Vegetative Growth of Vanilla Cuttings After Addition of Weed Clippings Mulch Under 2 Climatic Condition, Wet and Dry Seasons

by I Gede Ketut Adiputra

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Vegetative growth of vanilla cuttings after addition of weed clippings mulch under 2 climatic condition, wet and dry seasons

I G K Adiputra[1,3], I W Winaja[2] and I M Sumarya[1]

[1] Department of Biology, Faculty of Natural Science, University of Hindu Indonesia Denpasar

[2] Faculty of Post Graduate Study, University of Hindu Indonesia Denpasar. Jl.Sangalangit, Tembau, Penatih, Denpasar, Bali, Indonesia

E-mail: dr_gede_adiputra@yahoo.co.id

Abstract. Experiments were performed to determine the effect of allelochemicals released by weed clipping on the growth of vanilla cuttings. The objective of these experiments was to find out safe plant material for mulching vanilla plantation. The first experiment was conducted in the wet season, where two nodes cuttings were transplanted into 5 groups of pots containing topsoil and mulched with grass (G), fern (F), taro (T), dry leaf (DL) and without mulch as control cuttings (C). The second experiment was under dry season but using a similar procedure like that in the first experiment except dry leaf was replaced with coconut husk (CH). These two experiments were using 8 replicates and grown in a shaded greenhouse. Phenolic compounds in weed clippings were examined using the Folin-Ciocalteu method. This study found that fern and taro clipping decreased soil pH, delayed emergence of new root and bud burst in vanilla cutting. By contrast, grass clipping, dry leaf and coconut husk did not decrease soil pH and did not delay root emergence and bud burst. The growth rate of the new stem in grass added plant was 1.4 folds relative control but only 0.4 fold in taro added plants. It is concluded that vanilla plant resistant to phenolic compound released by grass clipping, but susceptible to phenolic compound released by taro clipping.

Keyword; Allelochemical, budburst, coconut husk, grass, root emergence, viability

1. Introduction

Vanilla is shallow-rooted plants that thrive well in moist growth medium containing high organic material (humus). The organic materials have a very important function as a nutrient source, maintain soil humidity, soil aeration and permitting unrestrained root growth [1]. According to [2], soil organic carbon can be maintained by decreasing loss or by the deliberate addition of organic materials. This implies that to maintain organic materials in vanilla plantations, a periodical addition of vegetative mulch is required.

Vegetative material for mulching vanilla plants is available in various forms, such as; fresh weeds or vegetable clipping, dry leaf or limb, coconut husk, etc. In theory, the fresh clippings more likely to contain higher nutrient than the dry fallen leaf of trees since most nutrient incorporated in macromolecules in a leaf is decomposed and redistributed to other plant parts during senescence [3]. Therefore, fresh weed clipping could enrich the soil with a higher nutrient rate than the fallen dry leaf.

[3] To whom any correspondence should be addressed (dr_gede_adiputra@yahoo.co.id)

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According to [4], 46% of initial N content in harvested mulch materials was released after 30 days and the other N content remains in the mulch by 250 days. So, increasing the application rate of harvested mulch would increase nutrient availability to support optimal growth. By using this type of biofertilizer, the use of chemical fertilizer can be reduced [5]. However, although fresh weeds clipping contains a higher nutrient, it may also contain toxic compounds which according to [6] could injure the shallow-rooted crop.

In vanilla plantation, various kinds of weeds also grew such as grass, fern and taro. Therefore, plant material from various species is available to maintain organic material in the vanilla plantation. However, some of these plant material could also contain compounds that toxic to vanilla plants. According to [7] the weed decrease crop yield via competition and allelopathy where phenolic is the predominant class of allelochemical. Besides, flavonoid and terpenoid are also regarded as allelochemical [8,9]. Therefore, by using fresh weed clipping for mulching, vanilla plants will encounter toxic compounds after the allelochemical is released. However, presuming that the vanilla plant resistant to the allelochemical, weed clippings would enrich the soil with organic materials and favor the growth of vanilla plants.

