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Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta*. 50th Annual Symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

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Thesis, Dissertation:

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Various antioxidant assays of agarwood extracts (*Gyrinops versteegii*) from West Lombok, West Nusa Tenggara, Indonesia

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Abstract. *Prihantini AI, Rizqiani K. 2019. Various antioxidant assays of agarwood extracts (*Gyrinops versteegii*) from West Lombok, West Nusa Tenggara, Indonesia. Asian J Agric 3: 1-5.* Agarwood extracts (Gyrinops versteegii) have not been widely explored as a source of natural products in particular antioxidant agents, which protect cells from damage caused by free radicals. The present study was aimed to evaluate antioxidant activities of agarwood extracts from West Nusa Tenggara using various antioxidant assays. The antioxidant activity of leaf, fruit and fruit bark extracts was investigated based on DPPH radicals scavenging activity, reducing power, and β-carotene bleaching assays. The total phenolic content was also investigated. The result showed that leaf extract revealed the strongest antioxidant activity on all assays performed such as DPPH radicals scavenging activity (IC_{50} 22.13±0.71 μg/mL); reducing power (251.85±0.03 mg QE/g dry extract); and β-carotene bleaching activity (IC_{50} 24.23±2.60 μg/mL). The total phenolic content (TPC) in the leaf was higher (184.90±0.76 mg GAE/g dry extract) than fruit bark and bark extracts. The high content of phenolic compounds in *G. versteegii* leaves indicated that these compounds might contribute to the antioxidant activities. In conclusion, these findings showed that *G. versteegii* leaves are potential for development as an antioxidant source.

Keywords: Agarwood, antioxidant, *Gyrinops versteegii*, West Nusa Tenggara

INTRODUCTION

Free radicals have an important role in our health. Reactive Oxygen Species (ROS) which are generated from oxygen, can lead to oxidative stress with the affection of various biological functions and structural changes during metabolism or other activities if it is increased and imbalanced between producing radical and antioxidant (Nimse and Pal 2015; Ramamoorthy and Bono 2007; Zima et al. 2001). This oxidative stress can cause a disturbance in numerous physiological processes and development to many degenerative disorders, such as cancer, cardiovascular, Alzheimer disease, neurodegenerative disease, asthma, autoimmune and gastrointestinal diseases, and the aging process (Siti et al. 2015; Arituluk et al. 2016; Wojtunik-Kulesza 2016).

The radicals can be overcome by antioxidants. Antioxidants are compounds that can inhibit ROS, reactive nitrogen species and other free radicals to prevent damage in normal cells, proteins and fats which ultimately prevent degenerative diseases (Arif et al. 2014). Presently, synthetic antioxidants commonly used such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) might affect genotoxic, carcinogenic effects, and hemorrhaging (Babbar et al. 2010; Salamah et al. 2011; Stankovic 2014). Due to this reason, natural antioxidant sources might be found to substitute synthetic antioxidants.

Agarwood is considered precious plant in the world, particularly in Asia, due to its high value of resin impregnated-heartwood from the family of *Thymelaeaceae* (Ismail et al. 2015). In Indonesia, agarwood is generally grown in Borneo (12 species), Sumatra (10 species), Nusa Tenggara (3 species), Papua (2 species), Sulawesi (2 species), Java (2 species), and Moluccas (1 species). The common main genus of agarwood in Indonesia are *Aquilaria malaccensis*, *A. microcarpa*, *A. beccariana*, *A. hirta*, *A. filaria*, *A. cumingiana* and *Gyrinops* (Hadi et al. 2011; Santoso et al. 2014). *Gyrinops versteegii* (Gilg.) Domke is an endemic agarwood that is in Lombok, West Nusa Tenggara Province. Most people in this area use it for resin production. The inoculation of fungi into the agarwood is conducted at the optimal age of the tree to produce optimal yield of resin. During waiting for the harvest of agarwood, certain communities use the agarwood leaves as a herbal tea which is believed that it is effective to cure various diseases including malaria, tumor, fatigue fever and flu symptoms (Nuringtyas et al. 2018). Mega and Swastini (2010) proposed that agarwood leaf extract contains secondary metabolites such as flavonoids, terpenoids and phenol compounds. These secondary metabolites were suspected to have antioxidant activities. Therefore, the aim of this study is to evaluate the antioxidant activities from leaves, fruits, and fruit barks of *G. versteegii* using various antioxidant assays.

MATERIALS AND METHODS

Extract preparation

Fresh and healthy leaves, fruits, and fruit barks of *G. versteegii* were dried at room temperature and blended. Approximately 20 g of the dried materials were extracted with methanol. The methanol extracts were then filtered and concentrated under a vacuum rotary evaporator and dried at room temperature prior to antioxidant assays.

DPPH radical scavenging activity assay

DPPH free radicals scavenging activity was evaluated according to Prihantini et al. (2014). Various concentrations of each sample were mixed with 0.5 mL of the 1 mM DPPH radical solution in methanol. For the control, a similar solution with the absence of sample was used. Samples were then incubated at room temperature under dark conditions for about 30 min. Absorbance (A) was measured with a spectrophotometer at 517 nm. The percentage of scavenging activity was determined by the following equation:

$$\text{Scavenging activity (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100\%$$

β-carotene bleaching assay

The β-carotene bleaching assay was performed according to Prihantini et al. (2014). Approximately 0.2 mg of β-carotene, 20 mg of linoleic acid and 200 mg of Tween40 were mixed in 0.5 mL chloroform, which was then removed using a rotary evaporator at 40°C. The resulting mixture was diluted with distilled water and mixed vigorously. The mixtures were made up to 50 mL and aliquots (4.8 mL) were added to test tubes containing various concentrations of each sample and methanol (control). A similar mixture without β-carotene was used for the background of the samples. The tubes were incubated at 50°C for 2 h. The absorbance at 470 nm was measured at 0 min either for the control (A_{c0}) or samples (A_{s0}). The absorbance of both the control (A_{c120}) and samples (A_{s120}) was measured after 120 min incubation. Furthermore, the antioxidant activity was evaluated with the following equation:

$$\text{Antioxidant activity (\%)} = 100[1-(A_{c0}-A_{s0})/(A_{c120}- A_{s120})]$$

Reducing power assay

The reducing power assay was performed according to Prihantini et al. (2014). Approximately 0.5 mL of various concentrations of samples in methanol was mixed with phosphate buffer (2.5mL, 0.2 M, pH 6.6) and potassium ferry-cyanide (2.5 mL, 1%). Trichloroacetic acid (2.5 mL, 10%) was added to the mixture after 20 min incubation at 50°C. Then, the mixture was centrifuged at 3000 rpm for 10 min. The upper layer solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride solution (0.5 mL, 0.1%). Absorbance was measured with a spectrophotometer at 700 nm. The reducing power assay was measured as quercetin equivalent.

Total phenolic content

The total phenolic content (TPC) of plant extracts was determined using Folin-Ciocalteu reagent (Prihantini et al. 2014). Approximately 500 µL of the extracts (1.0 mg mL^{-1}) was added with distilled water, made up to 8 mL and then mixed with 500 µL of 2 N Folin-Ciocalteu reagents. The mixture was allowed to stand for 8 min and 1.5 mL of 20% sodium carbonate was then added. The reaction mixture was incubated at room temperature for 2 h. Absorbance was measured at 765 nm and the phenolic content was determined using a calibration curve obtained from concentration of gallic acid.

Statistical analysis

All assays were performed in triplicate in independent three experiments. The data were expressed as the Mean+S.D value and analyzed by SPSS for windows followed by Tukey's post hoc test. Values with $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

There are increasing focuses on products extracted from nature to treat many human diseases. Antioxidant helps our body in prevention and healing process of oxidative stress caused by free radicals. A natural antioxidant can be extracted from plants (Chirag et al. 2013; Krishnaiah et al. 2011; Prihantini et al. 2015). However, due to the complex nature of phytochemicals, various antioxidant assays are necessary to evaluate antioxidant activity. Furthermore, total phenolic contents assay is also commonly used to complement antioxidant assays.

DPPH radicals scavenging activity

DPPH radicals scavenging assay is a common method to evaluate antioxidant activity of nature. The DPPH assay reflects a scavenging reaction between DPPH and the related samples. DPPH is a stable free radical, which turns from purple to yellow when it is scavenged by antioxidants as hydrogen donors. The delocalization of the DPPH radicals determines the occurrence of a purple color. When DPPH radical (2,2-diphenyl-1-picrylhydrazyl) reacts with a hydrogen donor, the stable molecule (2,2-diphenyl-1-hydrazine) is generated resulting in the discoloration of the purple color (Pisoschi and Negulescu 2011). The degree of discoloration indicates the scavenging potential hydrogen donating ability of the related samples.

