宮崎大学大学院

博士学位論文

Biodiversity conservation and clove oil productivity of mixed-culture systems in Indonesia

（インドネシアの混合栽培システムにおける生物多様性保全機能とクローブオイル生産性）

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ABSTRACT

Clove (*Syzygium aromaticum* L.) is an important cash-crop tree species in Indonesia as the producer of the essential oil represented by eugenol. Clove trees have been currently cultivated mostly in standard monoculture and partly in a traditional mixed-culture system as a kind of agroforestry with various trees and/or crops growing in a same site. Recently, agricultural production systems, including that for clove production, requires to play a role of conserving biodiversity as well as production efficiency for their sustainability. The present study aimed to develop the sustainable and efficient systems for clove and clove oil production from the following three aspects; 1) clove production systems desirable for biodiversity conservation with a special reference to the possible advantage of mixed-culture systems for conserving plant species diversity, 2) appropriate physical environments (light and water regimes) for clove seedlings required for successful establishment of mixed-culture systems, and 3) the optimal site conditions that assure the high eugenol yield for long-term production.

Firstly, I investigated the plant species occurrence in a typical mixed-culture (MIX) stand, and compared it with that in a clove monoculture (MON) stand in East Java, Indonesia, in order to examine the advantage of MIX in conserving plant species diversity. The occurrence of vascular plants in the understory were surveyed in the plots established for both MIX and MON with the microsite conditions. MIX and MON included 40 and 17 species, respectively, indicating far greater species richness in MIX with a diverse life form composition and large numbers of woodland and/or native species. The α- and the ß-diversities were higher in MIX than MON. These results suggested that the superiority of MIX for conserving plant species diversity compared to MON. Comparisons of microsite conditions revealed that the human disturbances to the soil surface associated with frequent clove litter collection from the whole stand reduced the plant species richness by inhibiting plant establishment and cancelling the positive effects of the variability in physical environment in MON. I concluded that the higher species richness of MIX was due to alleviating the effects of litter-collection disturbances, which facilitated the effects of the heterogeneous physical environment within the stand.

Secondly, I explored the growth responses of clove seedling to light and soil water regimes 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) x 4 watering treatments (1.0, 0.75, 0.5 and 0.25 liter/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 treatments. The results revealed that the growth of clove seedlings was generally more susceptible to water stress than to low light availability in particular for *IH*, *NB* and *LM*. From these results, I 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.

Third, I examined the influences of the same treatments in the second study on eugenol productivity to provide the basic information for the suitable site conditions for long-term essential oil production. The total leaf mass per tree (*LM*), eugenol content per unit leaf mass (*EL*) and the eugenol yield per tree (*EY*) were measured and compared between treatments of this study. The soil moisture deficit and the low light availability had negative and positive effects on the eugenol yield per tree, respectively. These results suggested that the relatively dryer site condition where moderate water stress is likely to occur is more suitable for planting clove trees from the aspect of the for long-term high productivity of eugenol, and that the high tree density which may results in a severe competition and a heavy mutual shading among clove trees should be avoided for maintaining high productivity for a long term.

These findings are thought to be useful in developing appropriate mixed-culture systems that can balance biodiversity conservation with clove oil productivity.

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Chapter 1. Introduction

1.1. Background: needs for developing appropriate and advanced systems for clove production

Clove (*Syzygium aromaticum* L.) is one of the important cash-crop trees in Indonesia as the producer of the essential oil represented by eugenol (Bhuiyan 2012; Kamatou et al. 2012; Nazrul et al. 2010; Pino et al. 2001; Razafimamonjison et al. 2014) used for medicines or other usages (Baietto 2014; Nurdjannah & Bermawie 2012; World Bank Group 2018). Clove trees have been currently cultivated in several ways such as standard monoculture or a traditional mixed-culture system as a kind of agroforestry with various trees and/or crops growing in a same site.

Recently, agricultural production systems, including that for clove production, have required to play a role for conserving biodiversity for the sustainability. There are several important points to be considered for developing efficient and ecologically desirable systems for clove production. First, monocultures for agricultural and timber production are efficient system in terms of the short-term productivity, but also have often suffer from the criticism because of their negative impacts on biodiversity and ecosystem services other than the provisioning service. Thus, it is needed to evaluate the functions of agroforestry or mixed-culture systems for clove production as the alternative to monocultures from the view point of biodiversity conservation. Second, in agroforestry or mixed cultures, evaluation of interactions such as competitions among planted crop species are key factors for establishing efficient production fields successfully. For this, it is needed to understand the growth response of clove trees to the limited resource availabilities under competitions such as soil water deficit due to root competition or low light regime by suppression from other plants. Third, as well as tree growth, the response of clove oil content and yield to the physical environmental factors is also crucial for assuring efficient and sustainable clove oil production in terms of the appropriate site selection for long-term high productivity.

1.2. Research history and current issues of clove and clove oil production

1.2.1. Biodiversity conservation in clove production or other agroforestry sites

Monoculture of tree plantations have negative impacts on biodiversity at stand (Felton et al. 2010) and also landscape levels compared to mixed culture conditions (Dey & Chaudhuri 2014; Iezzi et al. 2018). Monoculture system can eliminate all such functions due to the practice of only planting or rearing one type of crop (Zhang & Stanturf 2008). As a result, species loss due monoculture system against other drivers of climate change (Pawson et al. 2013) and environmental change (Naeem et al. 1996). Thus, there is no varieties of plant and range of insect species due to the lack of crop diversity that promotes insect biodiversity (Saunders 2016). It also means there are no naturally provide nutrients to the soil that can improve soil microorganism (Bini et al. 2013).

In contrast, agroforestry is a potential means to conserve biodiversity while continuing agricultural and forestry production (Wicaksono et al. 2011; Umar et al. 2019). Monocultures that often form densely closed canopies tend to eliminate plant species in the understory by heavy shade (Bekessy & Wintle 2008). In contrast, agroforestry, in which agricultural crops are grown beneath the canopy of planted trees, can provide more favorable microenvironments for the growth of many plants compared to monocultures (Jose 2012; Utomo et al. 2016; Umar et al. 2019). Similarly, mixed-culture systems can provide various habitats for plant species of different life-history traits by forming heterogeneous environments in the understory, such as different light or soil moisture regimes, due to the diverse canopy structures and root systems compared to those of monocultures. In addition to the physical environment in terms of resource availability, soil surface disturbance can also affect the ground vegetation in clove production fields, since fallen litter from clove trees is frequently collected for clove oil production (Pino et al. 2001; Kamatou et al. 2012; Nurdjannah & Bermawie 2012; Alighiri et al. 2018). Therefore, heterogeneity in soil surface disturbances associated with clove litter collection in mixed cultures can contribute to plant species diversity of ground vegetation compared to the presumably more uniform disturbances in clove monocultures. However, there is little information available for whether and how these physical environment and soil surface disturbance heterogeneities influence plant species diversity in ground vegetation.

1.2.2. Growth of clove trees

Clove trees is strictly a tropical plant and requires a warm humid climate having a temperature of 20 to 30^{0}C. Humid atmospheric condition and a well distributed annual rainfall of 1500 to 2500 mm are essential. It thrives well in all elevations ranging from the sea level up to an altitude of 1500 meters and also in places proximal to and away from sea. Deep black loam soil with high humus content found in the forest region is best suited for clove cultivation. It grows satisfactorily on laterite soils, clay loams and rich black soils having good drainage. Clove is propagated through seed, which is called mother clove. The nurseries are usually maintained daily with suitable environment to ensure uniform stand, then to make establishment and growth of clove trees the organic manure i.e. vermicompost proved to be the best propagating medium (Thankamani et al. 1996). The seedlings are ready for transplanting in the field when they are 18 to 24 months old and using mulch on seedling to reduce mortality during the two dry seasons of the first year after planting (Martin & Poultney 1992).

For ordinary clove plantations, scrubs are cleared before rainy season and pits of 60 to 75 cm^3 are dug at a spacing of 6x6 meters. The pits are partially filled with topsoil. After planting clove seedling, weeding is important for reducing competition with weed for nitrogen; severe competition has an impact the juvenile decline condition and causes a serious loss in production (Martin & Dabek 2008). Harvest of clove flower usually starts from the seventh or eighth year after planting, and full bearing stage is attained after about 15 to 20 years. Clove often experiences irregular or alternate bearing tendency. The clove tree is noted to be sensitive to wind and rain, so the weather conditions influence the flower production (Miraji 2013).

Recently, clove production has been conducted in clove monoculture systems because of its high production efficiency (Arimalala et al. 2019; Danthu et al. 2017; Martin 1991); however, the frequent litter collection associated with intensive soil surface disturbances in a whole area of the crop field 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 field.

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; Liu et al. 2018; Prasad et al. 2010; Pretzsch 2014). Thus, it is quite important to know the 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.

1.2.3. Clove oil content and productivity

Clove oil is extracted from flowers buds, leaves and stem of clove trees (Alighiri et al. 2018; Razafimamonjison et al. 2014); ca. 4-5 ton/ha leaf mass is collected per years for yielding eugenol. The clove tree is noted to be sensitive to the weather condition. When a well-maintained, full-grown tree under favorable conditions may give 300-450 kg/ha of dried flower buds for average annual yields after 15th year.

At the harvesting stage after establishing the clove stands, not only the plant growth but also the productivity of the essential oil per unit plant organ becomes the important matter (Kurniawan et al. 2009) irrespective of the monoculture or the mixed-culture systems. Even though the planted clove trees produce large amount of leaves, the low content of the essential oil could reduce the oil productivity per tree. Thus, it is also quite important to know how the clove trees change their essential oil content per plant organ according to various conditions. There are several reports on the variation of the eugenol content in relation to the different plant materials, such as clove varieties (Walus et al. 2019), their geographic origin (Razafimamonjison et al. 2014) and the different plant parts (buds, leaves and stems) (Razafimamonjison et al. 2013).

In addition to the plant materials, the environmental effects on the eugenol content are also important because they can provide basic and useful information for the suitable site conditions for long-term efficient production after the stand establishment. As an example of the few studies dealing with the environmental effects, Kurniawan et al. (2009) compared the eugenol content between the lowland and the highland. However, the detailed information on the response of the eugenol content of clove leaves to the environmental factors such as light or water availability are still lacking, and the actual site selection for efficient clove production is mostly depending on the empirical knowledge of the farmers without scientific evidences.

1.3. Objective of this study

The present study aimed to develop the sustainable and efficient systems for clove and clove oil production from the following three aspects; 1) clove production systems desirable for biodiversity conservation with a special reference to the possible advantage of mixed-culture systems for conserving plant species diversity, 2) appropriate physical environments (light and water regimes) for clove seedlings required for successful establishment of mixed-culture systems, and 3) the optimal site conditions that assure the high eugenol yield for long-term production.

In Chapter 2, I compared species occurrence of vascular plants between a typical mixed-culture and a monoculture growing clove trees in East Java, Indonesia, and explored the effects of heterogeneity of physical environments and soil surface disturbances on the plant species diversity to examine the advantage of mixed-culture system for clove production in conserving plants species diversity against for clove monocultures.

In Chapter 3, I conducted a field experiment with shading and irrigation treatments, and investigated the growth responses of juvenile plants of clove to light and soil water regimes at the early stage of their establishment. Based on the results, I discussed the appropriate methods for establishing mixed-culture stands for clove production.

In Chapter 4, I investigated the eugenol yield of the leaf samples from the same experiments conducted in Chapter 3. I compared the leaf mass production per tree, eugenol content per unit leaf mass, and the total eugenol yield per tree. Based on the results, I discussed the effects of water stress and shading on the eugenol yield, as well as predominant factor (leaf mass or eugenol contents) determining high eugenol yield per tree, in order to provide the scientific information of the suitable conditions for long-term essential oil production by clove trees.

Chapter 2. Biodiversity Conservation in Clove Production or Other Agroforestry Sites

2.1. Objectives

The present study aimed to examined the advantages of a mixed-culture system for clove production for conserving plants species diversity compared to clove monocultures. I compared the species occurrence of vascular plants between a typical mixed-culture and a monoculture field of clove trees in East Java, Indonesia, and explored the effects of physical environmental and soil surface disturbance heterogeneity on plant species diversity.

