watering treatment, B) comparison by the shading treatment. Different letters indicate significant differences between treatments ($p < 0.05$).
The leaf mass \( (\text{LM}) \) was also affected by the main effects watering and the shading treatments \((p<0.05 \text{ and } p<0.01, \text{ respectively})\) but not their interaction effect (Table 1). The watering treatment demonstrated a slight decrease along the watering rate with a significant difference of \( \text{LM} \) between W100 and W25 (Fig. 6A). However, differing from the response of \( \text{TM} \), a slight decrease of \( \text{LM} \) was also observed on the shading treatments, in which, significantly lower \( \text{LM} \) in S80 was observed than those in S0 and S60 (Fig. 6B).

The stem and branch mass \( (\text{SM}) \) was less affected by the treatments compared with the other parameters; only the shading treatment had a significant effect on \( \text{SM} \) (Table 1, Fig. 7), and the difference was only detected between S0 and S60 (Fig. 7B).

The root mass \( (\text{RM}) \) showed similar trends as \( \text{TM} \). A gradual \( \text{RM} \) decreases along the decrease of the watering rate was observed higher \( \text{RM} \) was observed at W100 than other three treatments with W50 and W25 as the lowest values (Fig. 8A). The effects of the shading treatment were also similar to that on \( \text{TM} \); a significantly lower \( \text{RM} \) was observed in S60 (Fig. 8B).

**Growth Trait of Young Clove Seedlings**

The study revealed that at juvenile stage (plant height < 150 cm), the growth performance was more sensitive to soil moisture stress induced by low watering rates than to low light condition due to shading treatment (Table 1). The findings were in accordance with Martin & Poultney (1992) that the mortality of clove seedling in the field was highest during the two dry seasons of the first year after planting, while clove can grow in semi-shade habitats (light woodlands).

Growth inhibition by water shortage was apparent in the seedling height. The increment ratio of seedling height \( (\text{IH}) \) was severely retarded in the treatment of W50 and W25 (Fig. 2A). Similar trends of growth reduction by water shortage were clearly observed in most of the examined parameters (number of the newly developed buds \( (\text{NB}) \) (Fig. 3A), leaf mass \( (\text{LM}) \) (Fig. 6A), root mass \( (\text{RM}) \) (Fig. 8A) and total plant mass \( (\text{TM}) \) (Fig. 5A). Although the difference in stem and branch mass \( (\text{SM}) \) among watering treatments were not significant, the plants given with less water (W50 and W25) also tended to have lower SM than W100 and W75 (Fig. 7A).

In contrast, the effect of the shading treatment appeared to be limited compared to that of the watering treatment. In \( \text{IH} \), the value differences among the treated plants were negligible (Fig. 2B). While, \( \text{SM} \) and \( \text{RM} \) as well as \( \text{TM} \) also did not show clear trends related with shading rate (Fig. 7B, Fig. 8B and Fig. 5B, respectively). We assumed that the water availability gave more effects on growth performance than shading rate of clove seedlings in terms of their height and biomass increment.

![Fig. 6. Leaf dry mass per plant \( (\text{LM}) \) under the different watering and shading treatments. A) comparison by the watering treatment, B) comparison by the shading treatment. Different letters indicate significant differences between treatments \((p<0.05)\).](image)
Among the examined growth parameters, \( LM \) showed a significant decline under the highest shading rate (S80) compared to S0 and S60 (Fig. 6B). Since the leaves are the major plant parts for the clove oil yield (Alighiri, Eden, Cahyono, & Supardi, 2018; Bhuiyan, Begum, Nandi, & Akter, 2010; Bustaman, 2011; Jirovetz et al., 2006; Kamatou, Vermaak, & Viljoen, 2012), the excessively low light can reduce the clove oil per plant when the leaves were harvested from the juvenile stage, even though the increment of height and other organs were not affected.