The study revealed that leaf extract of *G. versteegii* showed the highest activity than others with IC_{50} 22.13±0.71 µg/ml followed by fruit bark (76.87±3.64 µg/ml) and bark extracts (119.49±3.84 µg/ml), and it is significantly different activities ($p<0.05$) among the extracts (Table 1). The result indicates that leaf extract has the most potential ability on hydrogen donation. The higher activity of leaf extract was also reported by Mahdi-Pour et al. (2012).

Table 1. DPPH radicals scavenging activity and β-carotene bleaching activity of *G. versteegii*

Extracts	IC_{50} on DPPH radicals scavenging activity (μg/ml)	IC_{50} on β-Carotene bleaching activity (μg/mL)
Leaf	22.13±0.71[b]	24.23±2.60[a]
Bark	119.49±3.84[d]	104.97±0.1[c]
Fruit bark	76.87±3.64[c]	35.18±1.27[b]
Quercetin*	7.4±0.1[a]	n.a

Note: Data is expressed as Mean±S.D values, different letters in the same column indicate significant differences (p<0.05) at Tukey's post hoc test, n.a: Not available, *: Prihantini et al. (2014)

Table 2. Reducing power of *G. versteegii*

Extracts	Reducing power (mg QE/g dry extract)
Leaf	233.89±14.14[c]
Bark	99.44±5.50[a]
Fruit bark	122.78±7.38[b]

Note: Data is expressed as Mean±S.D values, different letters in the same column indicate significant differences (p<0.05) at Tukey's post hoc test, QE: Quercetin equivalent

However, in some cases, the assay gives incorrect results and recommendations. Some complications could be caused by partial ionization of the related samples, which affect the rate of their reaction with DPPH radicals (Tirzitis and Bartosz 2010). Therefore, other antioxidant assays are necessary to confirm and consider the recommendations.

β-carotene bleaching activity

β-carotene bleaching assay is another assay to evaluate antioxidant activity of nature. It performs discoloration in the absence of an antioxidant compound. Therefore, the presences of antioxidant molecules inhibit the extent of β-carotene bleaching caused by linoleic-free radicals. The radicals may attack the double bonds of β-carotene and cause discoloration. Antioxidants reduce the discoloration of β-carotene by stabilizing the linoleic-free radical and other radicals formed in the system (Jayaprakasha et al. 2001).

β-carotene bleaching activity of leaf, bark, and fruit bark extracts of *G. versteegii* is shown in Table 1. As DPPH radical scavenging activity, the leaf extract had the highest activity (IC_{50} 24.23±2.60 μg/mL) compared to other extracts. The result showed that the activity was statistically significantly different with fruit bark and bark at IC_{50} 35.18±1.27 and 104.97±0.1 μg/mL, respectively. It indicates that leaf extract compounds had high capability of neutralizing the free radicals generated in the system. It is known that β-carotene assay has a different mechanism involved in the method compared with DPPH assay (Prihantini and Tachibana 2017). However, the present study results in a similar order of the antioxidant activity. Therefore, the result of β-carotene bleaching activity supports the potential order of antioxidant activity as leaf > fruit bark > bark as the result of DPPH radicals scavenging activity.

Reducing power

Reducing power may serve as a significant indicator of the potential antioxidant activity. Compounds with reducing power indicate that they are electron donors. The presence of antioxidants causes the conversion of ferricyanide (Fe^{3+}) complex in the system to ferrocyanide (Fe^{2+}) by donating their electrons. The ferrocyanide then reacts with ferric chloride to form ferric ferrous complex (Pearl's Prussian blue). By measuring the formation of Pearl's Prussian blue at 700 nm, it is possible to determine the concentration of Fe^{3+} ion electrons (Jayanthi and Lalitha 2011). The result of reducing power of *G. versteegii* is shown in Table 2. The reducing power activity is shown as quercetin equivalent (QE). The higher quercetin equivalent indicates the higher reducing power of the extract. Extracts showing high reducing power activity indicate an enhanced capability to donate electrons.

The reducing power assay revealed leaf extract with the highest value, followed by fruit bark, and bark extracts (233.89±14.14; 122.78±7.38; 99.44±5.50 mg QE/g dry extract, respectively). A similar trend as DPPH radical scavenging activity and β-carotene bleaching activity, resulted in statistically significantly different at $P < 0.05$ among the extracts. Therefore, the result suggested that leaf extract of *G. versteegii* had high capability to donate electrons. Furthermore, the reducing ability was found to be concentration-dependent in which the reducing power increased with the increasing of the concentrations as shown in Figure 1. Higher absorbance of the reaction mixture indicates higher reduction ability (Jayanthi and Lalitha 2011).

Total phenolic content

Most phenolic compounds consist in the plant. Phenolic compounds are considered to play a role against a wide range of diseases (Ibrahim et al. 2012). The total phenolic content of *G. versteegii* is shown in Table 3. The results were expressed in gallic acid equivalent.

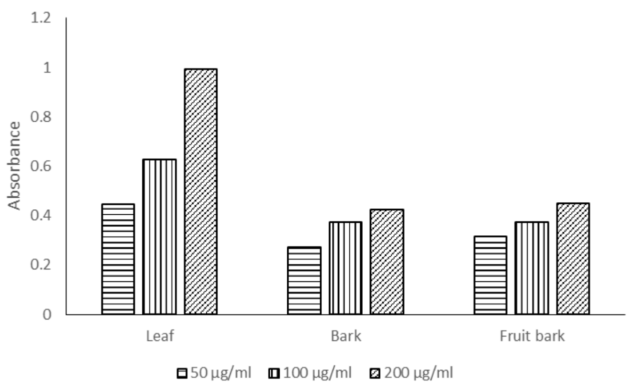

Figure 1. Reducing power assay of *G. versteegii* at different concentrations

Table 3. Total phenolic content of *G. versteegii*

Extracts	Total phenolic content (mg GAE/g dry extract)
Leaf	184.90±0.76[c]
Bark	67.65±0.23[a]
Fruit bark	113.02±0.38[b]

Note: Data is expressed as Mean±S.D values, different letters in the same column indicate significant differences ($p<0.05$) at Tukey's post hoc test, GAE: Gallic acid equivalent

The results showed that leaf extract had the highest total phenolic content, followed by fruit bark and bark extracts (184.90±0.76; 113.02±0.38; 67.65±0.23 mg GAE/g dry extract, respectively). It was statistically significant difference among the extracts at $P < 0.05$. The highest total phenolic content of leaf extract might be assumed that phenolic compounds present in the extract, gave contribution to the antioxidant activity in DPPH radicals scavenging assay, reducing power, and β-carotene bleaching assay. Phenolics can donate their electrons to stabilize radicals (Chanda and Dave, 2009; Seal, 2012). Furthermore, the donation of a hydrogen atom that stabilizes the free radicals is considered from phenolic hydroxyl groups (Prihantini et al. 2015). Other studies also reported that the phenolic hydroxyl group is excellent to scavenge free radicals (Jeong et al. 2007; Moalin et al. 2011). The electron donation is without resulting themselves reactive radicals, therefore suggesting that phenolics are considered as a good antioxidant. Furthermore, revealing a similar order of extract having the potencies in all assayed performed, the result is consistent with the findings of several studies in reporting correlation between antioxidant activity and total phenolic content. In conclusion, all the antioxidant assays investigated in the study revealed that leaf extract had the highest antioxidant activity. This indicates that *G. versteegii* leaves have potential to be developed as natural antioxidant sources.

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Review: Rice momilactones, potential allelochemical for weeds suppression

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Abstract. *Estiati A. 2019. Rice momilactones, potential allelochemical for weeds suppression. Asian J Agric 3: 6-15.* Weeds are one of the biological constraints declining the productivity and quality of rice. Among weeds, *barnyardgrass* is reported as the most destructive weed species. Synthetic herbicides are the preferred method to control weeds. However, the excessive and continuous use of synthetic herbicides can have a negative impact on the environment, health and even the emergence of herbicide-tolerant weeds. Therefore, another alternative to overcome weed problems has concerned scientists. Rice plants have been proven to be able to suppress the growth of weeds nearby by secreting secondary metabolites called allelochemicals. In this article, the achievements of research on rice allelochemicals at laboratory level will be reported. Among rice allelochemicals, momilactones are potential growth inhibitors. The biosynthetic pathway of momilactones and their corresponding genes have been extensively investigated in rice. *OsCPS4*, *OsKSL4*, *CYP99A2*, *CYP99A3* and *OsMAS* are genes that co-regulated in momilactones biosynthetic pathway and production, and they form a gene cluster which is located on chromosome 4. Reverse genetic approach by inserting genes knock-out of *OsCPS4* and *OsKSL4* into two rice cultivars from *Japonica* subspecies showed that insertional mutant lines harboring *cps4* or *ksl4* exhibited a significant loss in inhibition potential due to the lack of momilactones production.