2.2. Methods

2.2.1. Study site

The study was conducted in a mixed-culture stand (MIX) and a monoculture stand (MON) located in a lowland (80-150 m a.s.l.) in Watulimo District, Trenggalek Regency, East Java (Fig. 1A). The site is within a tropical monsoon climate with a dry season from May to October and a rainy season from November to April. The annual mean temperature and annual rainfall are 25.7 °C and 1981 mm, respectively. MIX and MON stands were established in 1997 and 1996, respectively (Table 1).

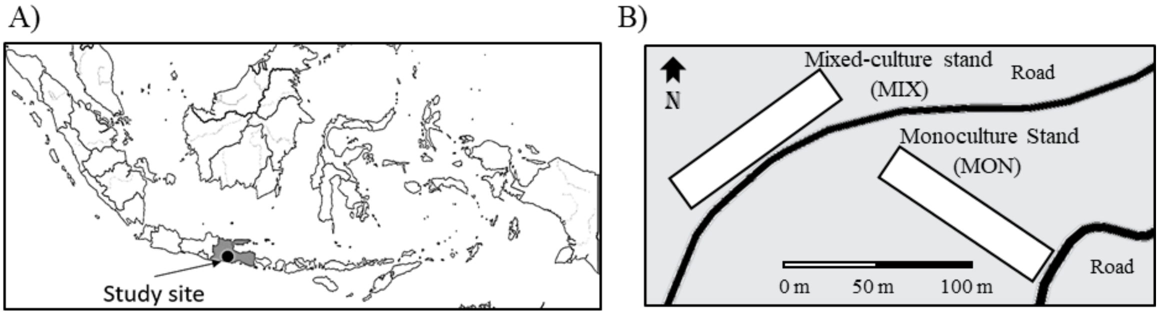


Figure 1. Location of A) the study site and B) the stands of the mixed-culture (MIX) and monoculture (MON)

Table 1. General description of the surveyed stands of the mixed culture (MIX) and the monoculture (MON)

	Mixed culture (MIX)	Monoculture (MON)
Planting year (clove trees)	1997	1996
Major crops [1)]	clove**, durian*, coconut***, dog fruits*, cassava*, ginger*, banana***	clove**
Spacing of planting crops	Not clear	ca. 6 m x 6 m
Weeding (brush cutting)	Once a year	Once a year
Fertilization	Once a year: organic and synthetic fertilizer for annual crops	Twice a year: organic and synthetic fertilizer
Clove tree density	48 trees/ha	240 trees/ha
Clove litter collection	Every 2-3 days	Every day
Clove litter productivity	1.1 ton/ha/year	4.6 ton/ha/year

[1)] Harvesting frequency: * once a year, ** twice a year, *** more than twice a year

[2)] Withered (naturally semi-dried) weight

In the MIX stand, more than 7 crop species including clove trees (48 trees/ha) were planted, while only clove was planted in MON with a density of 240 tree/ha (Fig. 2, Table 1). The two stands were ca.70 m distant from each other, situated in a mosaic landscape consisting of various kind of mixed cultures and monocultures with no adjacent natural forest patches. Both stands have been weeded once a year mainly by brush cutting, and fertilizer has been applied once and twice a year for MIX and MON, respectively. The fallen leaves (leaf litter) of clove trees are ordinarily collected every 2-3 days and every day in MIX and MON, respectively. Leaf litter collection are usually conducted by using a broom to gather the fallen litter beneath and around the canopy of clove trees in both stands (Fig. 3).

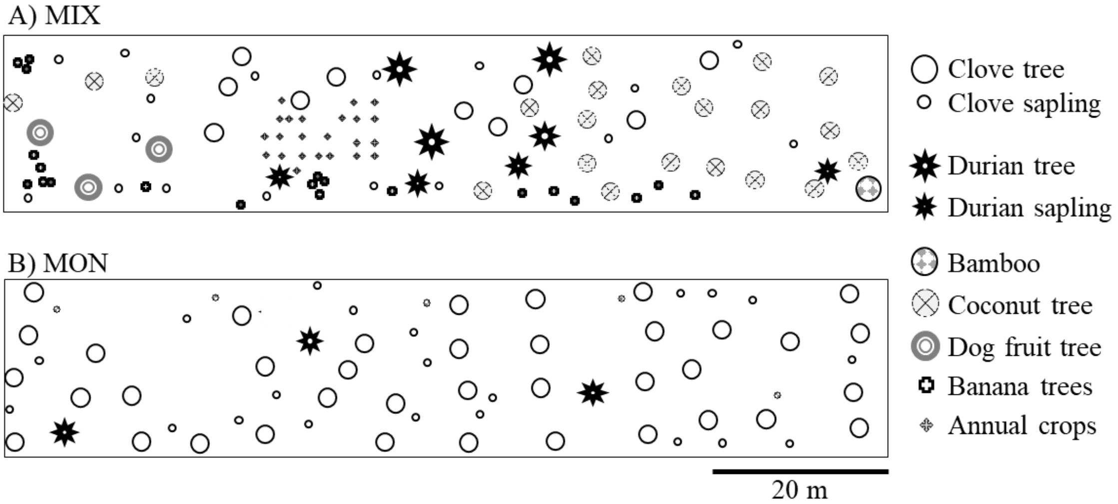


Figure 2. Positions of crops in A) mixed-culture (MIX) and B) monoculture (MON) stands

2.2.2. Field survey

The field survey was conducted in July 2018, the early dry season. Single 20 m × 100 m plots were established for each of the MIX and MON stands (Fig. 1B), and the distribution of crop plants and trees was mapped (Fig. 2). The plots were divided into 20 subplots (10 m × 10 m), respectively, and a 1 m × 1 m quadrat was systematically set at the corner of each subplot for the understory vegetation survey.

In each quadrat, all vascular plants identified in the understory were recorded, and the understory vegetation cover (*UVC*, %) and the litter cover (*LC*, %) on the ground surface of the quadrats were visually determined. Soil water content (*SWC*) and the sky factor (*SF*, the ratio of open canopy) were measured for each quadrat. Measurements of *SWC* were made using a TDR sensor (Hydrosense, Campbell) with 12-cm probes. Three or four measurements at each quadrat were averaged to determine the representative value. For measurement of SF, a hemispherical photograph was taken at the center of each quadrat (1 m above the ground surface) using a fish-eye lens (FC-E9, Nikon). SF was calculated by processing the hemispherical photographs.

Figure 3. A photograph showing the ordinary method of clove litter collection in the region

2.2.3. Analyses

In order to compare the plant species composition between MIX and MON, the plant species found in the quadrats were grouped in the following three ways: 1) five life forms (vine, shrub, grass, forb, and fern), 2) four kinds of their original habitats (woodland, wetland, grassland, and disturbed site), and 3) native or exotic species, based on descriptions in the literature (Setyawati et al. 2015; Rembold et al. 2017).

Species richness data obtained in each quadrat were evaluated by the additive portioning method (Wagner et al. 2000). The within-quadrat diversity (α-diversity) was calculated as the mean number of species per quadrat for each of MIX and MON. Then, the between-quadrat diversity (β-diversity) was obtained as the difference of the total number of species and the mean number of species per quadrat for each of MIX and MON.

In order to evaluate the heterogeneity of vegetation, the Sørensen-Dice index (*SDI*) was calculated for all combinations of 2 out of 20 quadrats in each stand, respectively, by the following equation:

$$SDI = 2a / (2a + b + c),$$

where parameters a, b, and c are the number of species common to both quadrats, the number of species unique to the first quadrat, and the number of species unique to the second quadrat, respectively.

SDI, *SWC*, *SF*, *UVC*, and *LC* were compared between MIX and MON by the Steel-Dwass test. The relationships between the number of species (total and native species) per quadrat and *SWC*, *SF*, *UVC*, and *LC* were examined for each of MIX and MON by Spearman's rank correlation test.

2. 3. Results

2.3.1. Occurrence of plant species

Altogether, 46 plant species were found in the two plots (Appendix 1). MIX and MON included 40 and 17 species, respectively, indicating far greater species richness in MIX than MON (Fig. 4). Though MIX and MON had similar number of forb species (15 and 11, respectively), the species numbers of the other four groups in MIX were larger than those in MON (Fig. 4A). In particular, 6 shrub species and 7 vine species occurred in MIX, while no shrub and only 1 vine species was found in MON. In MON, forbs occupied 65% of the total species number.

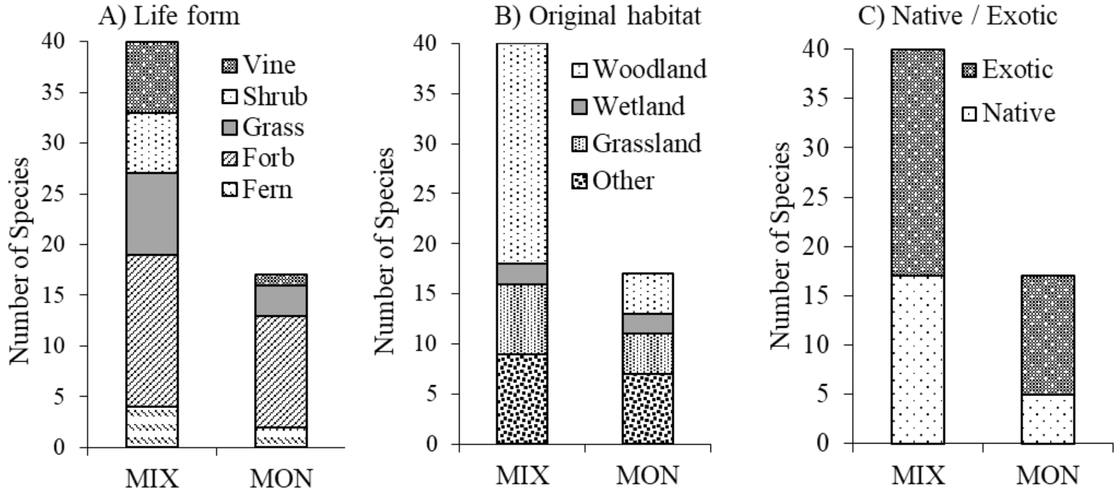


Figure 4. Number of species in the mixed-culture (MIX) and monoculture (MON) stands with reference to the species groups classified by A) life form, B) original habitat, and C) native or exotic

For the original habitats of the species, there was a large difference in the numbers of woodland species, which were 22 and 4 in MIX and MON, respectively (Fig. 4B). This difference (18 species) explained ca. 80% of the difference in the total species richness (23 species). The woodland species occupied more than half of the total number of species in MIX.

The number of native species in MIX (17) was more than three times that in MON (5) (Fig. 4C). The number of exotic species was also larger in MIX (23) than in MON (12); however, the proportion of the exotic species to the total species per plot was lower in MIX (57.5%) than in MON (70.5%).

2.3.2. Diversity and heterogeneity of plant species

In MIX, there were 8.25 species of the within-quadrat diversity (α-diversity), which was almost twice that in MON (4.45) (Table 2). The between-quadrat diversity (β-diversity) was far greater in MIX (31.75) than in MON (12.55). MIX showed significantly lower SDI (0.20 on average) than MON (0.47), indicating a higher spatial heterogeneity of plant occurrence compared to MON (Fig. 5).