Weeds and vegetables easily found in vanilla plantation have been reported to produce allelochemicals. [10] have reported that taro plants produce secondary metabolites, i.e. flavonoid and alkaloid. [11] has shown that fern produces sesquiterpene. Various kind of grasses has also been reported to contain allelochemical. [12] reported that root and aerial parts of *Echinochloacolona* and *Cyperusiria* reduce germination and suppress the early seedling growth of rice and soybean. [13] reported that Cynodonsp, a tropical forage grass, produces flavonoids. Since those allelochemicals can be released during residue decomposition [14,15], applying fresh weed clipping could easily injure the root system except vanilla plant resistant to the allelochemical.

According to [16], different allelopathic species have different activity and crop growth reduction depends upon its sensitivity. For example, [17] has examined the effect of aqueous and ethanol extract of various weeds on the growth of wheat. These authors found that aqueous extract of *Cyperusrotundus* and *Euphorbia hirta* reduce shoot length of the wheat where *Cyperus rotundus* has a stronger effect than *Euphorbia hirta*. By contrast, sesame plant leachate has been found to reduce the growth of *Cyperusrotundus* [18]. Indicating that different weeds could show a different effect on a certain species of crop or vice-versa.

The sensitivity of vanilla plant to allelochemical that released by weed clipping has received a little attention. It remains unclear which of the fresh weed clipping, commonly found in vanilla plantation, is safe for maintaining a sufficient level of soil organic material. Accordingly, this study examined the susceptibility of vanilla plants to the addition of weed clipping commonly found in vanilla plantation, i. e.fresh grass, fern and taro clippings.

2. Materials and methods

2.1. Experimental sites

This field study was located in Wongaya Gede, Tabanan, Bali, ca 700 m (above sea level), latitude and longitude are -8°22’39” and 115°6’92’’, respectively. This experiment was conducted twice where the first experiment was conducted from 9 December 2018 until 21 April 2019 in the wet season. The second experiment was commenced on 8 June 2019 in the dry season. Weather conditions during these growth experiments were collected from the internet, https://www.accuweather.com/en/id/ (figure 1A, B, C, D). Soil pH and soil moisture were observed using soil survey instrument (figure 4A, B).

2.2. Plant material and application of mulches to examined the effect of mulches on the growth of vanilla cuttings

Experiments to examine the effect of fresh weed clipping on the growth of vanilla cutting were conducted twice. The first experiment was conducted in a wet season where air temperature and daily precipitation was higher. In this experiment, two nodes vanilla cuttings were grown in 5 groups of pots,

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one cutting in 1 pot and each group consisted of 8 pots. So, a total of 40 cuttings were used in this experiment. One upper leaf of these 2 nodes vanilla cuttings were remained intact but the lower leaf was excised and all root systems on the cutting were excised. The growth medium for these cuttings was 0.5-liter topsoil collected from the local plantation and contained in 1-liter pots. The pots were then added mulch until it's full. The first group of the pots was mulched with freshly mixed-grass clippings comprises mainly of grass and euphorbia weed (G). The second, third and fourth groups were mulched with fern clippings (F), taro clippings (T) and dry leaf (DF). The fifth group was not mulched as control cuttings (C). Those fresh weed clipping were collected from locally raised weeds and applied into growth medium every 4 weeks, i.e. at day 0 (M1), day 28 (M2), day 56 (M3) and day 84 (M4). Dry leaf for mulching the cuttings was also collected from the local plantation and was applied only twice, at day 0 and day 56. These pots were mounted in a greenhouse without a roof to allow natural watering from rainwater. This greenhouse was shaded using shading net to reduce light intensity.