Keywords: Allelochemicals, growth inhibitor, momilactones, rice, weeds

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food in many regions around the world, especially in Asia. It globally provides approximately 20% of the caloric intake to more than 50% of the world's population (Khanh et al. 2013; Siddique and Ismail 2013; Amb and Ahluwalia 2016). With an increasing human population, rice production must increase to fulfill food security. However, cultivated rice is faced with problems that come from biotic and abiotic stresses, which lead to a decrease in productivity and quality. Weeds, insect pests, diseases, and abiotic stresses are major threats to rice production and climatic changes can worsen the situation (Farooq et al. 2013). Weed infestations are reported as the main biological constraint in reducing rice production. The loss of yield due to weed infestations is greater than the combined yield losses caused by insect pests and diseases (Asaduzzaman et al. 2010; Khang et al. 2016).

In paddy fields, cultivated rice and weeds always grow together although both plants compete for the same needs i.e., water, nutrient, light, space, and requirements for photosynthesis (Khanh et al. 2013). Weeds have competitive ability over cultivated rice since weeds grow taller and faster. Therefore, the presence of interfering weeds is a persistent problem for rice (He et al. 2012).

Worldwide, more than 1000 weeds species have been reported in paddy fields (Khanh et al. 2013). However, 12 of them are the important weeds species in Asia, i.e., *Cyperus difformis* L. (small flower umbrella), *Cyperus iria's* L. (rice flatsedge and umbrella sedge), *Echinochloa crus-galli* (L.) Beauv (*barnyardgrass*), *Echinochloa colona* (L.) Link. (jungle rice), *Eclipta prostrata* (L.) L. (false daisy), *Fimbristylis miliacea* (L.) Vahl (lesser fimbristylis), *Ischaemum rugosum* Salisb. (wrinkle duck beak), *Leptochloa chinensis* (L.) Nees (chinese sprangletop), *Ludwigia hyssopifolia*, *Schoenoplectus juncoides*, *Sphenoclea zeylanica* Gaertn (Gosseweed), *Oryza sativa* L. (weedy rice) (Rice Knowledge Bank). Among 12 weeds, *barnyardgrass* is reported as the most destructive weed and one of the most serious herbicide-resistant weeds (Tanveer et al. 2012; Zeinali et al. 2013). The presence of *barnyardgrass* together with rice plants in paddy fields can reduce rice production. When the ratio of rice plants to *barnyardgrass* in paddy fields is 10:1, this weed can reduce rice biomass and yields by 75% and 50%, respectively (He et al. 2012; Gealy et al. 2013; Yang et al. 2013).

Synthetic herbicides are believed to control weeds problems effectively, prevent crop yield loss and reduce labor in weeding. However, the use of synthetic herbicides can raise other problems such as high cost for crop production, the use of non-renewable energy resources, the excessive and overuse of herbicides may lead to the evolution of resistance in some paddy weeds to herbicides. Moreover, the negative effects on the environment and human health cannot be neglected (Fereira and Reindhart 2010; Bhadoria 2011; Mohammadi 2013). Thus, an alternative method to control weeds problem by minimizing the use of synthetic herbicide should be considered.

Allelopathy is reported as a self-defense mechanism of rice plants against weeds by suppressing weed growth. Although, in the rice fields, the weed-suppressive effect is more complex. It is a combination of competition for resources and the release of allelochemicals from rice varieties (Kato-Noguchi and Ino 2001; Kong et al. 2008). Laboratory bioassay is one of the appropriate methods for conducting preliminary studies on allelopathy because it is conducted under a controlled environment that can distinguish allelopathy effect from other competitive interference (Kato-Noguchi and Ino 2001). This paper reviews the published literature focused on allelopathy potential in rice plants and determines the allelopathy compounds that play an important role in inhibiting weed growth at laboratory level.

ALLELOPATHY, AN ALTERNATIVE TO SYNTHETIC BIOHERBICIDES

Allelopathy is defined as any direct or indirect harmful or beneficial effects on germination, growth, and development of one plant on another through the production of chemical compounds called allelochemicals that release into the environment (de Bertoldi et al. 2009; Bhadoria 2011; Bravo et al. 2013; Khanh et al. 2013). Although allelopathy has either a negative or positive effect, however many ecologists tend to interpret that allelopathy has only a negative effect from one plant to the surrounding plants which is considered as interfering plants by inhibiting their growth. These mechanisms occur to defend plants themselves (Bhadoria 2011; Bravo et al. 2013). The negative impacts of allelochemicals are harmful to the receiver plants but give a benefit to the donor plants (Bravo et al. 2013).

Many crops, including rice, have been reported to possess allelopathy properties (Dilday et al. 1989; Bhadoria 2011; Bravo et al. 2013; Amb and Ahluwalia 2016). To select rice accessions with strong allelopathic, International Rice Research Institute in the Philippines has developed well-designed bioassays at laboratory level under controlled environments called relay-seedling assay. This method eliminated the effect of resource competition between rice and test plants, thus the data obtained will represent only plant allelopathy (Kato-Noguchi 2004; Kato-Noguchi and Peters 2013). Relay-seedling assay has been routinely used in screening of hundreds of rice accessions and resulting in several rice accessions with strong allelopathic potential (Jensen et al. 2001; Olofsdotter et al. 2002).

By using relay-seedling assay, Olofsdotter et al. (2002) evaluated the effect of allelopathy from 50 rice cultivars on root elongation of *barnyardgrass* (one of the most destructive weed species) as a test plant. Thirty sterilized rice seeds were grown in Petri dish in two parallel rows and covered by Perlite. The distilled water flowed into Petri dish to maintain the moisture of Perlite. The Petri dishes were placed under 1300-3000 lx light density, with a photoperiod of 12 h and temperature between 29-33°C. On day 7, 20 *barnyardgrass* seeds as test plant were sown in between two rows of 7 -day-old rice seedlings in Petri dish. The root growth of *barnyardgrass* was measured at 17 days after incubation. The results exhibited that the roots growth of test plant were inhibited by 50 rice cultivars used with different reduction rate between 55%-81%. Data showed that 50 rice cultivars have different abilities in suppressing root growth of *barnyardgrass*, thus the activity of allelopathy is cultivars-specific. Based on this data, the 50 rice cultivars can be categorized as allelopathic cultivars and non-allelopathic cultivars (Olofsdotter et al. 2002). Microscope studies showed that secondary root growth of *barnyardgrass* was inhibited with the presence of allelopathic rice seedlings, meanwhile, no significant reductions were exhibited when grown together with non-allelopathic rice (Figure 1).

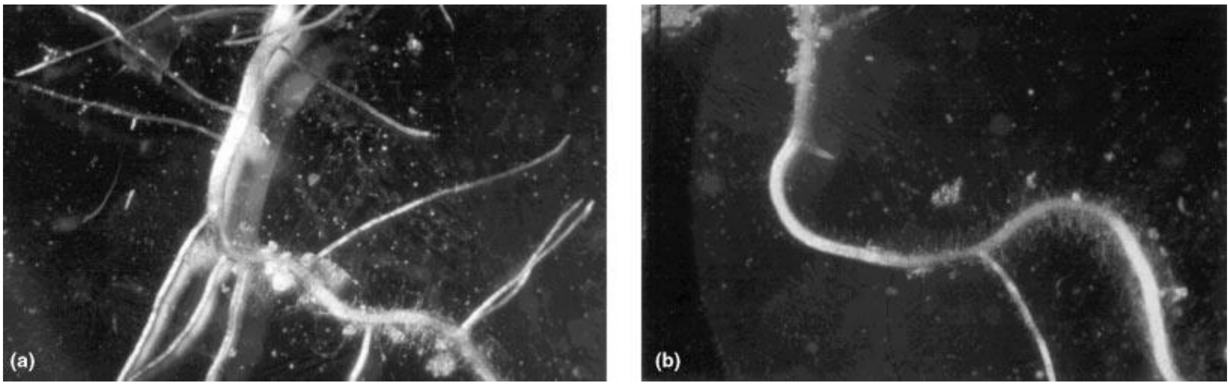


Figure 1. The secondary root growth of *barnyardgrass* when they grow together with rice seedlings of (a) non-allelopathic rice (*Aus 196*) and (b) allelopathic rice (*IR64*) in Petri dish. Secondary root growth of *barnyardgrass* was inhibited by the presence of allelopathic rice, *IR64* (Source: Olofsdotter et al. 2002).