Table 2. Within-quadrat (α-) and between-quadrat (β-) diversities of the mixed-culture (MIX) and monoculture (MON) stands calculated according to the additive partitioning method (Wagner 2000)

Stand	Number of total species (A)	Within-quadrat diversity (a-diversity) (B)	Between-quadrat diversity (b-diversity) (A - B)
Mixed-culture (MIX)	40	8.25^{a}	31.75
Monoculture (MON)	17	4.45^{b}	12.55

Different letters for the within-quadrat diversity indicate a significant difference (Steel-Dwass test, $p<0.05$)

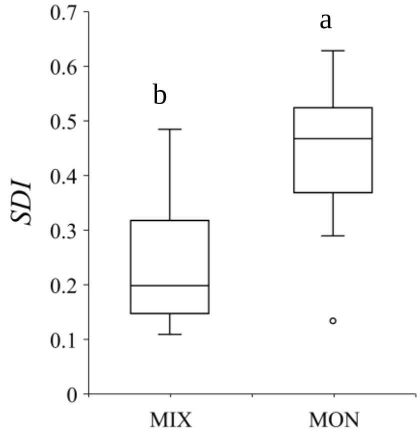


Figure 5. Comparison of Sørensen-Dice index (*SDI*) between the mixed-culture (MI) and monoculture (MN) stands. *SDI* was calculated for all combinations of 2 out of 20 quadrats in each stand, respectively. Different letters on the boxes indicate a significant difference (Steel-Dwass test, p<0.05)

2.3.3. Environmental and ground surface conditions

There was no significant difference in the soil water content (*SWC*) between MIX and MON (Fig. 6A), which had similar mean values (18.1% for MIX and 16.7% for MON) and ranges (12-25% for MIX and 10-25% for MON). The sky factor (*SF*) also did not differ significantly between MIX and MON (Fig. 6B), which had similar mean values (20.3% and 19.5%). In contrast to the two parameters of the physical environment (*SWC* and *SF*), the ground surface conditions significantly differed between MIX and MON. The understory vegetation cover (*UVC*) in MIX had a wide range of values (7-100%) with 60.7% on average, while that in MON was greatly low, ranging from 2% to 29% with 15.4% on average (Fig. 6C). The litter cover (*LC*) showed a similar trend as UVC (Fig. 6D). MIX showed a large variation of *LC* from 0% to 66%, while the maximum value of *LC* in MON was 6%. The average of *LC* was 19.6% and 2.0% for MIX and MON, respectively.

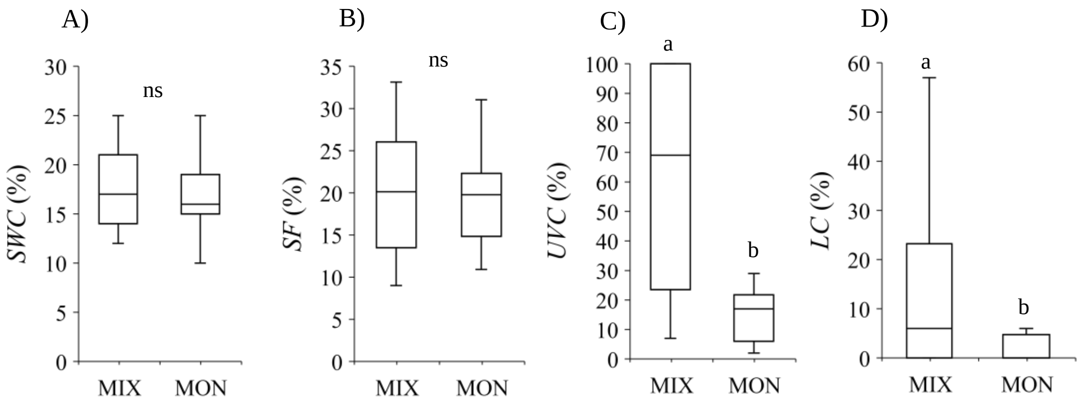


Figure 6. Comparisons of A) soil water content (*SWC*), B) sky factor (*SF*), C) understory vegetation cover (*UVC*) and D) litter cover of the ground surface (*LC*) between the mixed-culture (MI) and the monoculture (MN) stands. Different letters on the boxes indicate significant differences (Steel-Dwass test, $p<0.05$)

Correlation analyses among the four factors (Table 3) demonstrated significant positive correlations between *UVC* and *SF* both in MIX and MON, respectively. *LC* showed strong negative correlations with *SF* in MIX, but not in MON. Similarly, *LC* was correlated with *UVC* negatively in MIX, but not in MON. MON showed significant positive correlations between *SF* and *SWC*, and *UVC* and *SF*, respectively.

Table 3. Spearman correlation among soil water content (*SWC*), the sky factor (*SF*), understory vegetation cover (*UVC*) and litter cover of the ground surface (*LC*) in the mixed-culture (MIX) and monoculture (MON) stands.

		SWC		*SF*		*UVC*	
		r	*p*-value	*r*	*p*-value	*r*	*p*-value
Mixed-culture (MIX)	*SF*	0.357	0.123	-	-	-	-
	UVC	0.405	0.076	0.893	< 0.001	-	-
	LC	-0.339	0.143	-0.795	< 0.001	-0.839	< 0.001
Monoculture (MON)	*SF*	0.446	0.049	-	-	-	-
	UVC	0.221	0.350	0.535	0.015	-	-
	LC	0.138	0.562	0.279	0.233	0.325	0.162

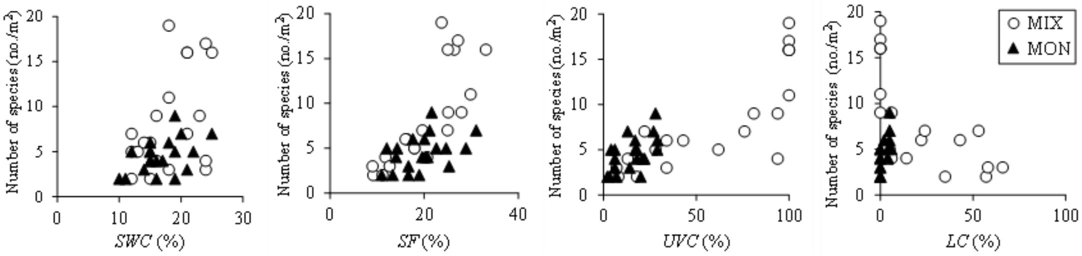
Among the relationships between the total number of species and the environmental factors or ground surface condition, significant correlations were found with regard to *SF*, *UVC*, and *LC* for both MIX and MON (Fig. 7A). The number of total species in MIX was correlated positively to *SF* (Table 4). The correlation in MON was also significant, but the number of species tended to be low compared to that in MIX at the higher range (ca. >20%) of *SF*.

The correlations with *UVC* were also significantly positive in MIX and MON. The number of species in MON was scattered and overlapped with that of MIX up to 30% of *UVC*, though MON had no quadrat of *UVC* higher than 30%. *LC* was negatively correlated to the number of species in MIX. In contrast, the correlation of *LC* in MON was positive, though the range of *LC* was narrow. The correlation of *SWC* to the total number of species was not significant for either MIX or MON, but the number of species in MON tended to be low over the whole range of *SWC*.

Table 4. Spearman's correlation analysis for total and native species number vs. soil water content (*SWC*), sky factor (*SF*), understory vegetation cover (*UVC*), and litter cover of the ground surface (*LC*)

	SWC (%)		*SF* (%)		*UVC* (%)		*LC* (%)	
	r	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value
All species								
Mixed-culture (MIX)	0.405	0.075	**0.853**	**< 0.001**	**0.868**	**< 0.001**	**-0.802**	**< 0.001**
Monoculture (MON)	0.424	0.062	**0.490**	**0.028**	**0.487**	**0.029**	**0.709**	**< 0.001**
Native species								
Mixed-culture (MIX)	0.368	0.109	**0.774**	**< 0.001**	**0.864**	**< 0.001**	**-0.651**	**< 0.001**
Monoculture (MON)	0.370	0.107	**0.455**	**0.043**	0.414	0.069	**0.709**	**< 0.001**

Regarding the native species, the species number showed similar correlations to environmental factors (Fig. 7B, Table 4) as found for total species (Fig. 7A), except for *UVC* in MON, in which the correlation to the number of native species was not significant. The separated scattering of the quadrats of MON from that of MIX in the diagrams of *SWC* and *SF* was more apparent for the native species (Fig. 7B) compared to those for total species (Fig. 7A).


A) Total species

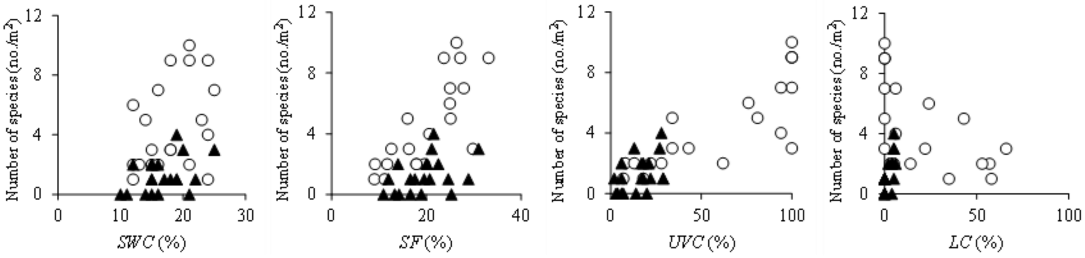

B) Native species

Figure 7. Number of a) total species and b) native species per quadrat in relation to soil water content (*SWC*), sky factor (*SF*), understory vegetation cover (*UVC*), and litter cover of the ground surface (*LC*). Open circles and solid triangles indicate the quadrats in the mixed-culture (MIX) and monoculture (MON) stands, respectively

2.4. Discussion

2.4.1. Advantage of the mixed-culture system in conserving plant species diversity

The results obtained in this study indicated that, for clove production, the mixed-culture (MIX) system can hold a far higher plant species richness compared to the monoculture (MON) system. In addition to its larger number of total species, MIX had a diverse life form composition (Fig. 4A), while two-thirds of the species in MON were only forbs. Further, the high total species richness of MIX was closely related to the richness of the woodland species (Fig. 4B). This suggested that MIX has a higher value for conserving woodland species even as an agricultural production site compared to MON, which had only a few woodland species in spite of its 'woodland' structure.

In agricultural lands, frequent human disturbances often promote the dominance of disturbance-dependent exotic plants (MacDonald 2004; Lundholm & Marlin 2006; Scales & Marsden 2008; Pejchar & Mooney 2009; Boreux et al. 2016; Cordeiro et al. 2018). However, the MIX stand examined in the present study consisted of 23 native species that occupied more than half of the total species (Fig. 4C). This also suggests a conservation advantage for MIX compared to MON for clove production.

Similar advantages in conserving plant species diversity have been reported for the typical agroforestry systems where crops are cultivated beneath canopy trees planted for timber production (Roshetko et al. 2013; Deheuvels et al. 2014; Matinahoru 2014; Liu et al. 2018; Sabastian et al. 2019). The present study revealed that the mixed-culture system is also effective for conserving plant species in an agriculture-forestry landscape.

2.4.2. Limiting factors of plant occurrence and heterogeneity in MIX and MON

The high plant species richness of MIX was reflected not only by its high α-diversity as the mean species richness at the quadrat level, but also by the β-diversity, which is the spatial heterogeneity or dissimilarity of species occurrence among the quadrats within each plot (Table 2 and Fig. 5). This suggested that the higher variabilities of the microsite conditions within the MIX stand might have strongly affected its high species richness.

Among the microsite conditions examined in the present study, soil water content (*SWC*) and sky factor (*SF*) showed no significant difference between MIX and MON, and had similar ranges of values (Fig. 6A and 6B), indicating that MON maintained the variability of *SWC* and *SF* equivalent to that of MIX. This might have been partly due to the low planting density of clove trees in the MON plot (Table 1) that allowed for an unclosed canopy (high *SF*) and somewhat low root density of clove trees. The tree spacing of ca. 6 m × 6 m of the MON investigated in the present study is ordinary and considered of intermediate density in this region for clove monocultures (Kaya et al. 2002; Sutriyono & Ali 2018; Arimalala et al. 2019). Thus, similar spacing's of clove trees as in the MON of the present study would not cause *SWC* and *SF* to be too uniform.

Compared to *SWC*, the correlation of *SF* with the species richness at each quadrat was apparent for both the total species and the native species numbers (Fig. 7, Table 4), suggesting that the light environment rather than soil moisture was the predominant factor determining species occurrence in the understories of MIX and MON in the present study. In particular, the large variation of *SF* in the MIX directly explains the high mean quadrat species richness (α-diversity) and may also explain its spatial heterogeneity (β-diversity) (Table 2 and Fig. 5).

Although *SF* was correlated with the number of species both in MIX and MON, the relationship was weaker in MON than in MIX (Fig. 7, Table 4), indicating less effect of the light environment on species richness. In particular, the number of native species in MON was strongly restricted over the whole range of *SF* as well as of *SWC* (Fig. 7B). These results were attributed to differences in the soil surface conditions (Fig. 7, Table 4) presumably caused by the different frequencies and extents of soil surface disturbance between MIX and MON. Litter collection in MIX was infrequent and partial beneath and around the canopy of the scattered clove trees, which must have resulted in infrequent and partial soil surface disturbances compared to those in MON where litter collection was conducted more frequently over the whole stand.