In addition, NB also showed a significantly lower value under heavy shade (S80) up to half of those under S0 and S60 (Fig. 6B). This might be due to the decrease of the number of leaves per plants which might result in the decrease of axillary buds. The less number of leaves and buds could be resulted from the inhibited stem and branch growth. However, since the \( SM \) in S80 did not differ from those in S0 and S60 (Fig. 7B), the decline of NB and \( LM \) are thought to be due to the fewer developed buds per unit stem/branch mass rather than the chances of elongation or branching rates.
The buds of clove, as well as the leaves, are also important plant parts for clove oil yield (Frohlich et al., 2019; Gaylor et al., 2014; Guan, Li, Yan, Tang, & Quan, 2007; Pino, Marbot, Agüero, & Fuentes, 2001). The previous study has reported that buds can produce a high-quality essential oil in terms of multiple useful chemicals other than eugenol (Alma, Ertaş, Nitz, & Kollmannsberger, 2007; El Ghallab et al., 2020; Lee & Shibamoto, 2001; Moemenbellah-Fard, Abdollahi, Ghanbariasad, & Osanloo, 2020; Moyler, 1993), though the dry mass of buds per plant is smaller than leaves. Thus, the decline of NB under a heavy shade should be considered if the clove oil yield from buds was desired at the juvenile stage of their establishment. Further, number of buds would potentially influence the further development of the individual tree through branching. Therefore, long-term heavy shaded condition should be avoided to ensure the better subsequent growth in terms of vigorous branching and leave formation, although the short-term effect of the heavy shade on height and total biomass increment was not clearly found in this study.

Implication to Clove Production in Mixed Culture Systems

Based on the study, it is suggested that dense planting of clove seedling with other competitive crops such as coconut, banana, nutmeg and cinnamon (Arimalala et al., 2019; Pandey, Lata, Venkatesh, & Medhi, 2006), which may lead severe inter-species competition for soil water, should be avoided in order to ensure the fast growth of clove seedling at the establishment stage. In particular, on the sites where water stress easily occurs due to unfavorable topographic and soil conditions, excessive root competition is desired to be avoided. The dense canopy is also known to reduce rainwater input into the soil due to the canopy interception and evaporation loss of rainwater (Calder, 1996; Murakami, 2006). Thus, sparse planting would be preferable for establishing the mixed-culture stands to reduce the root competition and the interception loss of rainwater.

The moderate shade has been reported to alleviate the negative impacts of severe water stress in some tropical crop species such as Robusta coffee (Coffea canephora) (DaMatta & Cochicho Ramalho, 2006). In cocoa, low-density shade trees positively affected their biomass (Isaac, Timmer, & Quashie-Sam, 2007; Isaac, Adjei, Issaka, & Timmer, 2011). On the other hand, for Arabica coffee (Coffea arabica), Cavatte et al. (2012) reported that shading did not alleviate the negative impacts of drought on the coffee tree. In the present study, the interaction effect of the watering and the shading treatments was not clear (Table 1, Fig. 4 & Fig. 9 to 13). Thus, the role of the moderate shading (S60: 60%, in the present study) in terms of alleviating the water stress is expected to be less effective for clove seedling’s as reported in the case of Arabica coffee.

![Fig. 9. Comparison of the increment ratio of seedling height (IH) under the 12 treatments with different watering and shading levels.](image-url)
Fig. 10. Comparison of total dry mass per plant (TM) under the 12 treatments with different watering and shading levels.

Fig. 11. Comparison of leaf dry mass per plant (LM) under the 12 treatments with different watering and shading levels.

Fig. 12. Comparison of stem and branch dry mass per plant (SM) under the 12 treatments with different watering and shading levels.
CONCLUSION

We explored the growth responses of clove seedling to light and soil water regimes by a field experiment through shading and irrigation treatments to clarify their growth traits at the early stage of plantation establishment. We concluded that dense planting of clove seedling with other competitive crops should be avoided to insure the fast growth of clove seedlings at the establishment stage.

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