Since cultivated rice has defense mechanisms toward weed species through the production of allelochemicals, these allelopathic compounds are considered plant-produced herbicides (Colquhoun 2006). And, since rice allelopathy is environmentally friendly and secreted from rice itself, utilizing allelopathy to suppress weeds growth surrounding cultivated rice as a promising approach for biological control of weeds in sustainable agriculture practice (Fang et al. 2013; Khanh et al. 2013).

RICE MOMILACTONE INVOLVEMENT IN ALLELOPATHY EFFECT

Most allelochemicals are classified as secondary metabolites, which are not required in primary metabolic processes (growth, development, and reproduction) of the allelopathic organisms (Ghahari and Miransari 2009; Belel and Rahimatu 2012). Many secondary compounds such as phenolic acids, hydroxamic acids, fatty acids, terpenes, and indoles were identified both in rice root exudates and rice residues as potential allelochemicals (Kato-Noguchi et al. 2010; Kato-Noguchi 2011; Kato-Noguchi 2012). However, although many secondary compounds have been reported as potential allelochemicals, only compounds that are released from the plants into environment and can inhibit the growth of neighboring plants that are considered as allelochemicals (Kato-Noguchi 2008; Kato-Noguchi et al. 2010).

Phenolic acid such as *p*-coumaric acid, *p*-hydroxybenzoic acid, ferulic acid and vanillic acid are found in aqueous extracts of rice straws, roots and residues and are often mentioned as the most important among potential allelochemicals (Kato-Noguchi 2004; Hui-Li et al. 2010; Siddique and Ismail 2013). However, their growth inhibition was relatively weak (Kato-Noguchi and Ino 2005a).

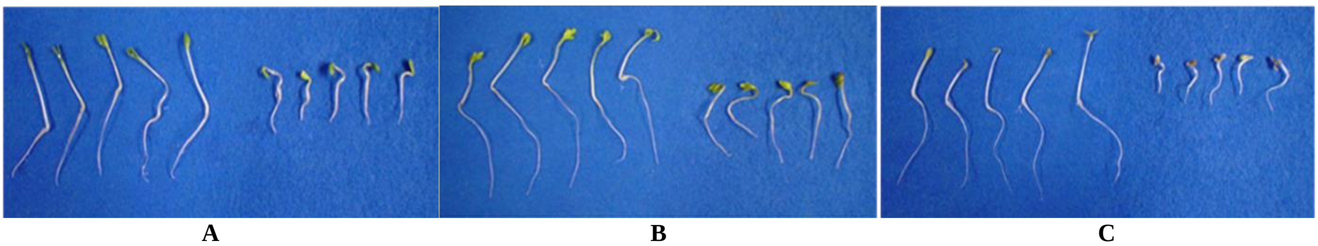
To investigate which secondary compounds possess allelopathic property in rice plants, eight *Japonica* type cultivars from *Kinuhikari*, *Hinohikari*, *Nipponbare*, *Sasanishiki*, *Yukihikari*, *Norin 8*, *Kamenoo*, and *Koshihikari* as donor plants were investigated on three sensitive plants to allelochemicals i.e., alfalfa (*Medicago sativa* L), cress (*Lepidium sativum* L.) and lettuce (*Lactuca sativa L.*) as receiver plants/ test plants. In this experiment, the effects of resource competition between donor and test plants such as water, nutrients and light were eliminated. Sterilized sixth day-rice seedlings were put on filter paper in Petri dish, moistened with phosphate buffer. Each of the three-day old test plants seedlings as described above, were then grown together with rice seedlings in Petri dish at 25°C and 12 h- photoperiod. The control experiment was conducted by growing each test plant without rice seedlings. All experiments were replicated seven times in complete randomized block designs. Three days after incubation, the lengths of roots and shoots and fresh biomass of the three test plants were measured. The results showed that all eight rice cultivars could inhibit the growth of the roots, shoots, and fresh mass of three test plants with different inhibition indexes, where the greatest inhibition index was reported from *Koshihikari* cultivar with the percentage of inhibition indexes on alfalfa, cress and lettuce as 60.6, 63.2 and 71.9, respectively (Kato-Noguchi and Ino 2001; Kato-Noguchi 2004; Kato-Noguchi and Peters 2013) (Table 1; Figure 2).

To find an allelochemical in rice root exudates that possess the greatest allelopathic potential, rice seedlings of *Koshihikari* cultivar were hydroponically grown for 14 days. The culture solution was then purified by chromatographic fractionations and finally, the putative compound with the inhibitory effect was isolated and the chemical structure of the putative inhibitor was determined by spectral data as momilactone B (3,20-epoxy-3α-hydroxy-synpimara-7,15-dien-19,6β-olide, $C_{20}H_{26}O_4$) (Kato-Noguchi 2004; Kato-Noguchi and Ino 2005b).

Table 1. Inhibition index of rice seedlings on alfalfa, cress, and lettuce seedlings (Kato-Noguchi and Ino 2001).

Rice cultivars	Inhibition index (%)*		
	Alfalfa	Cress	Lettuce
Norin 8	43.9	53.7	51.4
Kamenoo	22.3	46.6	53.2
Nipponbare	16.3	31.4	30.5
Sasanishiki	29.6	48.2	51.5
Kinuhikari	22.6	49.2	43.9
Koshihikari	60.6	63.2	71.9
Hinohikari	7.4	33.5	51.1
Yukihikari	27.1	30.4	39.9

Note: * The inhibition indexes were calculated on average of inhibition rate of root length, shoot length and fresh mass. Inhibition rate of root length, shoot length and fresh mass was scored so that those of control plants were 100%.



A B C

Figure 2. The growth of hypocotyls and roots of test plants i.e. (A) alfalfa; (B) cress and (C) lettuce when grown with and without rice seedlings cv. *Koshihikari*. The growth of test plants without rice seedlings/control (*left*); the growth of test plants with the presence of rice seedings/incubated with rice (*right*) (Kato-Noguchi 2004; Kato-Noguchi and Peters 2013).

Table 2. I_{50} values of momilactone A and B on root and shoot growth of four weeds species (Kato-Noguchi and Ota 2013).

Cultivars	Momilactone A		Momilactone B	
	Root	Shoot	Root	Shoot
Timothy	76.5±6.3	157±12	5.6±0.4	7.9±0.8
Barnyardgrass	91.2±7.2	145±11	6.7±0.4	6.3±0.4
Jungle rice	66.7±5.4	238±21	7.2±0.5	11.6±0.8
Crabgrass	98.5±7.3	275±19	9.5±0.6	12.4±1.1

In addition, another potential allelochemicals that were found in rice exudates of *Koshihikari* cultivar were identified as momilactone A. However, the ability of momilactone A to suppress the growth of neighboring plants is weaker compared to momilactone B (Kato-Noguchi and Ino 2005a).

RICE MOMILACTONES POTENTIAL FOR WEEDS SUPPRESSION

To study the effectiveness of momilactone A and B against weed species, an experiment using four weeds species had been conducted by Kato-Noguchi and Ota (2013). Four weeds species, i.e., *timothy* (*Phleum pratense* L.), *barnyardgrass* (*Echinochloa crus-galli* (L) Beauv), *jungle rice* (*Echinochloa colona* L. Link) and *crabgrass* (*Digitaria sanguinalis* L.) were subjected to bioassay as test plants. Momilactone A and B were isolated from husks of rice cv. *Koshihikari* and dissolved in methanol and added to two sheets of filter paper in a Petri dish and allow to evaporate. The filter paper in Petri dish was moistened with 3 ml of 1 mM MES buffer. The final concentrations of momilactone A and B were 0.01, 0.03, 0.1, 0.3, 1,3, 10, 30, 100, 300, 1000, 3000 and 10000 µM. Sterilized ten germinated of each test plant seedlings were put on filter paper in Petri dish containing momilactones solution with different concentrations as above. The length of shoots and roots of test plants seedlings were measured after 48 h of incubation in the darkness at 25°C. For the control experiment, sterilized ten germinated seeds of each test plant were placed on filter paper in Petri dish containing MES buffer without momilactones filtrate. Percentage of inhibition was calculated following the formula: [(control plant length-plant length incubated with momilactone A or B)/control plant length] x 100. The results showed that momilactone A and B can inhibit the growth of shoots and roots of all test plants. The concentrations of momilactone A and B required for 50% growth inhibition (I_{50}) for shoots and roots of all test plants are presented in Table 2.