In MIX, understory vegetation cover (*UVC*) and litter cover (*LC*) were correlated negatively (Table 3), suggesting that vegetation and litter were covering the ground surface complementary each other under the less soil surface disturbances. The range of *UVC* and *LC* in MIX were large enough to explain the large variation in the number of total and native species; higher *UVC* with lower *LC* supported the occurrence of more plant species (Fig. 7). These results suggested that the higher *UVC* indicates the more opportunities for plant species being established, and consequently facilitated the effects of heterogeneous *SF* (and probably *SWC*) to increase the species number at the stand level. On the other hand, the strong negative correlation of the species number with *LC* might indicate that *LC* act as a constraint for establishment of plant species under infrequent litter collection. Similar result, that litter cover reduced the plant species number in ground vegetation's, has been observed in other agroforestry cases in particular for large and thick leaf litter such as teak (*Tectona grandis*) in the same region (Umar et al. 2019).

Conversely, *UVC* and *LC* of MON were restricted within quite narrow ranges up to 30% and 6%, respectively, showing the evidence for the higher degree of soil surface disturbance associated with the frequent collection of clove litter from the whole MON plot. No significant correlation between *UVC* and *LC* in MON (Table 3) indicated that they were not complementary in this plot, but influenced similarly by litter collection and associated soil surface disturbances. Since the correlation of *UVC* to the total species number in MON was similarly positive as in MIX (Table 4), and the total species number per quadrat of MON was scattered against *UVC* similarly as in MIX (Fig. 7), at least in the same range of *UVC*, it can be interpreted that *UVC* in MON had influenced the species number similarly as in MIX. In other word, the species number in MON was supposed to be strongly restricted by the reduced *UVC* due to human disturbances. Considering these results together with the large variations of *SWC* and *SF* (Fig. 6A and 6B) and their weaker correlation with the species number (Fig. 7, Table 4) in MON, it is supposed that frequent and extensive human disturbances on the soil surface had a role of cancelling the positive effects of the heterogeneity of physical environments on species richness at the stand level by reducing severely the opportunities for plants establishment.

The species number in MON showed a positive correlation with *LC*, differing from that in MIX (Fig. 7, Table 4). This result could be interpreted that, in MON, *LC* with small values and a narrow range indicated the degree of escaping from the disturbance associated with litter collection, rather than the degree of disturbance itself.

Appendix

Appendix 1. Characteristics and occurrence of plant species found in the study site

Species	Life form	Original habitat	Native/ Exotic	Occurrences in each site	
				MIX	MON
Ageratum conyzoides	Forb/Herb	Woodland	Exotic	+	+
Axonopus compressus	Graminoid	Grassland	Exotic	+	
Borreria verticillata	Forb/Herb	Others	Native	+	+
Brachiaria ramose	Graminoid	Grassland	Native	+	
Cardiospermum halicacabum	Vine	Other	Exotic	+	
Centella asiatica	Forb/Herb	Wetland	Exotic		+
Centrosoma pubescens	Vine	Woodland	Exotic	+	
Chromolaena odorata	Forb/Herb	Woodland	Exotic	+	
Commelina diffusa	Forb/Herb	Other	Exotic	+	
Costus speciosus	Shrub	Woodland	Native	+	
Crassocephalum crepidioides	Forb/Herb	Other	Exotic		+
Cyclosorus truncatus	Fern	Woodland	Native	+	
Cynodon dactylon	Graminoid	Grassland	Exotic	+	+
Cyperus kyllingia	Graminoid	Grassland	Exotic		+
Cyperus rotundus	Graminoid	Grassland	Exotic	+	
Derris elliptica	Vine	Woodland	Native	+	
Diplazium esculentum	Fern	Woodland	Native	+	+
Elephantopus scaber	Forb/Herb	Grassland	Exotic	+	+
Eleusine indica	Graminoid	Grassland	Exotic	+	
Emilia sonchifolia	Forb/Herb	Other	Exotic		+
Ficus septica	Shrub	Woodland	Native	+	
Flemingia semialata	Shrub	Woodland	Native	+	
Heliotropium indicum	Forb/Herb	Other	Exotic		+
Hyptis capitate	Shrubs	Other	Exotic	+	
Imperata cyindrica	Graminoid	Grassland	Exotic		+
Ipomoea muricata	Vine	Others	Exotic	+	+
Ischaemum indicum	Graminoid	Grassland	Native	+	
Lantana camara	Shrub	Woodland	Exotic	+	
Leucas martinicensis	Forb/Herb	Woodland	Native	+	
Lindernia crustacea	Forb/Herb	Wetland	Native	+	+
Lygodium circinatum	Fern	Woodland	Native	+	+
Mikania cordata	Vine	Woodland	Exotic	+	
Mimosa pudica	Vine	Other	Exotic	+	
Momordica balsamina	Vine	Woodland	Exotic	+	
Neomarica longifolia	Forb/Herb	Woodland	Exotic	+	
Oldenlandia corymbosa	Forb/Herb	Woodland	Exotic	+	
Oplismenus burmannii	Graminoid	Other	Native	+	
Osbeckia stellate	Forb/Herb	Woodland	Native	+	
Oxalis barrelieri	Forb/Herb	Woodland	Exotic	+	
Peperomia pellucida	Forb/Herb	Woodland	Exotic	+	+
Phyllantus ninuri	Forb/Herb	Other	Native	+	+
Pteris biaurita	Fern	Woodland	Native	+	
Scleria sumatrensis	Graminoid	Wetland	Native	+	
Solanum torvum	Shrub	Woodland	Exotic	+	
Stachytarpheta jamaicensis	Forb/Herb	Other	Exotic	+	+
Xanthosoma sagittifolium	Forb/Herb	Woodland	Exotic	+	

Chapters 3. Growth of clove trees

3.1. Objective

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

3.2. Materials and Methods

3.2.1. Study site and experimental design

The experiment was conducted in a private nursery located in Kesamben Distric, Blitar Regency, East Java, Indonesia (190 m a.s.l) (Fig. 8). 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.

Figure 8. Location of the study site

I established 36 plots sized 2.5 m × 2 m. Twenty clove seedlings (18 months old, ca. 120 cm in average height) were planted in each plot on February

7, 2017. From one year after planting (July 4, 2018), three levels of shading treatments using by shading net (0%, 60% and 80% shading; hereafter S0, S60 and S80, respectively) and four levels of watering treatments (1.0, 0.75, 0.5 and 0.25 l/m2/day; hereafter W100, W75, W50 and W25, respectively) were applied independently with three replications for each of combination of the shading and the watering treatments as the split-plot design (Fig. 9). The experiment was continued ca. 6 months until January 2, 2019.

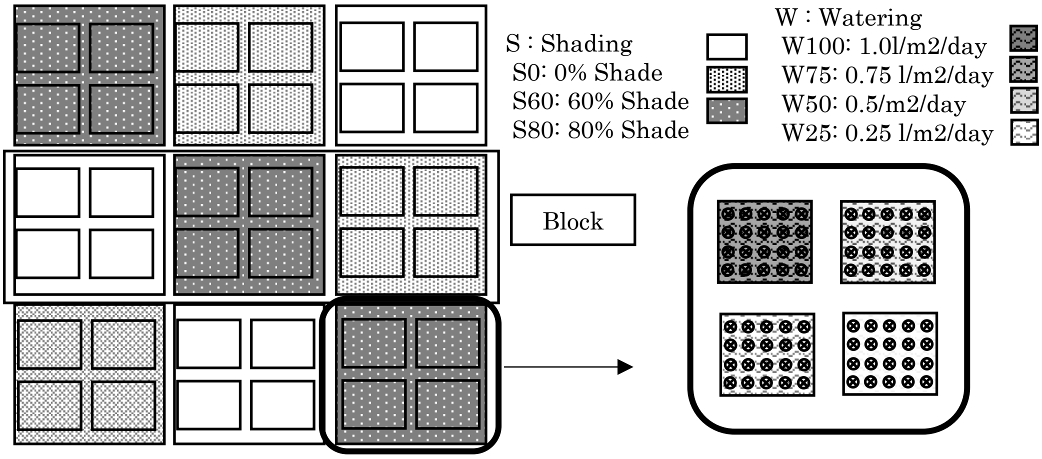


Figure 9. Schematic drawings of the experimental design combining the shading treatment and the watering treatment

3.2.2. Measurements

During the experiment (January 2, 2018), height of the seedlings was measured almost every month except for October. Number of buds were also counted at the beginning and the end of the treatments to determine the number of newly created buds during the treatments. At the end of the experiments, all the seedlings were harvested to determine the dry mass of leaves, stems and branches, and roots. Dry mass of each organ was measured for each seedling separately after drying up by using dry oven (48 hours, 72°C).

3.2.3. Data analyses

To compare the height growth of seedlings that had different initial sizes, the increment ratio of seedling height (*IH, m/m*) was calculated by dividing the final size measured at the end of the treatments (January 2, 2018) by the initial size measured at the beginning of the treatments (July 04, 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 ω-procedure.

3.3. Results

3.3.1. Height growth

Seeding height in treatments W50 and W25 tended to show slower growth compared to the adequately watered treatments (W100 and W75) throughout the experimental period, though the variation of seedling height at each measurement was quite large within treatment (Fig. 10). Since the initial height was slightly differed among the treatments, height growth was compared by the increment ratio of seedling height (*IH*). The watering treatments had significant effects on *IH* ($p<0.001$), while the shading treatments and their interaction were not significant ($p>0.05$) (Table 5). The treatment W100 showed the highest value of *IH*, followed by W75 with significantly higher values than W50 and W25 (Fig. 11A).

Table 5. 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

Figures denote F-value.

*, ** and *** indicate the significance level ($p<0.05$, $p<0.01$ and $p<0.001$, respectively)

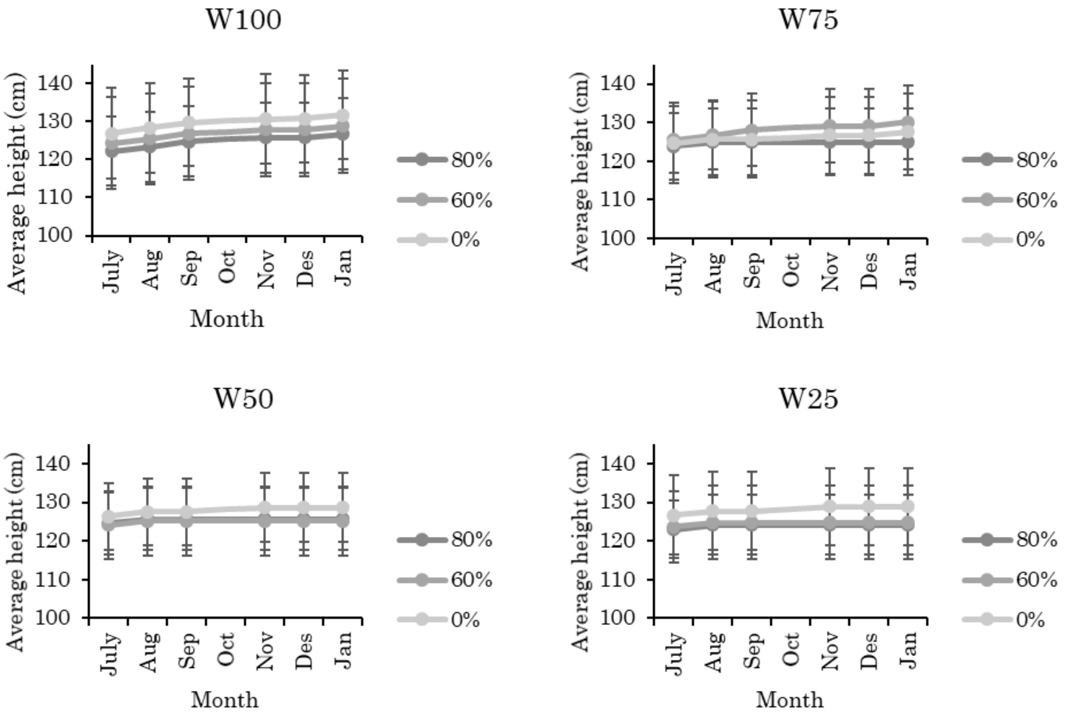


Figure 10. Changes of seedling height during the experimental periods. Vertical bars indicate standard errors