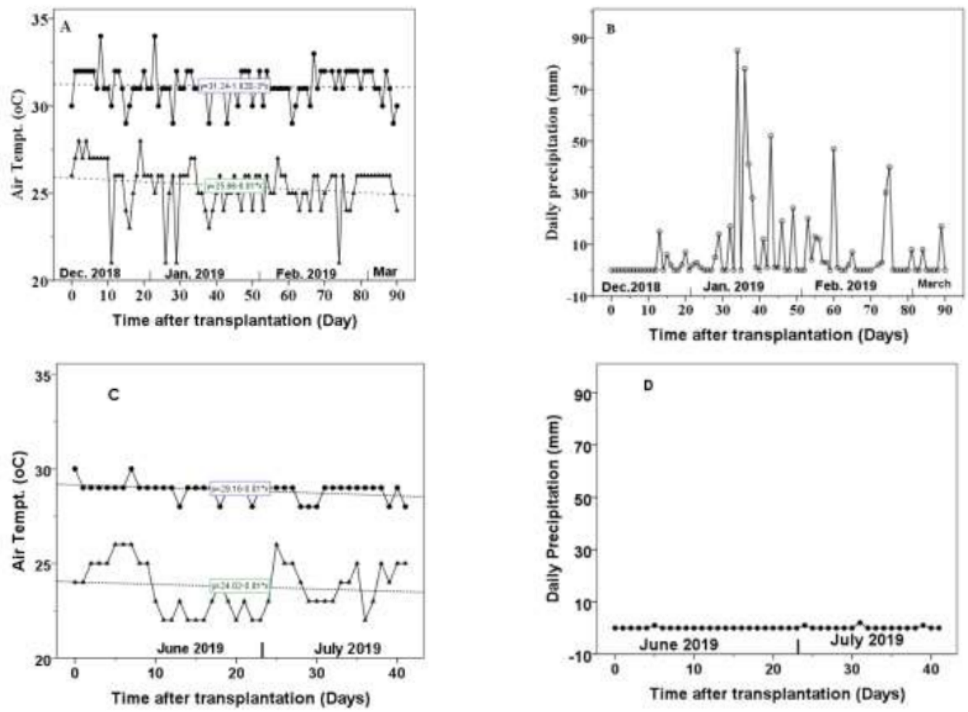

Figure 1. A: Weather condition in Wongaya Gede during the wet season in experiment 1. Average high temperature and low temperature (°C) were 31.0 ± 0.8 and 25.3 ± 1.2. **B:** Daily precipitation during experiment 1. The average daily precipitation (mm) was 6.9 ± 14.3. **C:** Weather condition in Wongaya Gede during experiment 2. Average high and low air temperature (°C) were 28.9 ± 0.4 and 23.8 ± 1.4. **D.** Daily precipitation during experiment 2. The average daily precipitation (mm) was 0.4 ± 0.2.

The second experiment was conducted during the dry season where air temperature and precipitation were lower. Plant material and application of mulch were performed using a similar procedure like that in experiment 1, except that dry leaf mulch was replaced by coconut husk (CH) and grass clippings were not mixed-grass but only Oplismenus sp. Watering was performed every week using drip irrigation systems. The emergence of new root and the bud burst were observed every week commenced on day 7 after the time of transplantation. The number of leaves produced in the newly growing stem was also

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observed every week. Statistical analysis for data on the growth parameter was processed using IBM SPSS data editor.

2.3. Preparation of weed extract

Method for preparation of extract was modified from a method described by [12]. Grass (*Oplismenus hirtella*), fern (*Athyrium sp*) and taro (*Colocasia esculenta*) leaves were collected from a plantation. These plants were chopped into pieces with a knife before oven-dried at 48°C for 72 hours. The dried material was then powdered using a blender. Fifteen-gram powder of each plant material was suspended in 150 ml aquadest for 24 hours. The extract was then filtered using filter paper (Whatman number 40). The filtrate obtained from this extraction is assumed to be 100 g/L extract.