Table 2 showed that the I_{50} values of momilactone A on four weeds species were 66.7-98.5 µM and 145-275 µM for roots and shoots, respectively, meanwhile, the I_{50} values of momilactone B were 5.6-9.5 µM and 6.3-12.4 µM for roots and shoots, respectively. By comparing the I_{50} values of momilactone A and B on roots and shoots of four weeds cultivars, it showed that inhibitory activity of momilactone B on the roots of *timothy*, *barnyardgrass*, *jungle rice* and *crabgrass* were 13.7, 13.6, 9.26, and 10.4-fold higher than that of momilactone A. The inhibitory activity of momilactone B on the shoots of timothy, *barnyardgrass*, jungle rice and crabgrass were 19.9, 23.0, 20.5, and 22.2-fold higher than that of momilactone A. Therefore, it could be suggested that momilactone B plays a more important role in weeds growth inhibition than momilactone A (Kato-Noguchi and Ota 2013; Kato-Noguchi and Ino, 2005b).

In addition, an experiment using lettuce and Chinese cabbage has proven that the concentration of momilactone B at 10 µM drastically could inhibit the growth of hypocotyls of lettuce and Chinese cabbage seedlings. Meanwhile, the application of momilactone A at 100 µM exhibited only slightly inhibited both of test plants (Toyomasu et al. 2008). This data explained that momilactone B can suppress the growth of test plants with a concentration at 10^{-1} of momilactone A. In other words, momilactone B only requires low concentration to produce a high inhibition ability against surrounding plants (Trezzi et al. 2016). An extensive study on momilactone A reported that the function of momilactone A is more as a defense mechanism against fungal pathogen, which is termed as phytoalexin (Kato-Noguchi and Peters 2013; Atawong et al. 2002). Thus, momilactones have dual functions i.e., 1) as germination and seedling growth inhibitor (allelochemicals) and 2) as antimicrobial natural products (phytoalexins) (Wang et al. 2011; Toyomasu et al. 2008).

RICE MOMILACTONES POTENTIALLY CAN INHIBIT THE GROWTH OF *BARNYARDGRASS*

Most allelopathy studies were focused on one of the most noxious weeds in rice fields i.e., *barnyardgrass* (Khan et al. 2013; Kato-Noguchi and Peters 2013; Ma et al. 2014). To understand the correlation between allelopathic activity and momilactones secretion of eight rice cultivars, bioassay on *barnyardgrass*, using a method called donor-receiver bioassay, was conducted by Kato Noguchi (2010a). In this research, the interfering effects such as nutrients, light and pH were eliminated during the period of bioassay, thus the inhibitor effect of rice toward *barnyardgrass* was solely due to an allelopathy effect.

Sterilized seeds of eight rice cultivars i.e., *Kinuhikari*, *Hinohikari*, *Nipponbare*, *Sasanishiki*, *Yukihikari*, *Norin 8*, *Kamenoo*, and *Koshihikari* were germinated on a moist filter paper in Petri dish and incubated in growth chamber at 25°C and 12 h photoperiod. Four days after incubation, seedlings of each of the eight rice cultivars with uniform roots and shoots length were transferred into Petri dishes containing two sheets of filter paper moistened with MES buffer and allowed to grow for another three days. In the meantime, sterilized seeds of *barnyardgrass* were grown on a moist filter paper in Petri dish at 25°C in a darkness condition for three days. Further, *barnyardgrass* seedlings with uniform roots and shoots length were then moved into Petri dish that already contained 7-day-old rice seedlings and were incubated at 25°C and 12 h photoperiod. To maintain moisture, MES buffer was added into Petri dish at 12 h interval. The control experiment was conducted with the same protocol as above, but the weeds seedlings were grown without rice seedlings.

Three days after incubation, the length of roots and shoots of *barnyardgrass* were measured. Percentage of inhibition was calculated following formula: [(control plant length-plant length incubated with rice) /control plant length] x 100. The liquid growth medium and filter paper in Petri dish were collected at the end of bioassays for further assay to determine momilactone A and B concentrations. The results showed that all rice cultivars inhibited the roots and shoot growth of *barnyardgrass* seedlings with different percentages of inhibition. The highest inhibition activity on the growth of *barnyardgrass* seedlings came from *Koshihikari* cultivar as well as the highest concentrations of momilactone A and B in their medium. Meanwhile, the lowest concentration of momilactones and the lowest activity of inhibition were found in *Kinuhikari* cultivar. Momilactone A and B in the medium of *Koshihikari* cultivar were 6.9-and 5.8- fold higher than *Kinuhikari* cultivar. The inhibition of roots and shoots growth of *barnyardgrass* seedlings by *Koshihikari* were 3.9- and 3.2-fold greater than *Kinuhikari* cultivar. Thus, *Koshihikari* cultivar released the highest concentration of momilactones and possess the highest inhibition on the growth of *barnyardgrass* seedlings (Table 3 and Table 4). Therefore, it could be suggested that there was correlation between the secretion level of momilactones and allelopathic activity. Increasing production of momilactones, increasing allelopathic activity. In addition, the secretion level of momilactones might be cultivar-dependent (Kato-Noguchi et al. 2010a).

Table 3. The inhibition of shoots and the roots growth of barnyardgrass seedlings when grown together with eight rice cultivars seedlings. Shoots and roots length of control plants were 19.7±0.9 and 23.5±1.8 mm, respectively. There were three replications per cultivar and the experiment was repeated seven times with three Petri dishes for each experiment. Different letters indicate significant differences ($P<0.05$) for each column according to Tukey's test (Kato-Noguchi et al. 2010a).

Rice cultivars	**Growth inhibition of *E. crus-galli* (%)**	
	Shoot	**Root**
Kinuhikari	17.1a	15.6a
Hinohikari	18.5a	18.5a
Nipponbare	28.8b	25.5b
Sasanishiki	38.8c	29.5b
Yukihikari	45.6d	38.2c
Norin 8	48.8d	47.1d
Kamenoo	51.8de	54.8de
Koshihikari	54.5e	60.3f

Table 4. Concentration of momilactone A and B in the culture medium of the donor-receiver bioassay as determined by LC/MS/MS analysis. Means from seven independent experiments with three replicates per cultivar. Different letters indicate significant differences ($P<0.05$) for each column according to Tukey's test (Kato-Noguchi et al. 2010a).

Rice cultivars	**Concentration (µmol/L)**	
	Momilactone A	**Momilactone B**
Kinuhikari	0.21a	0.66a
Hinohikari	0.27b	0.71a
Nipponbare	0.29b	1.12c
Sasanishiki	0.34bc	1.41c
Yukihikari	0.40c	2.14cd
Norin 8	1.03d	2.73d
Kamenoo	1.17d	3.23df
Koshihikari	1.45f	3.84f

RICE MOMILACTONE B PLAY AN IMPORTANT ROLE IN INHIBITING THE GROWTH OF *BARNYARDGRASS* AND THEIR SECRETION WAS INDUCED BY THE PRESENCE OF WEEDS

Rice momilactone B is reported to play a more important role in weeds growth inhibition than momilactone A. To study the presence effect of *barnyardgrass* on the concentration of secreted momilactone B from rice seedlings and its inhibitor activity as well, bioassay, where rice seedlings were incubated with (mixed-incubation) and without (mono-incubation) *barnyardgrass* seedlings, had been conducted by Kato Noguchi (2011b). Fifty sterilized seeds of rice cv. *Koshihikari* and 50 *barnyardgrass* seeds were grown separately on moist filter paper in a growth chamber at 25°C and 12 h photoperiod for 10 days. Ten-day-old uniform rice and *barnyardgrass* seedlings were then transferred onto a holed plate of polystyrene foam that was floated on Hoagland medium with pH 6.0 in plastic container at 25°C and 12 h photoperiod. After 10 days of incubation, rice seedlings were harvested for *barnyardgrass* bioassay and momilactone B was determined.