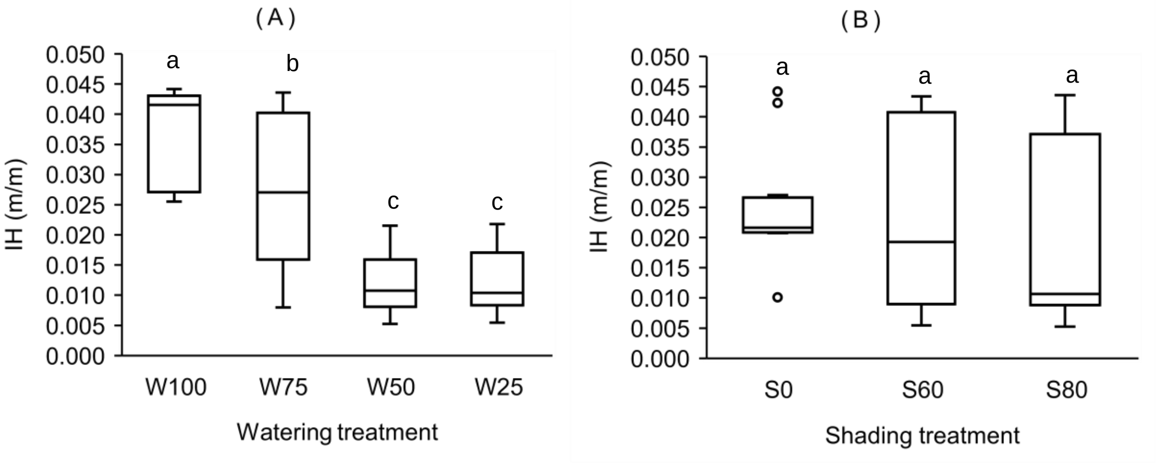


Figure 11. The increment ratio of seedling height (*IH*) under the different watering and shading treatments. A) comparison by the watering treatment, B) comparison by the Shading treatment. Different letters indicate significant differences between treatments ($p< 0.05$)

3.3.2. Number of buds

Number of the newly created buds (*NB*) was affected by both the watering and the shading treatments as well as their interaction ($p<0.001$) (Table 5). *NB* demonstrated similar results as in *IH*, showing the highest *NB* in W100, followed by that in W75 (Fig. 12A). For the shading treatments, S80 had significantly lower *NB*, while there was no significant difference between S0 and S60 (Fig. 12B). The effects of the watering treatments were most apparent under no shading (S0), showing the far higher *NB* in W100 and W75 than in W50 and W25 (Fig. 13). *NB* in the severest watering treatment (W4) between full light (S0) and moderate shade (S60) was significantly different, but the difference was small (3.1 and 4.0 for S0 and S60, respectively) (Fig. 13).

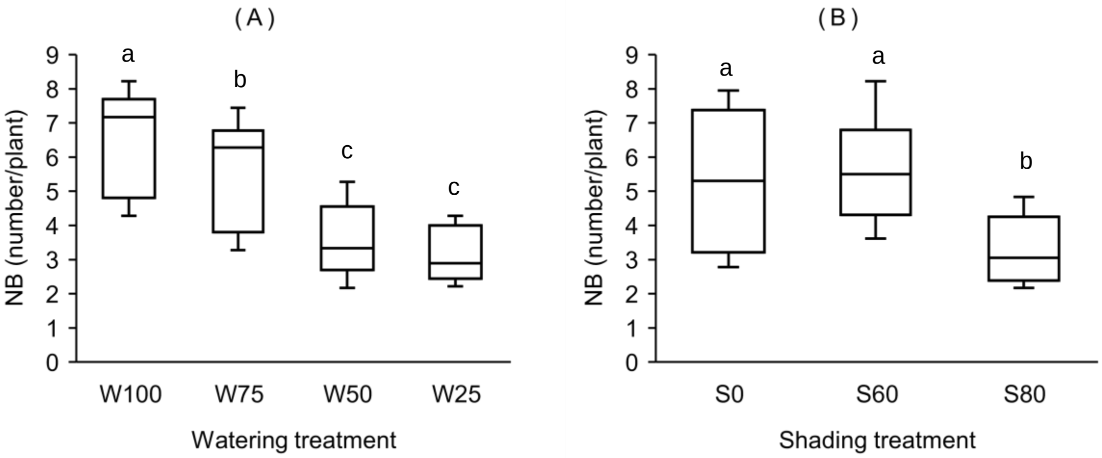


Figure 12. Number of the newly created buds (*NB*) under the different watering and shading treatments. A) comparison by the watering treatment, B) comparison by the shading treatment. Different letters indicate significant differences between treatments ($p < 0.05$)

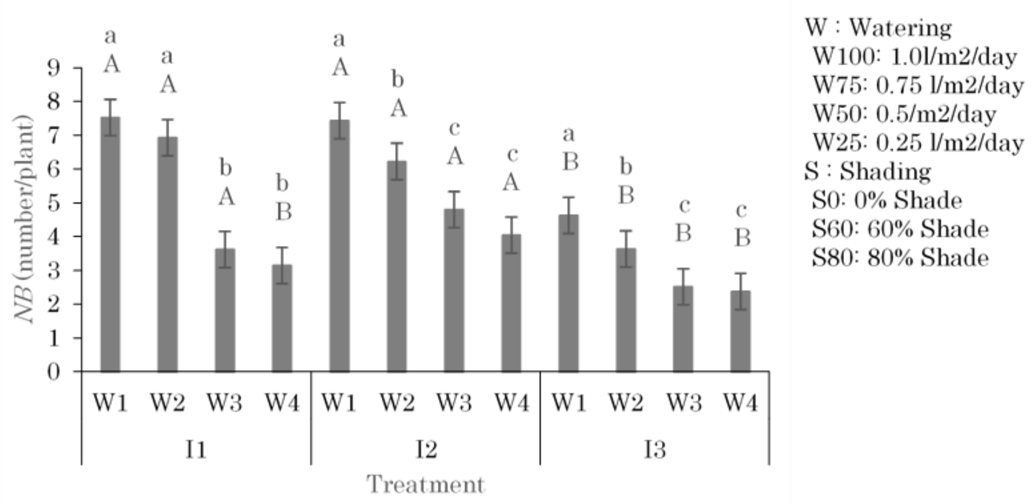


Figure 13. Comparison of number of the newly created buds (*NB*) under the 12 treatments with different watering and shading levels. Different letters indicate significant differences ($p < 0.05$) between treatments according to Tukey's test. Lowercase and uppercase letters denote the comparison among the shading and watering treatments, respectively

3.3.3. 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 5). The effect was more apparent for the watering treatments; *TM* showed a decreasing trend along the decrease of the watering rates, with significantly higher *TM* in W100 and W75 than in W50 and W25 (Fig. 14A). In contrast, the shading treatments did show a simple decrease; S60 had significantly lower *TM* than the other two treatments, while S0 and S80 did not differ significantly (Fig. 14B).

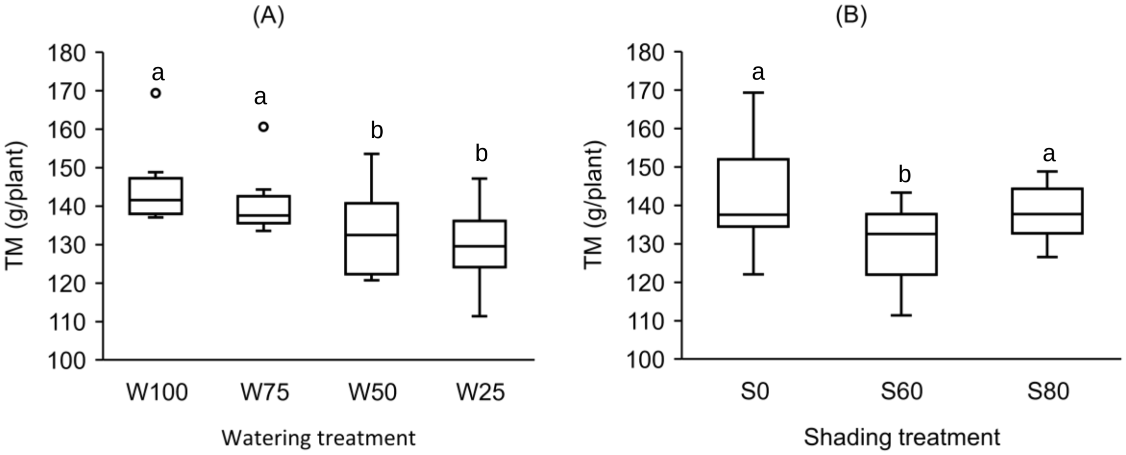


Figure 14. Total dry mass per plant (*TM*) under the different watering and shading treatments. A) comparison by the watering treatment, B) comparison by the shading treatment. Different letters indicate significant differences between treatments ($p < 0.05$)

The leaf mass (*LM*) was also affected by both the watering and the shading treatments ($p<0.05$ and $p<0.01$, respectively) with no interaction effect (Table 5). The watering treatment demonstrated a simple decrease along the watering rate with a significant difference of *LM* between W100 and W25 (Fig. 15A). However, differing from the response of *TM*, a simple decrease of *LM* was also observed by the shading treatment, showing significantly lower *LM* in S80 compared those in S0 and S60 (Fig. 15B).

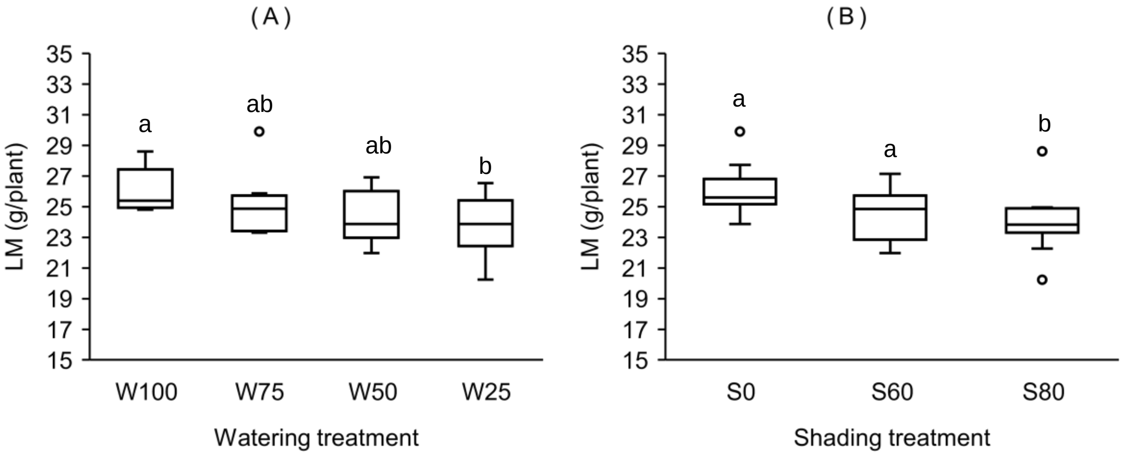


Figure 15. Leaf dry mass per plant (*LM*) under the different watering and shading treatments. A) comparison by the watering treatment, B) comparison by the shading treatment. Different letters indicate significant differences between treatments ($p < 0.05$)

The stem and branch mass (*SM*) was less affected by the treatments compared with the other parameters; only the shading treatment had a significant effect on *SM* (Table 4, Fig.16), and the difference was only detected between S0 and S60 (Fig. 16B).