2.4. Determination of phenolic compound in weed extract

Total phenolic content in the weed extract which is regarded as allelochemical was determined in Food-Health Laboratory, Denpasar. 5 ml of the plant extract (0.1 g/ml) were diluted into 25 ml aquadest in a calibrated flask. 0.5 ml of this diluted extract were then pipetted into a 25 ml flask before added 9.5 ml aquadest and 1 ml Folin-Ciocalteu reagent. After vortexed for 5 minutes, 10 ml Na_2CO_3 solution (7%) was added and incubated for 90 minutes at room temperature. Absorbance was then measured using spectrophotometer at a wavelength of 750 nm.

3. Results

3.1. The emergence of the new root system and the bud burst after addition of mulches under the condition of the wet season

The addition of grass clipping mulch at day 0 (M1) caused the vanilla cutting to produce the root system on day 14 and was not increased until days 28 when the second mulch was added (M2). After the addition of this second mulch, vanilla cutting was showing further growth at day 35 until day 49 (figure 2A). However, the addition of mulch at day 54 (M3) did not make the cutting to show further growth even though 25% of cutting has not grown. Unlike grass clipping, the addition of fern clipping at day 0 (M1) caused the cutting to show root growth at day 21 and increased until day 28. When the second addition of mulch was applied (M2), there was no cutting showing further growth for 7 days before then grew until days 49. Further addition of mulch at day 54 (M3) and day 84 (M4) did not affect cutting to initiate root emergence. The addition of taro clipping caused the vanilla cutting to show later root growth than the addition of fern clippings. After the addition of taro clipping at day 0 (M1), root growth was firstly found on day 28. The addition of taro clipping at day 28 (M2) did not inhibit further growth for 2 weeks until day 42. The addition of M3 and M4 also did not affect further growth of root systems. The addition of dry leaf mulch caused the vanilla cutting to show root growth from day 14 to day 28. Further growth was not found until the termination of the experiment. Unlike root growth in those mulched cuttings, control plant firstly showing root emergence on day 14 and continued until 100% of cutting showing growth on day 35.

Under the condition of the wet season, the addition of grass clipping at day 0 (M1) caused the cutting to show budburst on day 28 (figure 2B). When M2 was added, further budburst was observed for 2 weeks until day 42. The addition of grass clipping at day 56 (M3) did not alter 6 week lag period until further cutting showing budburst at day 84. The addition of fern clipping at day 0 (M1) caused the cutting to show bud burst also at day 28. However when (M2) was applied, bud burst was ceased for a week before then resumed bud burst until day 49. Similar to that found in grass added cuttings, the addition of fern clipping at day 56 (M3) did not alter lag period until day 77. Addition of taro clipping at day 0 (M1), day 28 (M2) and day 56 (M3) caused the cutting to begin budburst at day 70, i.e. 14 days after addition of M3. The addition of dry leaf at day 0 (M1) caused the cutting to show budburst on day 28. Although dry leaf was not added at day 28, cutting was not showing bud burst in the ensuing 1 week period before a higher number of cutting were showing bud burst until day 42. Cutting added dry leaf then underwent a lag period for 7 weeks. In control cutting, bud burst was began at day 28 and the number was continuously increased until day 56 when all cutting has shown bud burst.