Barnyardgrass bioassay was conducted by growing five-day-old uniform *barnyardgrass* in Petri dishes containing rice extracts, which is homogenized from 10 g fresh weight of rice seedlings. The control experiment did not contain rice extracts. The length of roots and shoots of *barnyardgrass* were measured 48 h after incubation in the darkness at 25°C. Inhibitor activity (%) was calculated by the formula: [(control plant length-plant length treated with rice extract) /control plant length] x 100. To determine momilactone B, 10 g of fresh weight of rice seedlings were homogenized with aqueous methanol and homogenate was filtered through filter paper No.2. This procedure was repeated two times to have an aqueous residue and further loaded into a column of synthetic polystyrene adsorbent, purified and finally momilactone B was calculated following method described by Kato-Noguchi et al. (2002).

Bioassay where rice seedlings were incubated without (mono-incubation) *barnyardgrass* seedlings was conducted in the container. *Barnyardgrass* root exudates resulted by growing *barnyardgrass* seedlings onto a holed plate of polystyrene foam for 10 days. Then, the medium in the container was collected and filtered and the filtrate was loaded onto a column of synthetic polystyrene adsorbent, eluted with methanol, evaporated, and dissolved with methanol again and added to filter paper in incubation container. In the next step, 1L of Hoagland nutrient solution was poured into the incubation container with different final concentrations of *barnyardgrass* root exudates in the medium i.e., 0, 10, 30, 100, 200 and 300 mg/L. With the uniform rice seedlings being transferred onto a holed plate of polystyrene foam and floated in the container containing different concentrations of *barnyardgrass* root exudates as explained above for 10 days. At 10 days after incubation, the rice seedlings were then harvested and used for *barnyardgrass* bioassay and momilactone B was also quantified. *Barnyardgrass* bioassay and determination of momilactone B in rice seedlings were performed by the same methods as above.

The results from mixed- and mono-incubation showed that the extracts resulting from mono-incubated rice could inhibit the roots and shoots of *barnyardgrass* as 15% and 12%, respectively. Meanwhile, the extracts from mixed-incubated rice could inhibit 79% and 75% of roots and shoots growth of *barnyardgrass*, respectively. Thus, mixed-incubation induced 5.3-fold increase in roots inhibition and 6.3-fold increase in shoots inhibition compared with mono-incubated rice. In line with allelopathy activity, the concentration of momilactone B was also higher in mix-incubated rice at 18.6 nm/seedling than in mono-incubated rice at 2.7 nm/seedling. This number showed that the concentration of momilactone B was 6.9-fold higher in mixed-incubated rice than in mono-incubated rice. Thus, the concentration and inhibitor activity of momilactone B were higher with the presence of *barnyardgrass* seedlings (Kato-Noguchi. 2011; Trezzi et al. 2016) (Figure 3). This condition was presumably because rice can detect root exudates of *barnyardgrass* which is contain signaling molecules that can induce the increased production and secretion of momilactone B into the environment (Kato-Noguchi and Peters 2013; Zhang et al. 2018; Kato-Noguchi 2011b).

In rice plants, momilactone B was released into the environment during their life cycle and the released level was increasing until flowering initiation and afterward was decreased (Kato-Noguchi 2008; Kato-Noguchi et al. 2013). The concentration level of momilactone B at day 80 (around flowering) was 58- fold higher than at day 30 (Figure 8) and the level of momilactone B in the shoots was 3.8-fold greater than in the roots (Kato-Noguchi et al. 2011a; Kato-Noguchi and Ino 2005b). In high allelopathic rice varieties, the concentration of momilactone B per day can reach 2-3 µg (Soltys et al. 2013).

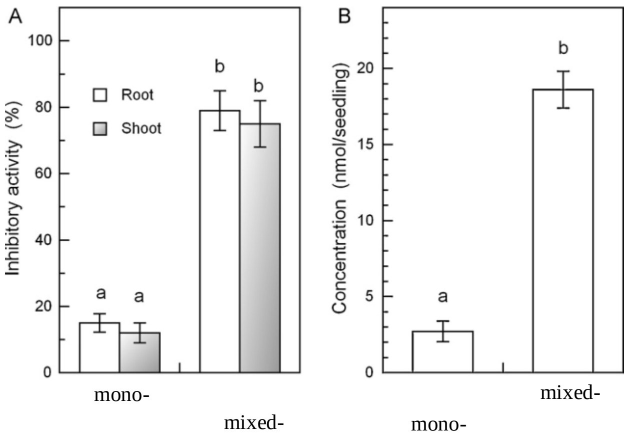


Figure 3. The inhibitor activity of allelopathic (A) and the concentration of momilactone B (B) in rice seedlings when grown together with (mixed-) and without (mono-) *barnyardgrass* seedlings (Kato-Noguchi 2011b).

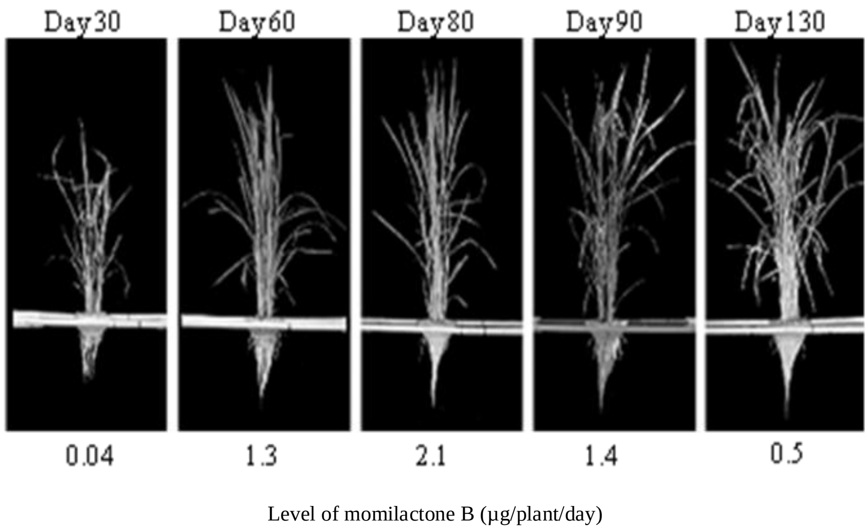


Figure 4. The secretion level of momilactone B from rice plants at different stages of growth (Kato-Noguchi and Ino, the regional institute online publishing)

Biosynthesis of momilactones and identified genes responsible for momilactones production

The biosynthetic pathway of momilactones and their corresponding genes have been extensively investigated in rice (Okada et al. 2016). Genetic evidence is provided indicating that they form a gene cluster for momilactones production, located on chromosome 4 which includes copalyl diphosphate synthase 4 (*OsCPS4*), kaurene syntase-like 4 (*OsKSL4*), two cytochromes P450 (CYP) genes (*CYP99A2* and *CYP99A3*), and a short chain alcohol dehydrogenase (SDR) (Kato-Noguchi and Peters. 2013; Miyamoto et al. 2014; Wang et al. 2011) (Figure 4). CYPs genes and a putative dehydrogenase gene (*AK103462/ OsMAS*) are involved in the downstream steps of momilactones biosynthesis (Shimura et al. 2007; Xu et al. 2012; Miyamoto et al. 2014).

Momilactone biosynthesis is initiated by dual cyclization reactions (Figure 5). The initial cyclization of common diterpenoid precursor (*E,E,E*)-geranylgeranyl diphosphate (GGPP) to *syn*-copalyl diphosphate (*syn*-CPP) is catalyzed by copalyl diphosphate synthase *OsCPS4* (Xu et al. 2012; Okada et al. 2016). Further cyclization of *syn*-CPP to (9β-H)-pimarane-7,15-diene is catalyzed by (9β-H)-pimarane-7,15-diene synthase *OsKSL4*. Cytochromes P450 is involved in the downstream oxidation of the (9β-H)-pimarane-7,15-diene. The involvements of one of cytochromes P450, *CYP99A3* was found to catalyze consecutive oxidations of the C_{19} methyl group of the momilactone precursor, (9β-H)-pimara-7,15-diene to form sequentially (9β-H)-pimara -7,15-dien -19-ol, (9β-H)-pimara-7,15 dien -19-al, and (9β-H)-pimara-7,15 dien -19-oic acid. Then, the (9β-H)-pimara -7,15 - dien 19- oic acid give rise to 3 β -hydroxy - (9β-H)-pimara-7,15 dien- 19,6 β -olide and finally, the 3 β -hydroxy - (9β-H)-pimara-7,15 dien- 19,6 β -olide is converted to momilactone A by *AK103462* (*OsMAS*) (Okada et al. 2009; Zhao et al. 2018). To produce momilactone B, it is proposed to form from momilactone A through C_{20}-hydroxylation and hemiketal ring closure (Wang et al. 2011; Zhao et al. 2018). In this biosynthetic pathway, *CYP99A2* is one of cytochromes P450 is involved in a lesser extent compared with *CYP99A3* (Zhao et al. 2018).