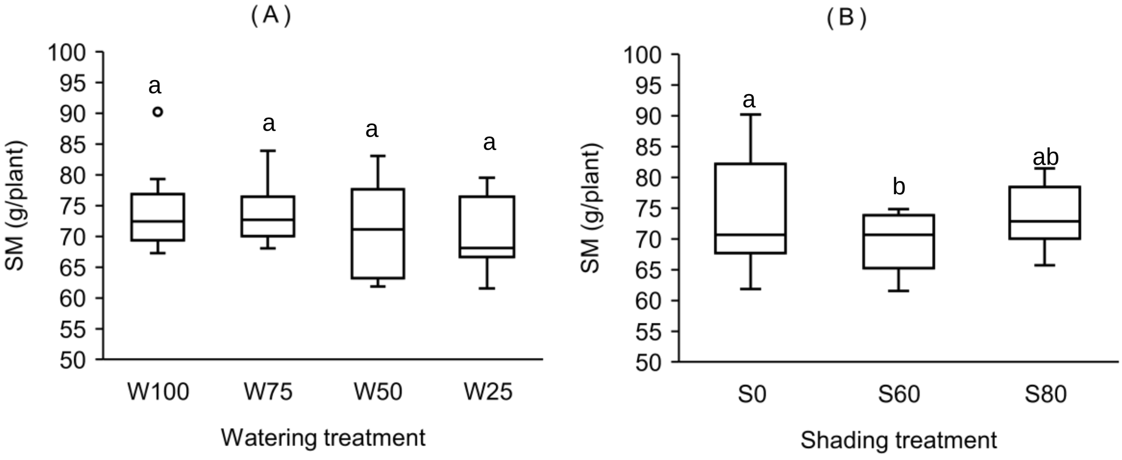


Figure 16. Stem and branch dry mass per plant (*SM*) under the different watering and shading treatments. A) comparison by the watering treatment, B) comparison by the shading treatment. Different letters indicate significant differences between treatments ($p < 0.05$)

The root mass (*RM*) showed similar trends as *TM*. A gradual simple decrease along the decrease of the watering rate was observed with significantly higher *RM* in W100 than in the other three treatments, and W50 and W25 showed lower values than W100 and W75 (Fig. 17A). The effects of the shading treatment were also similar to that on *TM*; a significantly lower *RM* was observed in S60 (Fig. 17B).

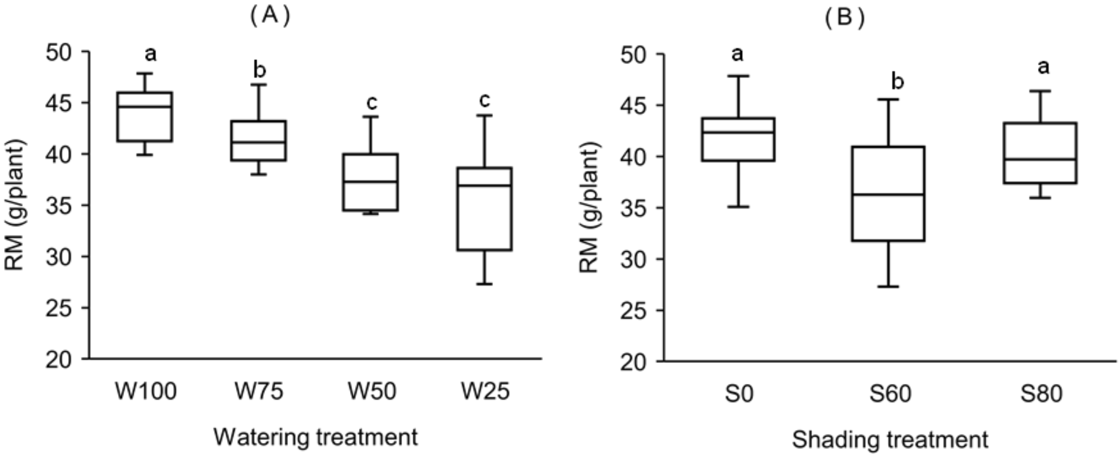


Figure 17. Root dry mass per plant (*RM*) under the different watering and shading treatments. A) comparison by the watering treatment, B) comparison by the shading treatment. Different letters indicate significant differences between treatments ($p < 0.05$)

3.4. Discussion

3.4.1. Growth trait of young clove seedlings

Our experiments in this study revealed that clove plants at the juvenile stage (seedling height < 150 cm) had a growth trait which was generally more susceptible to soil moisture stress induced by low watering rates than to low light availability induced by shading (Table 5). This was consistent with the field observation of the same species reported by Martin & Poultney (1992) where 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 (*IH*) was severely reduced by the treatments W50 and W25 (Fig. 11a). Similar trends of growth reduced by water shortage were clearly indicated in most of the examined parameters (number of the newly created buds (*NB*) (Fig. 12a), leaf mass (*LM*) (Fig. 15a), root mass (*RM*) (Fig. 17a) and total plant mass (*TM*) (Fig. 14a). Although the difference in stem and branch mass (*SM*) among watering rate was not significant, the lower watering rate (W50 and W25) tended to show the lower value of *SM* than W100 and W75 (Fig. 16a), indicating the almost universal negative effect of water shortage to the growth parameters.

In contrast, the effect of the shading treatment appeared to be limited compared to that of the watering treatment. The difference among the shading rates was not significant in *IH* (Fig. 11b). *SM* and *RM* as well as *TM* did not show the change of one-direction decrease along the increased shading rate (Fig. 16b, Fig. 17b and Fig. 14b, respectively). Thus, I concluded these results that the water availability is the major factor limiting the growth of clove seedlings in terms of their height and biomass increment.

Among the examined growth parameters, however, *LM* showed a significant decline under the highest shading rate (S80) compared to S0 and S60 (Fig. 15B). Since the leaves are the major plant part for the clove oil yield (Alighiri et al. 2018; Bhuiyan 2012; Bustaman 2011; Jirovetz et al. 2006; Kamatou et al. 2012), this result means that 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 is not inhibited.

In addition, *NB* also showed a significantly lower value under the heavy shade (S80) which was less than half of those in S0 and S60 (Fig. 15B). This might be due to the decrease of the number of leaves per plants which might result in the decrease of axillary buds. Numbers 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. 16b), the decline of *NB* and *LM* are thought to be due to the lowered leaf/bud density 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 (Razafimamonjison et al. 2014; Guan et al. 2007; Pino et al. 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 et al 2007; Lee & Shibamoto 2001; Moyler 1993), though the dry mass of buds per plant is far smaller than that of 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 future branching. Therefore, long-term heavy suppression should be avoided to insure the better future growth in terms of vigorous branching and foliage production, although the short-term effect of the heavy shade on height and total biomass increment was not apparent in this study.

3.4.2. Implication to clove production in mixed culture systems

Based on the results of the present 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 et al. 2006), which may lead severe inter-species competition for soil water, should be avoided in order to insure the fast growth of clove seedling at the establishment stage. In particular, on the sites where water stress easily occurs because of unfavorable topographic and soil conditions, excessive root competition is desired to be avoided. The closed 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 & Ramalho 2006). In cocoa, low-density shade trees positively affected their biomass (Isaac et al. 2007; Isaac et al. 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 5, Fig. 13 and Fig. 18 to 22). 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 seeding's same as in the case of Arabica coffee.

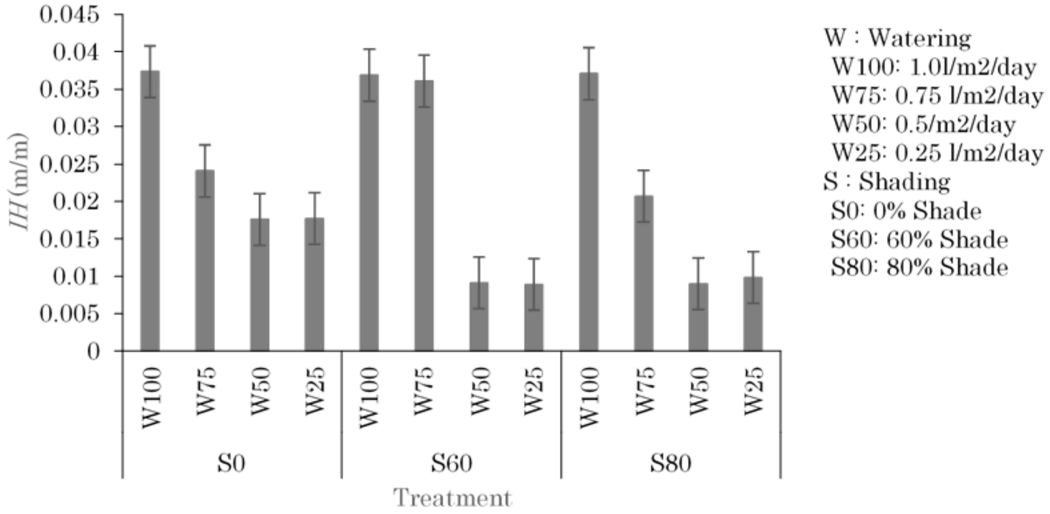


Figure 18. Comparison of the increment ratio of seedling height (*IH*) under the 12 treatments with different watering and shading levels

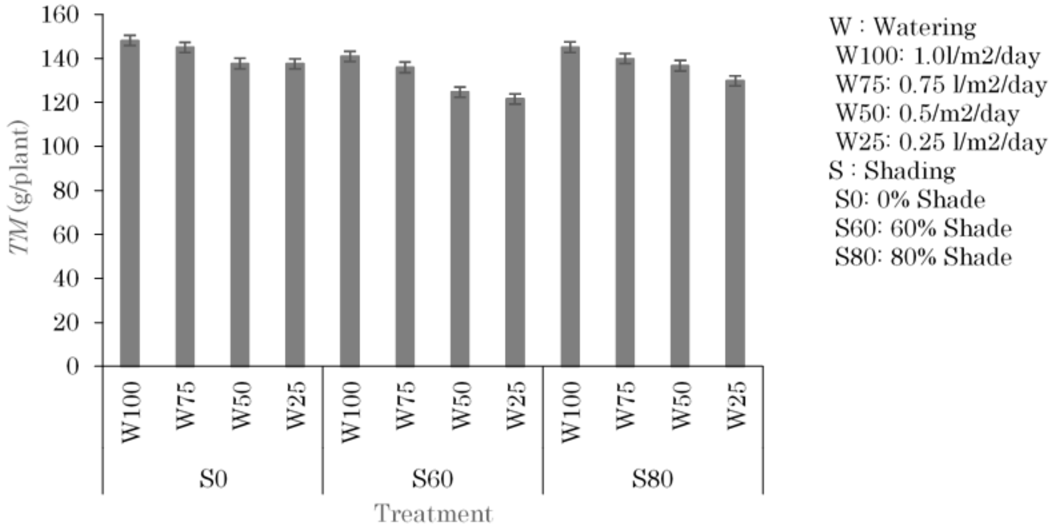


Figure 19. Comparison of total dry mass per plant (*TM*) under the 12 treatments with different watering and shading levels

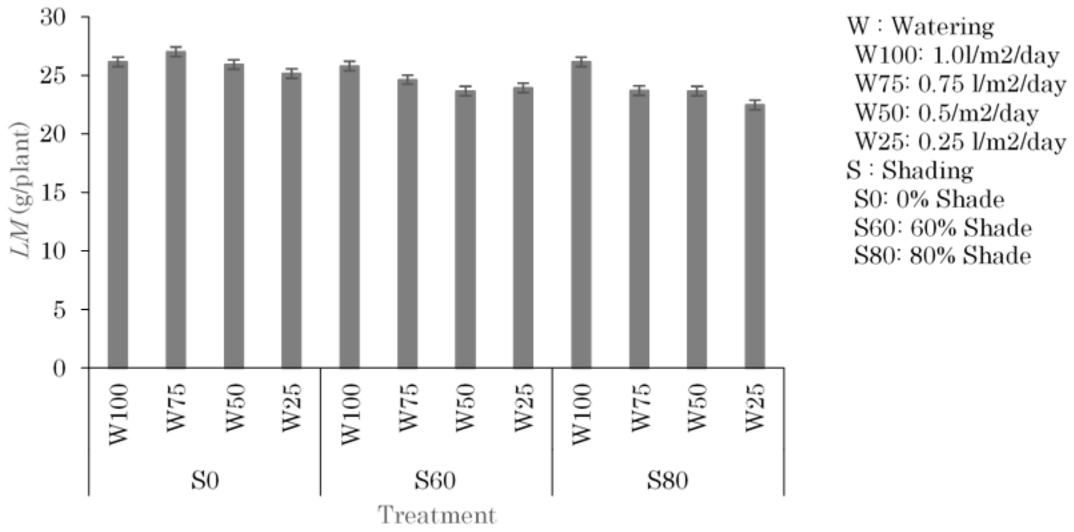


Figure 20. Comparison of leaf dry mass per plant (*LM*) under the 12 treatments with different watering and shading levels