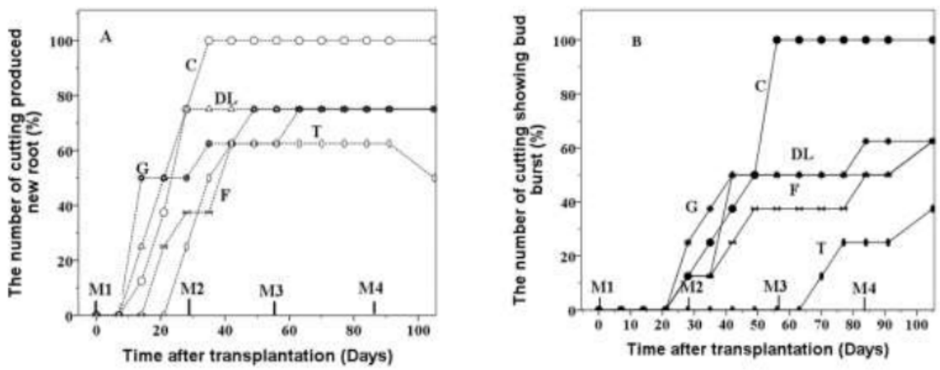

Figure 2. A: The initial growth of the root system in 2 nodes vanilla cutting after the addition of mulch under the condition of the wet season. C, Control; DL, Dry leaves; G, Grass; F, Fern; T, Taro. **B:** Budburst in 2 node vanilla cutting after addition of mulches under the condition of the wet season.

3.2. The emergence of the root system and bud burst under the condition of the dry season

Under the condition of dry season, vanilla cutting mulched with grass clipping, coconut hush and control at day 0 (M1) caused the cutting to commence new root production on day 22 (figure 3A). This root production is about 7 days later than in the wet season. In grass clipping added cutting, the production of the new root was continued until day 36. The addition of coconut husk showed similar production of new root like that found in control where a high number of cutting firstly showing new root growth on day 22 and continued until day 28 before underwent lag period. The addition of taro clipping caused the cutting to begin showing new root production at day 28 and continued until day 36.

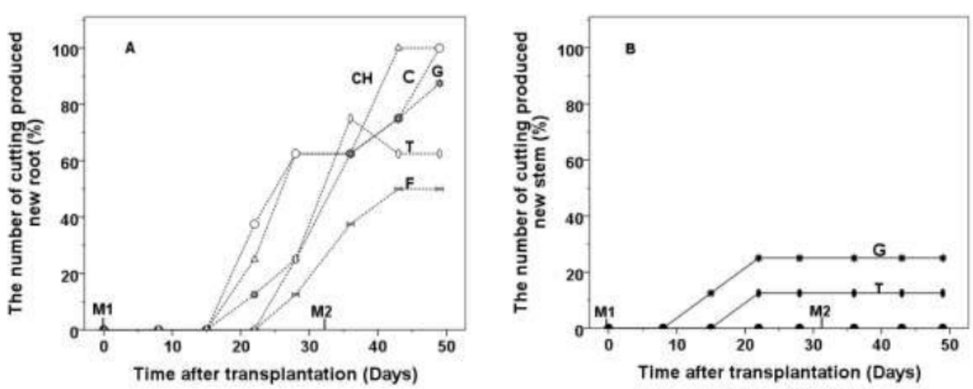

Figure 3. A: The emergence of the root system in 2 nodes vanilla cutting after the addition of mulches under the condition of dry season. C, Control; CH, Coconut husk; G, Grass; F, Fern; T, Taro. **B:** Budburst in 2 nodes vanilla cutting after addition of mulches under the condition of dry season.

Unlike in the wet season, in the dry season, some cutting has been found to show bud burst before the production of the root system (figure 3B). This early budburst was found in grass and taro added cutting. At day 15, vanilla cutting mulched with grass clipping has shown 12.5% bud burst although this cutting produced new root at day 22. In taro added cutting, 12.5% budburst was found on day 22, although this cutting produced root on day 28. Even though, the number of cutting showing budburst was not found increased until days 36. Vanilla cutting added coconut husk, fern and control cutting has not been found to show budburst at day 36.

3.3. Soil pH and soil moisture after addition of mulches

Relative to the control growth medium, the addition of coconut husk changed pH from 6.01 into 6.03 which is very small (0.02) if it is compared to the addition of fresh mulches (figure 4A). The addition of fern and taro decreased pH into 5.87 and 5.90. This decreased accounted for about 0.14 and 0.11. Although statistically was not significant, $p = 0.163$ and 0.277 for fern and taro, these changes are strong enough to delay the emergence of a new root system. By contrast, the addition of grass clipping was found to increase pH into 6.23 and statistically significant ($p = 0.031$). However, although pH changes in soil were higher than fern and taro, the addition of grass did not delay the production of the new root system (figure 3A).