The involvements and the important role of *OsCPS4* and *OsKSL4* in momilactones biosynthetic pathway and momilactones production, had been proven by selective removal of those genes using gene knock-out experiments (Xu et al. 2012). Reverse genetic approach by insertion genes knock-out of *OsCPS4* and *OsKSL4* in Zhonghua 11 and Hwayoung rice cultivars has been conducted (Xu et al. 2012). Bioassays to determine the effect of removing *cps4* or *ksl4* on plant suppression were conducted by growing homozygous insertional mutant lines either harboring *cps4* or *ksl4* together with lettuce (the sensitive plant species) or *barnyardgrass* (noxious weed) in Petri dishes without any supplied nutrients to avoid any confounding. The control experiment was conducted by growing *barnyardgrass* or lettuce together with wild-type rice seedlings. Parameters observed in this experiment were the length of roots and hypocotyls after 6 or 12 days after incubation for lettuce and *barnyardgrass*, respectively.

The results showed that homozygous insertional mutant lines harboring either *cps4* or *ksl4* exhibited the decreasing in allelopathy potential on both lettuce and *barnyardgrass* as shown in Figures 6 and 7. The length of roots and hypocotyls of lettuce and *barnyardgrass* were longer when they were grown together with mutant rice lines seedlings compared with their wild type of rice. In other words, the wild type has an allelopathy effect greater than mutant lines. It is suggested that in mutant rice lines, they lose their ability to suppress the growth of neighboring plants due to the loss of *OsCPS4* or *OsKSL4* gene expression which directly influences momilactones production (Xu et al. 2012; Kato-Noguchi and Peters 2013; Kato-Noguchi and Ota 2013). Thus, it is proved that momilactones have a direct role in allelopathy ability. However, phenotypically, those mutant lines exhibited normal growth through all stages of development (Xu et al. 2012).

In addition, it was reported that the OsTGAP1is an elicitor-inducible rice basic leucine zipper (bZIP) transcription factor which plays an important role in momilactones biosynthesis and regulates the expression of all five genes in the cluster (Figure 4). Knock-out mutant line of *OsTGAP1* showed no expression of *OsCPS4*, *OsKSL4*, *CYP99A2*, *CYP99A3* and *OsMAS* and the absence of momilactones production. Therefore, *OsTGAP1*is proven as a key regulator for elicitor-inducible production of momilactones and to coordinate the transcription of all five genes essential for momilactones production (Okada 2009; Zhao et al. 2018).

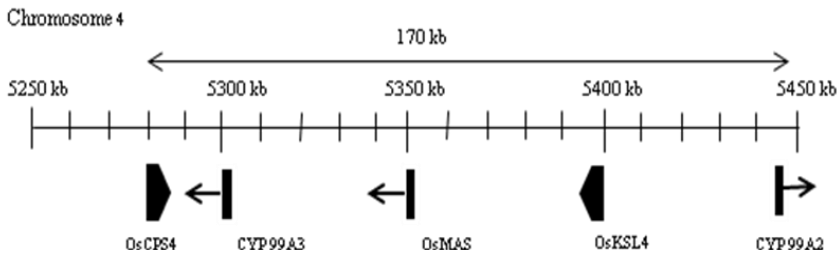


Figure 4. Rice momilactones biosynthetic gene cluster (Xu et al. 2012).

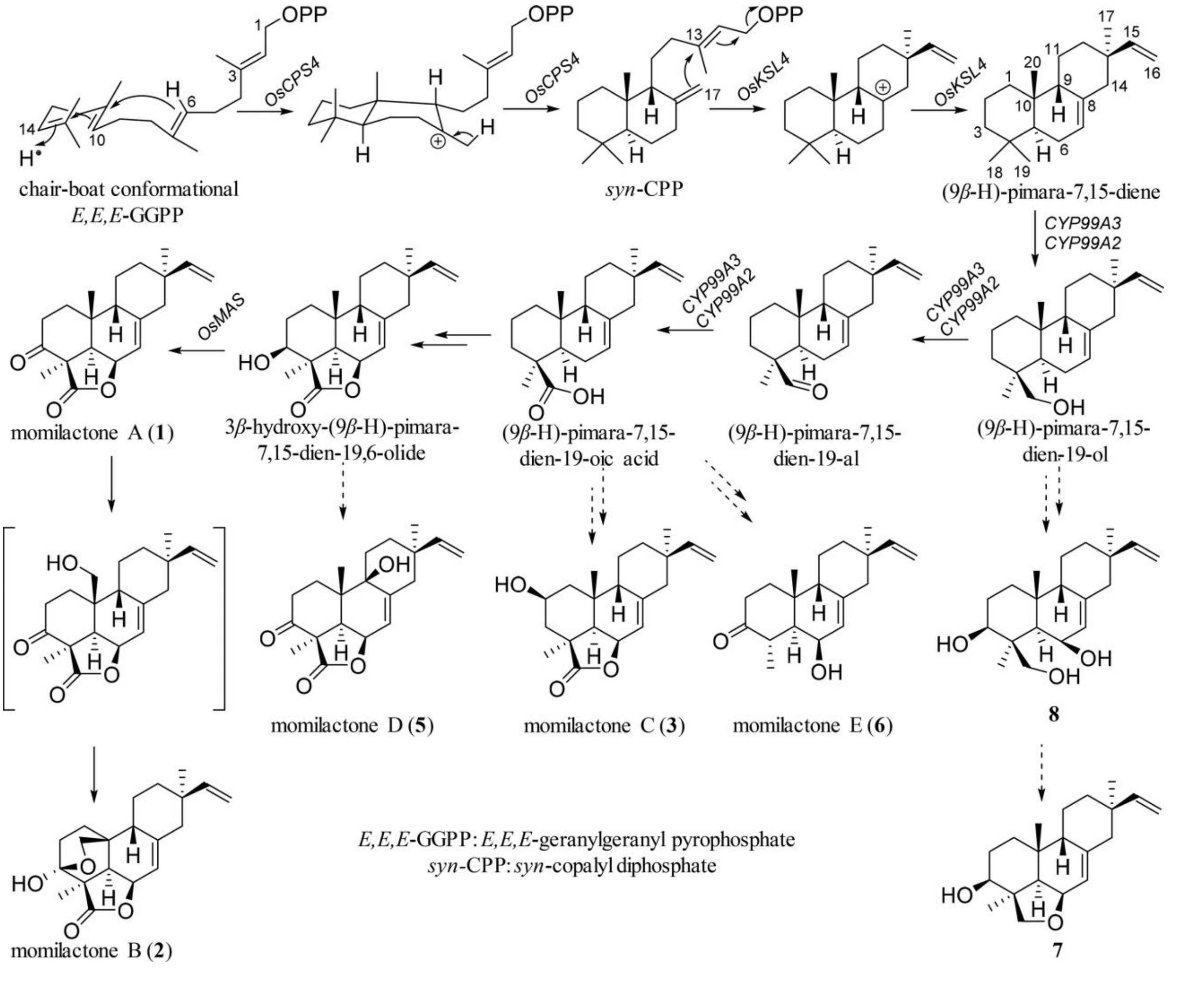


Figure 5. Biosynthesis pathway of momilactone A and B (Zhao et al. 2018).

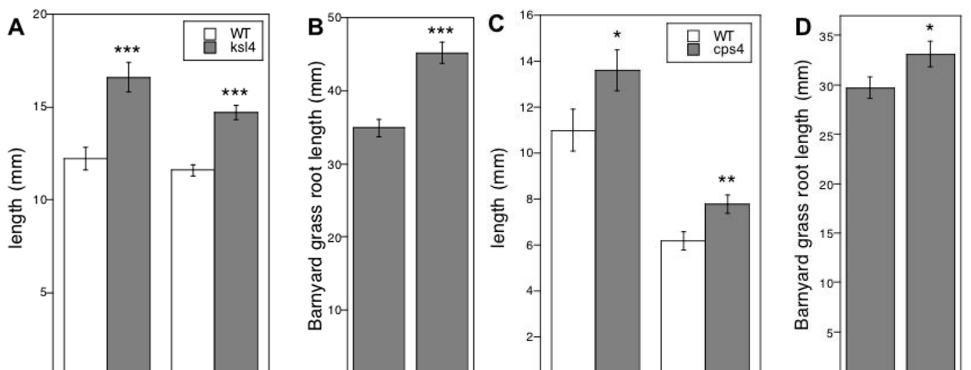


Figure 6. Effect of *OsKSL4* and *OsCPS4* knock-out mutants rice (*ksl* and *cps4*) on allelopathy potential against lettuce (A and C) and *barnyardgrass* (B and D). Comparison of the length of roots and hypocotyls seedlings of lettuce and *barnyardgrass* when they were grown together with mutant rice versus their wild type of rice seedlings (Xu et al. 2012).