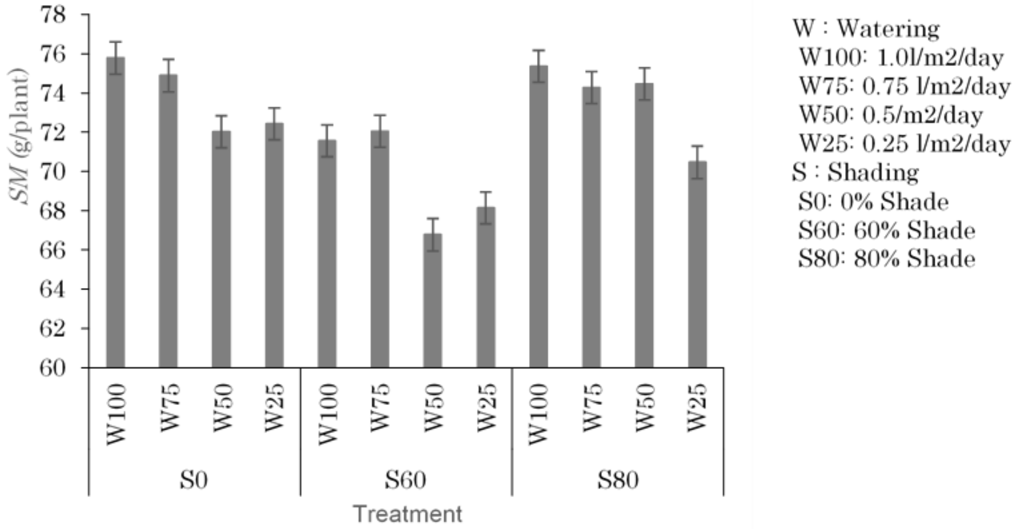


Figure 21. Comparison of stem and branch dry mass per plant (*SM*) under the 12 treatments with different watering and shading levels

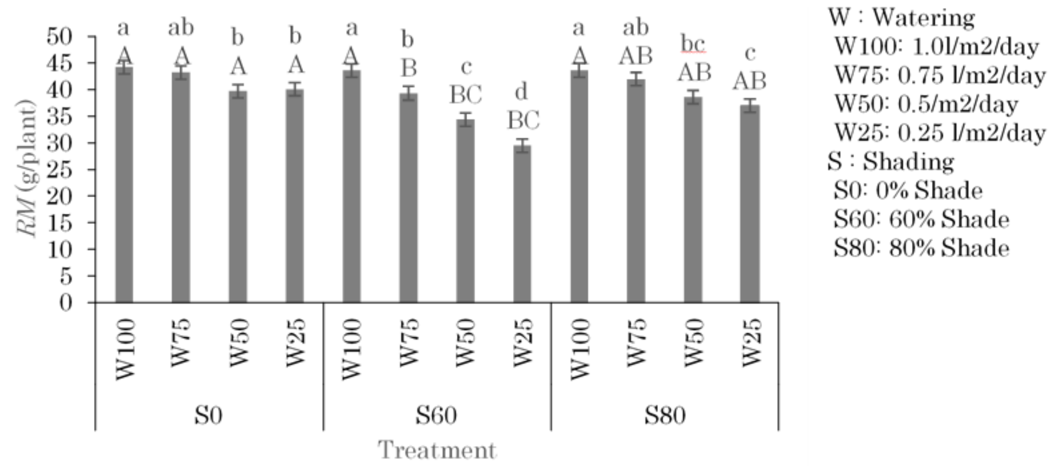


Figure 22. Comparison of root dry mass per plant (*RM*) under the 12 treatments with different watering and shading levels. Different letters indicate significant differences ($p < 0.05$) between treatments according to Tukey's test. Lowercase and uppercase letters denote the comparison among the shading and watering treatments, respectively

Chapter 4. Eugenol content of Clove oil and productivity

4.1. Objective

This study aimed to examine the hypothesis that 1) the positive effect of water stress increasing the eugenol content per unit leaf mass exceeds the negative effects of reducing leaf mass, and results in increased eugenol yield per plant, and 2) the low light condition reduces the eugenol yield by reducing eugenol content as well as leaf mass. In order to examine these hypothesis, I conducted an experiment of different watering and shading treatments applied to the clove seedlings, and compared their leaf mass production, eugenol content per unit leaf mass, and the total eugenol yield per tree to discuss the effects of water stress and shading on the eugenol yield. Based on the results, I discussed the suitable cite conditions for essential oil production by clove trees.

4.2. Materials and Methods

4.2.1. Experimental design of shading and watering treatments

The plant materials used in this study were grown in a private nursery located in Kesamben Distric, Blitar Regency, East Java, Indonesia (Fig. 8 in Chapter 3). In May 2018, I planted 18 months old clove seedlings (18 months old, ca. 120 cm in average height) in the nursery, and grew them for ca. 6 months under 12 combinations of three different shading treatments (0%, 60% and 80% shading; hereafter S0, S60 and S80, respectively) and four watering treatments (1.0, 0.75, 0.5 and 0.25 l/m2/day; hereafter W100, W75, W50 and W25, respectively). Each combination was replicated by three plots consisting of 20 seedlings respectively, that is, the total number of the plots and the seedlings used in the experiment were 36 plots (12 treatments x 3 replications) and 720 seedlings, respectively (Fig. 9 in Chapter 3). The details of the experimental design were described in Chapter 3.

4.2.2. Relative water content and total leaf mass per tree

During January 5 to 8, 2019, I harvested all seedlings. Five out of 20 seedlings were selected randomly to determine the total leaf mass per tree (LM), that is, oven-dried (48 hours, 72°C) weight of total leaves base on Chapter 3 result.

The other 15 seedlings per plot were used for the analyses of eugenol content. The leaves of the 15 seedling samples were mixed for each plot hanged in the shade room for five days until air-dried (withered). The leaves of 300 g (air-dried weight) per plot were re-sampled from them and subjected to the further analyses of eugenol content. The other leaves of the 15 sample seedlings were used to determine the relative water content. After measuring the air-dried weight (AW), the leaves were oven-dried (48 hours, 72°C), and the dry weight (DW) were measured. Relative water content of the leaves (RWC) was calculated by the following equation:

$$RWC = (AW - DW)/DW,$$

and used for estimating the DW of the leaf samples subjected to the analyses of eugenol content.

4.2.3. Extraction of essential oil solution

After measuring the *FW*, the leaves sampled for analyses of eugenol content were subjected to the steam distillation method (Alighiri et al. 2018; Kamatou et al. 2012; Lee et al. 2009) to extract the essential oil including eugenol. The distillation experiment was conducted in the laboratory of the University of Brawijaya on January 10-16, 2019, by using a distillation equipment (3-liter capacity for materials, and 2-liter capacity for water). The mixture of water and the material leaves were distilled under a condition of 1 bar and 130 °C for 6 hours. The extracted solutions including the essential oil were measure for their weight and volume, and 10 ml of sample solution per plot were brought to the laboratory of the University of Miyazaki.

4.2.4. Measurement of eugenol content

Contents of eugenol (*EC*: w/w) in each essential oil sample were measured as follow. One ml of each sample solution was dried by the addition of small amount of anhydrous sodium sulfate, then diluted with toluene (approximately 10 mg/g). Diluted samples were analyzed by GC-flame ionization detector (GC–FID). GC-FID analysis was performed with a Shimadzu GC-2010 Plus gas chromatograph under the following conditions; a SUPELCO SPB-50 capillary column (15 m × 0.32 mm inside diameter; 0.25 μm film thickness); a column temperature ranging from 50 (1 min) to 290 °C (2 min) at 20 °C/min; an injection temperature of 270 °C and a detection temperature of 300 °C; and helium was used as the carrier gas.

4.2.5. Eugenol content per unit leaf mass and eugenol yield per tree

Eugenol content per unit leaf mass (EL) for each plot was calculated by the following equation:

$$EL = EC \times TW / DWs,$$

where TW is the total weight of solution, and DWs is the dry weight of sample leaves subjected to essential oil extraction which was estimated from their air-dried weight (AWs: 300 g) and RWC as,

$$DWs = AWs / (1 + RWC).$$

Then eugenol yield per tree (EY) for each plot was calculated as follows:

$$EY = EL \times LM.$$

4.2.6. Statistical analysis

The total leaf mass per tree (LM), the eugenol content per unit leaf mass (EL) and the eugenol yield per tree (EY) were compared between treatments were compared by Two-way ANOVA (p<0.05). The treatment means were compared by using the Tukey's ω-procedure. For LM, the analyses have already been performed in the Chapter 3 and I cited the results in the present study.

4.3. Results and Discussion

Both the different watering and shading treatments significantly affected all of the leaf dry mass per tree (LM), the eugenol content per unit leaf mass (EL) and the eugenol yield per tree (EY) (Table 6). The interaction between the watering and the shading treatments was significant for EL and EY (Fig. 24 & 26), but the effect of interaction was not clear (Table 6).

Table 6. Summary of Two-way ANOVA performed for leaf dry mass per tree (*LM*), eugenol content per unit leaf mass (*EL*) and eugenol yield per tree (*EY*) under different watering and shading treatments

Treatment	Leaf dry mass per tree (*LM*) [1]	Eugenol content per unit leaf mass (*EL*)	Eugenol yield per tree (*EY*)
Watering	3.78 *	31.52 ***	75.22 ***
Shading	6.60 ** [2]	22.18 ***	411.60 ***
Watering x Shading	0.88	2.99 *	12.57 ***

[1] Sited from Tabel 4

[2] Values indicate F-value. ***, p<0.001; **, p<0.01; *, p<0.05.

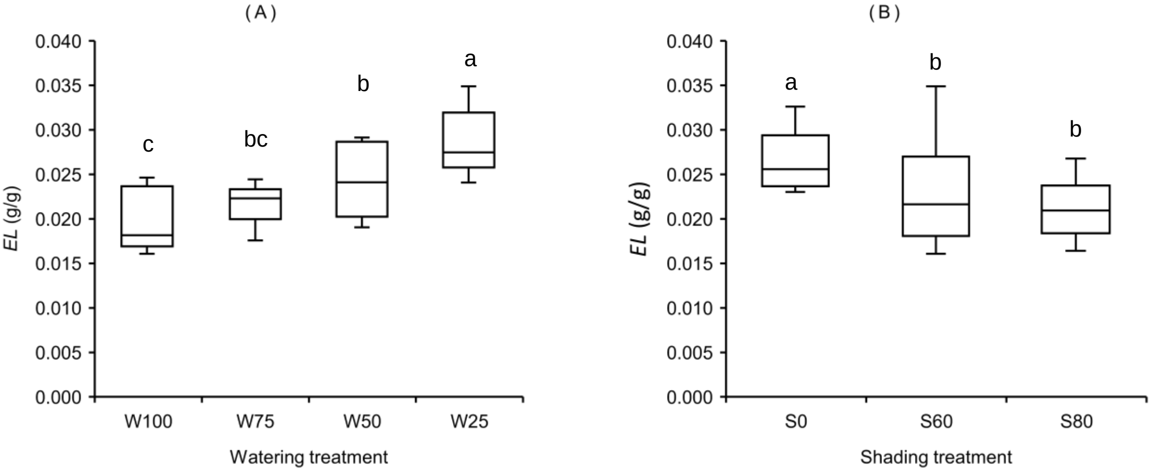


Figure 23. Eugenol content per unit leaf mass (*EL*) under the different watering and shading treatments. A) comparison by the watering treatments, B) comparison by the shading treatments. Different letters indicate significant differences between treatments ($p < 0.05$)