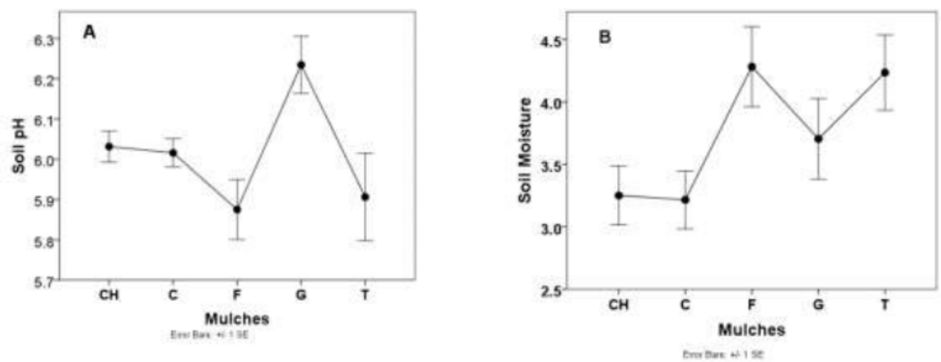

Figure 4. A: Soil pH in the growth medium under the condition of the dry season in experiment 2. C, Control; CH, Coconut husk; G, Grass; F, Fern; T, Taro. **B:** Soil moisture in the growth medium under the condition of the dry season.

Relative to the control growth medium, the addition of fern and taro mulch significantly increased soil moisture ($p = 0.009$ and 0.013 for fern and taro) but not coconut husk and grass ($p = 0.932$ and 0.230), figure 4B.

3.4. Viability of vanilla cutting after addition of various mulches during the wet season

Under the condition of warmer and wetter weather conditions, all cutting transplanted into the control growth medium was showing growth until the experiment was terminated on day 133 (figure 5). Cutting transplanted into growth medium with addition of taro and fern clipping was firstly showing rotten symptoms and unable to produce new stem. In the ensuing period, vanilla cutting added grass clipping and dry leaf was also showing rotten stem. At the end of observation, vanilla cutting added taro clipping was showing the lowest viabilities.

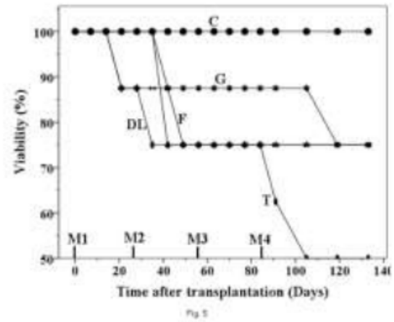

Figure 5. Viability of Vanilla cutting transplanted into topsoil growth medium with addition of various mulch. C, Control; DL, Dry leaf; G, Grass; F, Fern; T, Taro.

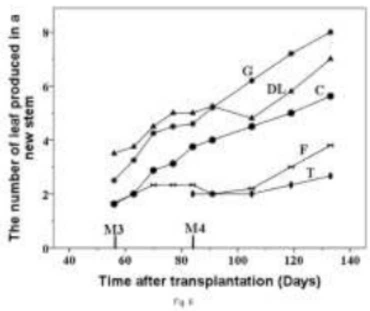

Figure 6. The average leaf number produced by the new stem in the growing cuttings. C, Control; DL, Dry leaf; G, Grass; F, Fern; T, Taro.

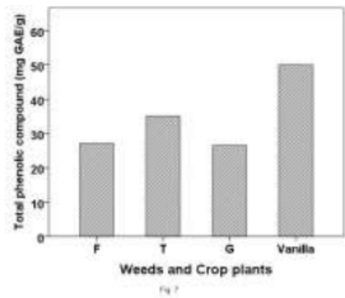

Figure 7. The total phenolic compound in weed and vanilla plants.

3.5. Production of leaves in the new growing stem

After the cuttings have ceased to show new root emergence or budburst, in the wet season, leaf growth was then clearly visible in growing cuttings (figure 6). Relative to control plants, the rate of leaf production was higher in cutting added grass clippings and dry leaf mulch, but the addition of fern and taro clipping was showing slower leaf production. Based on the number of leaves produced on day 133, the growth rate of the new growing stem was increased by the addition of grass and dry leaf mulch but repressed by the addition of taro and fern mulches (table1).

Table 1. The number of leaves produced in the new stem of growing cutting on day 133.

Mulch-type	N	The number of growing cutting (%)	The number of leaf in the new stem	Growth rate (leaf day^{-1})
Taro	3	37.5	2.6^{a}	0.023
Fern	5	62.5	3.8^{ab}	0.033
Control	8	100	5.6^{bc}	0.049
Dry leaf	5	62.5	7.0^{c}	0.061
Grass	5	62.5	8.0^{c}	0.070

The growth rate in grass added plants was 1.4 fold relative control but only 0.46 fold in taro added plant. Statistically, the number of leaves produced by cutting added grass clipping was significantly higher than control ($p = 0.04$). By contrast, the number of leaves produced by cutting added taro clipping was significantly lower than control. Cutting grown with the addition of taro and fern clipping was not different significantly.

3.6. The total phenolic compound in weed and vanilla plant

By using the Folin-Ciocalteu method, a total phenolic compound in weeds was 26.6, 27.2, 35.1mg GAE/g for Grass (Oplismenussp), Fern (Athyrium) and Taro (Colocasia), respectively. Vanilla leaf as crop plants were found to contain a much higher total phenolic compound than taro leaf, i.e. 50.09 (figure 7).

4. Discussion

Developing vanilla plantation in the agroforestry system is promising and challenging. Since vanilla bean is regarded as the most expensive spices, smallholder farmers in rural areas could enhance their income by developing vanilla plantation. However, vanilla plants encounter serious constraint during the dry season because it has a very shallow root system which makes this plant receiving the impact of drought in the first instant [6]. In order to mitigate this constraint, the addition of vegetative mulch is required to lengthen soil moisture [1,2]. Fortunately, there is plenty of plant material available in an agroforestry system for mulching. However, some of this plant may release allelochemical during its decomposition [14,15] and subsequently could injured vanilla plants. This present study found that different mulch affected the emergence of new root and bud burst differently, in agreement with conclusion [8]. This evidence indicates the possible nutrient and allelochemical release from weed clipping and other mulches which affected the growth of vanilla cutting.

In order to determine which of the mulches favor growth in vanilla cutting, firstly it is assumed that the mechanism of initial growth in vanilla cuttings is similar to that occurred in seeds. According to [19], the initial growth of new root and stem in a seed is preceded by the transport of low molecular products into an embryo from macromolecular stored reserves. This process involving a growth regulator and enzymes that sensitive to external abiotic conditions. In this present study, it was found that the addition of fresh fern and taro clipping delayed initial growth of new root system and bud burst (figure 2A, B). However, this delay was not found in cutting added fresh grass clipping, dry leaf or coconut husk. This indicated that the addition of fern and taro clipping could change the abiotic factor and inhibit at least the transport of previously-stored nutrients into meristematic tissues. This indication is supported by data found in this present study where the addition of fern and taro mulch substantially decreased soil pH (figure 4A). By contrast, the addition of grass clipping and coconut husk did not decrease soil pH. Since growth regulator and enzymes is generally regarded as sensitive to pH, lowering pH in soil growth medium most likely repressed the activity of whether enzymes or growth regulator that involved in nutrient reserve mobilization.