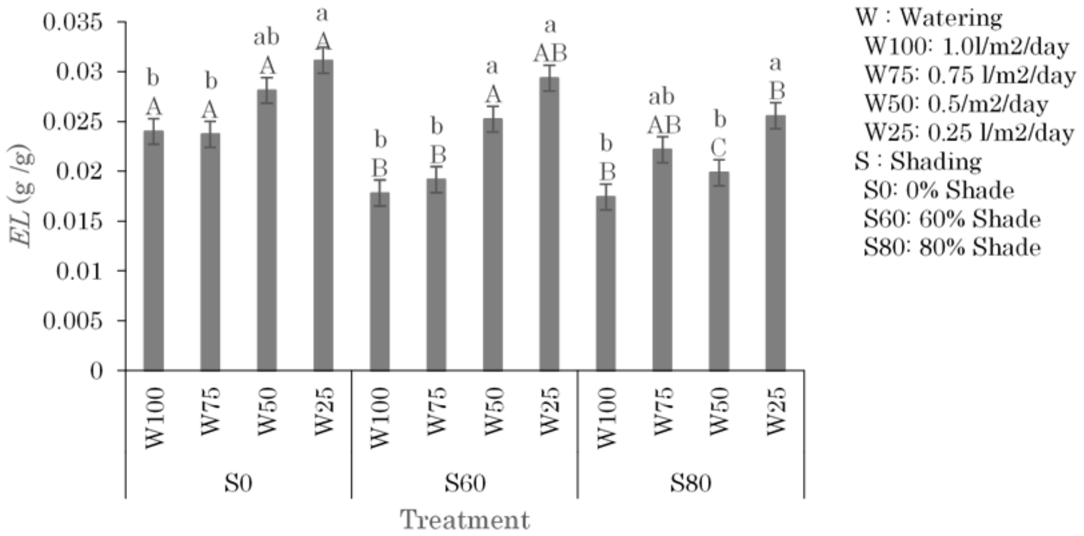


Figure 24. Eugenol content per unit leaf mass (*EL*) under the 12 treatments with different watering and shading levels. Different letters indicate significant differences ($p < 0.05$) between treatments according to Tukey's test. Lowercase and uppercase letters denote the comparison among the shading and watering treatments, respectively

As reported in Chapter 3, *LM* showed gradual decline along the decrease of the watering rate (Fig. 15A), indicating that the sites where soil water deficit is hard to occur are more suitable for growing clove trees at least from the view point of their growth. Similarly, the increased shade also resulted in lower *LM* (Fig. 15B) which in the S80 treatment (24.0 g/tree) was lower than that in S0 (26.0) slightly but significantly. This suggested a need of avoiding too high planting density which would cause a severe competition and the mutual shading among clove trees.

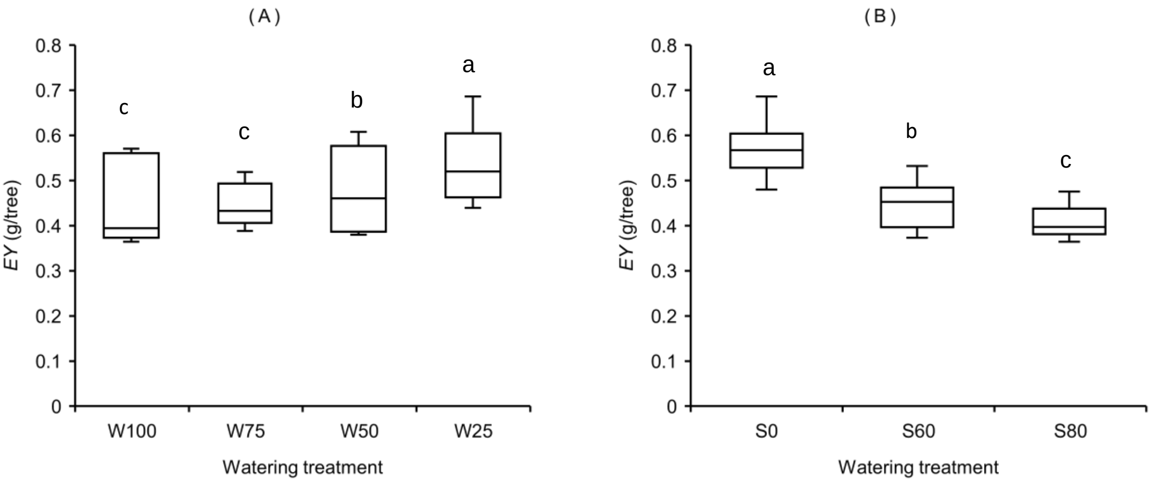


Figure 25. Eugenol yield per tree (*EY*) under the different watering and shading treatments. A) comparison by the watering treatments, B) comparison by the shading treatments. Different letters indicate significant differences between treatments ($p < 0.05$)

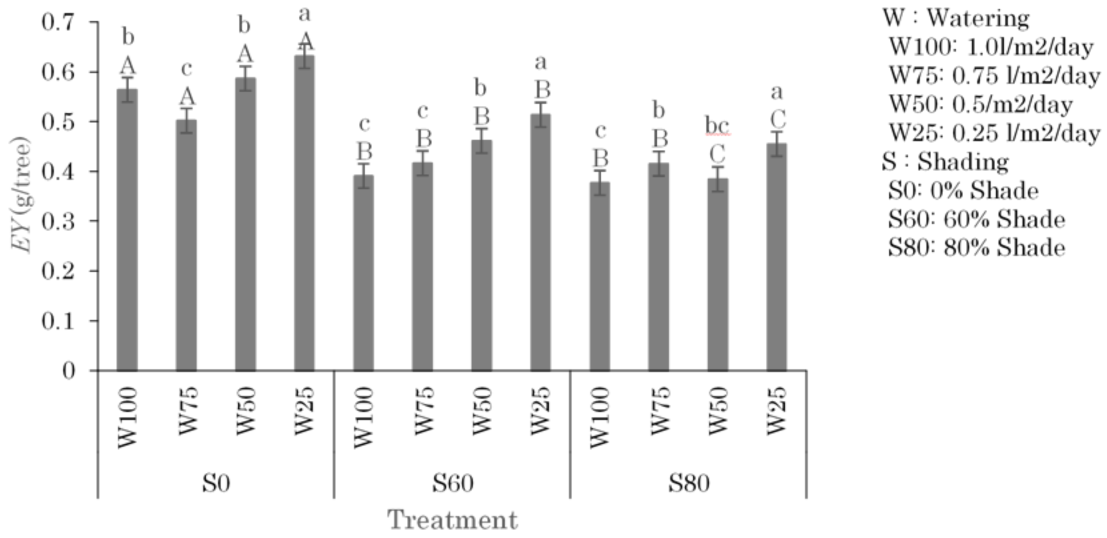


Figure 26. Eugenol yield per tree (*EY*) under the 12 treatments with different watering and shading levels. Different letters indicate significant differences ($p < 0.05$) between treatments according to Tukey's test. Lowercase and uppercase letters denote the comparison among the shading and watering treatments, respectively

In contrast to *LM*, *EL* was increased with the decrease of watering rate drastically (Fig. 23A). *EL* in W25 (0.028 g/g) ca. 47% larger than that in W100 (0.019 g/g), while *LM* in W25 (23.8 g/tree) was decreased with only 8% compared to that in W100 (26.0 g/tree) (Fig. 15A). Thus, the positive effect of water stress on *EL* was thought to be stronger than its negative effect on *LM*. On the other hand, the effect of the shading on *EL* was negative (Fig. 23B), being consistent with that on *LM* (Fig. 15B). Both S60 and S80 had lower *EL* compared with that in S0, indicating that the heavy shade reduces the eugenol productivity from clove leaves by reducing both *LM* and *EL*.

The behavior of *LM* and *EL* under the different watering and shading treatments resulted in the change of *EY* as shown in Fig. 25. The relatively weaker negative effect of the low irrigation rates on *LM* (W50 and W25 in Fig. 15A) was counteracted by the stronger positive effects on *EL*, resulting in higher *LY* in the less watered treatment (W50 and W25) (Fig. 25A). In particular, *EY* in W25 (0.53 g/tree) was 20% larger compared to that in W100 (0.44 g/tree).

In the Chapter 3, I found that the water stress at the juvenile stage of plant establishment reduce the leaf mass per plant, which could reduce the eugenol yields from clove leaves under water stress when the eugenol contents would not change. However, Farahani et al. (2020) reported that water stress increased the concentration of the terpenoid including eugenol in damask rose (*Rosa damascena*). Similar phenomena of enhanced secondary metabolites production under water stress have been reported in other plants (Jaafar et al. 2012; Mandoulakani et al. 2017). Thus, these studies suggested a possibility that, if the positive effect of water stress which increase the eugenol content per unit leaf mass can exceeds its negative effect reducing the leaf mass per plant, the eugenol yield per plant would be enhanced under a water-stressed conditions. The present study revealed that, the positive effects of water stress in terms of enhancing the eugenol production per unit leaf mass is stronger than the negative effect reducing the tree growth and leaf mass, resulting in more eugenol yield of clove at plant level under moderate water stress.

In contrast to the effects of water stress, the low light condition has been reported to reduce the essential oil production in many plants (Hälvä et al. 1992; Fernandes et al., 2013; Kumar, Sharma, & Pathania 2013). Thus, it is expected that the low light conditions would reduce the yield of essential oil via decline in both plant mass and oil concentration, though this has not also been examined for eugenol of clove trees.

From this result, is was suggested that the xeric sites (e.g., on thinner top soils or upper slopes) are rather suitable for long-term high productivity of eugenol from clove leaves, though the water stress should be avoided at the establishment stage of planted seedlings (The findings in Chapter 3). On the other hand, the low light availability by shading reduced *EY* markedly with the combined effects on *LM* and *EL* (Fig. 25B). *EY* in S60 (0.44 g/tree) and S80 (0.41 g/tree) was smaller with 23% and 28% compared to that in S0 (0.57 g/tree). These results suggested that the planting density and the management practices for avoiding the heavy shade to clove trees are crucial for clove efficient oil production.

Chapter 5. Conclusion

This study argues for a broad concept provided by mixed culture practices as a key pathway for the reorientation of agricultural systems in East Java, Indonesia toward sustainable production that harmonize productivity and biodiversity conservation. Mixed culture examined in this study will be an effective strategy to link environmental conservation with economic realities, while enhancing the livelihoods of smallholders and traditional communities.

I concluded from Chapter 2 that, in the clove monoculture stand investigated in the present study, it is not the homogenous physical environment but rather human disturbance of the soil surface associated with frequent and intensive clove litter collection over the whole stand that reduced the plant species richness by inhibiting plant occurrence and cancelling the positive effects of the varied physical environment. In contrast, the higher species richness of the mixed-culture stand was due to less litter-collection disturbances, which facilitated the effects of the heterogeneous physical environment within the stand.

To optimize productivity benefits from mixed culture, more information is needed to better suitable practices to the environment and to capitalize on above and belowground processes that improve function performance of plant such as light and soil water content. Therefore, in Chapter 3, I explored the growth responses of clove seedling to light and soil water regimes by a field experiment with shading and irrigation treatments to clarify their growth traits at the early stage of plantation establishment. I concluded these results 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.

In Chapter 4, I experimentally examined the effect of the watering and the shading treatments on eugenol productivity of clove seedlings, and found that the soil moisture deficit and the low light availability had negative and positive effects on the eugenol yield per tree, respectively. The results suggested that the relatively dryer site condition where moderate water stress is likely to occur is more suitable for planting clove trees from the aspect of the for long-term high productivity of eugenol, and that the high tree density which may results in a severe competition and a heavy mutual shading among clove trees should be avoided for maintaining high productivity for a long term.

Combination results of this research show that importance to careful pattern/design and proper management, mixed-species plantations with three or four main species provide an advantage in increasing species richness under tree stands. Too many species and narrow space will directly make it difficult for juvenile clove plants to produce and thrive due competition for water and light, consequently reducing clove growth and productivity. In addition, it is necessary to arrange plants that will provide sufficient space to grow and have more advantages in biodiversity, economy and forest health over monoculture.

Mixed-culture are proven ecologically to increase biodiversity in one site. therefore, this pattern widely applied in many place or area with various cropping patterns and different characters. The results of this study can be used jointly in all models competing with cropping patterns and commodities grown because it depends on interactions in agroforestry practices over time, space, and planting options related to production benefits and management strategies. Therefore, in the future it is necessary to identify a combination of trees and plants and their management that can provide better ecological services, including micro-climate modification to support biodiversity and production.

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