During the decomposition process, the fern and taro clippings could release a sufficient amount of allelochemical which could also inhibit the mobilization process. As shown by data in figure 7, total phenolic content in taro extract is much higher than in grass extract which implying that the potential release of allelochemical is higher in taro than grass clipping. Even though, the total phenolic content did not proportional to the delay of root emergence and bud burst after the addition of M1. For example, total phenolic in grass extract was 75% relative to the amount in taro extract (figure 7), but the lag period of root emergence after the addition of grass clipping was only 50% relative taro mulch (figure 8A). The lag period of budburst after addition of M1 was even shorter, i.e. only 40% relative to the addition of taro clipping (figure 8B). Importantly, the lag period in grass added cutting similar to that found in cutting added dry leaf, coconut husk and control. This evidence is strongly indicated that phenolic released by taro clipping is more toxic than that release by grass clippings. The toxicity of compounds released by taro clippings is also evidenced by the viability of cutting after addition of mulch (figure 5). In cutting added taro mulch, the viability of cutting was only 50%, but in grass added mulch viability was 75%. This could also indicate that vanilla plants resistant to phenolic compounds released by grass clippings but susceptible to phenolic compound release by taro mulch.

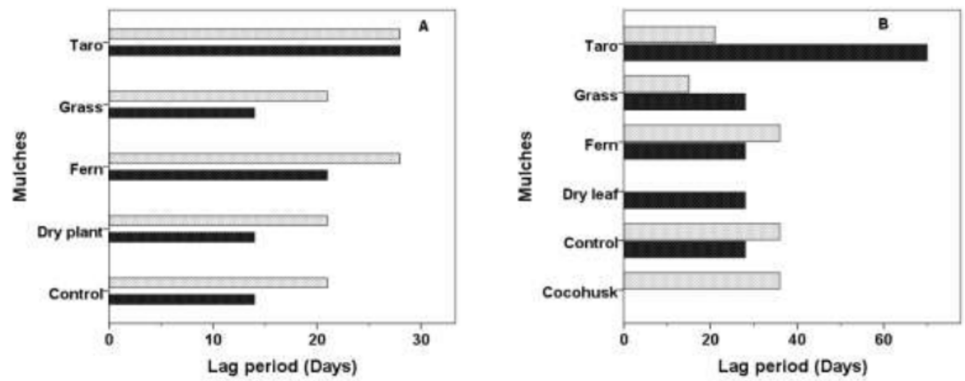

Figure 8. A: Lag period in root emergence after the addition of mulches at day 0 (M1). □, the lag period during the dry season; ■, the lag period during the wet season. **B:** the lag period of budburst after the application of mulches at day 0 (M1). □, the lag period during the dry season; ■, the Lag period during the wet season.

Other than allelochemical, mulches could also release nutrients that enhanced the growth of cuttings. Under the condition of the wet season, bud burst and further growth were higher in cutting added grass clipping and dry leaf rather than in fern and taro mulches (figure 2B). Since budburst was occurred two weeks after the emergence of the root, the growth of new stem in the cutting most likely has involving nutrient uptake from the soil via the new root system. As shown in figure 2B, bud burst was much higher in cutting added grass rather than in taro added cutting which indicating that grass released nutrients into growth medium and taken up by new growing root system. This indication is supported by the growth rate of the new stem where cutting in G and DL showed a much higher rate than in C, F and T (figure 6). Statistically, the number of leaves produced in G cutting was significantly higher than in T cuttings (Table 1).

5. Conclusion

The optimal high-low air temperature required by vanilla to grow is 31-25°C and daily precipitation 6.9 mm. Under weather condition in a tropical country, grass clipping, dry leaf and coconut husk is an important resource for the vanilla plant. Firstly because vanilla plants acquired nutrients previously released by the decomposition of grass clipping, dry leaf and coconut husk. Secondly, vanilla plant tolerant to phenolic compound released by the grass clippings. By contrast, although nutrients may also release during decomposition of fern and taro clipping, vanilla plants are highly susceptible to phenolic compounds released by taro clippings. Thus, to sustain vanilla plantation, fresh grass clipping, dry leaves and coconut husks are found relatively safe for mulching but not fern and taro clipping. By returning grass clipping and fallen dry leaves of broadleaf plants to the plantation, establishment of vanilla plantation could then become much cheaper for smallholder farmers in a rural area.

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