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Figure 7. Comparison of allelopathy potential in mutant rice lines versus wild-type rice on germinated lettuce seedlings (Xu et al. 2012).

Discussion

One of the most important factors that play a role in the declining yield and the quality of rice are weeds. In paddy fields, cultivated rice and weeds always grow together for the same needs. Among weed species, *barnyardgrass* was reported as the most destructive weed and the most serious herbicide-resistant weed (Zhang et al. 2018). Farmers control weeds by using synthetic herbicides. However, the overuse and continuous use of synthetic herbicides can lead to the emergence of herbicide-resistant weeds and moreover give negative effects on the environment and human health. Therefore, an alternative method to control the weed problem is by minimizing the use of synthetic herbicides should be considered.

Rice has self-defense mechanisms by secreting phytotoxic compounds into the environment that can suppress the germination and growth of weeds nearby. This kind of self-defense is called allelopathy (Heidarzade et al. 2012; Zhang et al. 2018). Allelopathy is important to prevent rice plants from interfering with weeds. Allelopathy is defined as any direct or indirect harmful effects of one plant on another through the production of chemical compounds that releases into the environment called allelochemicals. There are many secondary compounds that are potential allelochemicals. Researchers are interested in studying which allelochemicals are the most responsible compounds for inhibiting weed growth. This article summarized published literature to determine the important compounds of rice allelochemicals that play an important role in suppressing weed growth. All experiments have been conducted in laboratories to eliminate other resources competitive interference. Therefore, the data obtained from the experiments solely reflects the allelopathic effect.

Although rice has the ability to suppress root growth of *barnyardgrass*, its abilities, however, vary among cultivars. Thus, the activity of allelopathy is cultivars-specific. From microscopic studies, rice cv. *IR64* with potential allelopathic can inhibit the growth of secondary roots of *barnyardgrass* but not with non-allelopathic rice plant cv. Aus 196 (Olofsdotter et al. 2002). A study explained that among allelochemicals, momilactone A and B are the most important compounds with allelopathic potential, although momilactone B plays a more important role in weeds growth inhibition than momilactone A (Kato-Noguchi and Ota 2013; Kato-Noguchi and Ino 2005a). Momilactone A function is reported more as defense mechanism against fungal pathogen called phytoalexin.

The genes involved in momilactones biosynthetic pathway and thus directly affecting momilactones production, form a gene cluster consisting of *OsCPS4*, *OsKSL4*, *CYP99A2*, *CYP99A3* and *OsMAS* located on chromosome 4 and all these five genes are coordinately regulated by OsTGAP1. To prove the involvement of *OsCPS4* and *OsKSL4* genes in momilactones biosynthetic pathway and production, a reverse genetic approach by using gene knock-out of both genes has been conducted by Xu et al. (2012). The homozygous *cps4* or *ksl4* knock-out lines exhibited a significant loss in suppressing the growth of lettuce and *barnyardgrass.* This data is direct evidence that the loss of allelopathic ability in mutant lines to suppress the neighboring plants is due to the loss of *OsCPS4* and *OsKSL4* genes expression as well as momilactones production (Xu et al. 2012). The same results were obtained in knock-out mutant line of *tgap1* which exhibited no expression of *OsCPS4*, *OsKSL4*, *CYP99A2*, *CYP99A3* and *OsMAS* and no momilactones production. Thus, it could be concluded that momilactones is played an important role in allelopathy activity.

In the future, momilactones act as natural plant growth suppressants that are environmentally friendly, could be contributed as an alternative to the use of synthetic herbicides in weed management strategy. However, bioassays in greenhouse that imitate the situation in the field and continued with field studies to demonstrate the efficacy of momilactones still need to be carried out.

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The feasibility and farmer perception of true shallot seed technology in Sigi District, Central Sulawesi, Indonesia

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Abstract. *Rahayu HSP, Muchtar, Saidah. 2019. The feasibility and farmer perception of true shallot seed technology in Sigi District, Central Sulawesi, Indonesia. Asian J Agric 3: 16-21.* Shallot is one of the horticultural commodities that play a significant role in both the national and regional economies around Indonesia. A fluctuating supply of shallot influences the inflation level. Shallot production is currently facing many problems, including high production costs. The production cost mostly goes to expenses for labor and seed, while Indonesian shallot is mainly produced from the bulb seed. This high-cost production causes lower shallot competitiveness. Therefore, introduction of True Shallot Seed (TSS) technology, which lowers the cost of shallot seeds, could be an ideal option to improve the shallot competitiveness in Indonesia. However, the shallot farming feasibility and the farmer's perception of this technology are two critical aspects that need to be considered in the adoption of this new technology. This research aimed to study the potency of true shallot seed development in Central Sulawesi based on the TSS's farming feasibility and farmer perception of TSS. The research was conducted in Sigi District, Central Sulawesi. The results showed that the farming of shallot using TSS was feasible, and within 14.9 $t.ha^{-1}$ productivity, the Revenue-Cost Ratio was 3.15 while the Benefit-Cost Ratio was 2.15. The perception was examined based on three aspects namely technical, economic, and social aspects. The results showed that farmers were interested in planting true seed of shallot based on its high productivity, lower production cost, and market acceptance of the product; while in the social aspect, the extension and farmer group's support still need to be improved for development of TSS.

Keywords: Central Sulawesi, perception, shallot, seed cost

INTRODUCTION

Shallots are one of the vegetable commodities that have high economic value in terms of meeting the national consumption, farmers' sources of income, and as one of the potential foreign exchange earners. Besides being used in cooking, shallot also can have health promoting properties. Shallot can be cultivated in a wide range of agroecosystems, from lowland to highland regions. In Sigi Regency, shallot is cultivated by farmers in the lowlands. Dolo and Sigi Biromaru sub-districts are among the shallot production centers in Sigi District. Bima and Tajuk are shallot varieties mainly cultivated by farmers in these two sub-districts.

Shallot production in Indonesia is commonly based on crops generated from bulb seeds. However, most farmers usually sell the bulbs immediately after harvest as they need immediate cash, in addition to their inability to store the bulbs safely in a large quantity. Consequently, there has been a shortage of blubs that are usually used as seeds at the peak of planting time. Furthermore, farmers are forced to buy bulb seeds in the market at a very high price, which in most cases, are transported from long distances such as East Java. Some farmers keep their own bulb seeds by tightening them into bunches and hang them for two to three months. This method is also used to break the seed dormancy problem. However, some bulbs decrease in quality with the storage time, especially if the storage system is not properly managed. In addition, the use of bulbs as planting materials can also promote diseases caused by pathogens such as viruses, fungi, bacteria, and nematodes (Currah and Proctor 1990).

For tackling all problems related to the use of bulb seed, a research effort was made to grow shallot from the true seed of shallot (TSS). This was a good option for the grower to avoid storage problems, since TSS can be stored for a longer time and less space is needed. Furthermore, fewer seeds are required and the TSS is easily handled, including its transportation to the farm. Shallot production using bulbs, need a high amount of seed of around 1 to 1.5 tons of bulb seed per hectare. While using true shallot seed, one would need only approximately 4-6 kg per hectare.

Another advantage of TSS is that shallot crops are grown from true seeds also have a lower seed cost. Since the cost for seeds from bulbs is the highest in shallot farming (Putter and Adiyoga 2013), the price of shallot bulb seed is an important consideration. In Central Sulawesi, the price range of bulb seeds is anywhere from IDR 25000 to IDR 60000, depending on the fluctuation of shallot bulbs price for consumption purposes. Brick and Basuki (2010) found that at the farmers' level, the use of TSS as planting material was cheaper than that of bulb seeds. Furthermore, the plant density in seedlings will determine which one is more costly, between TSS seedling and bulb seed. The cost of TSS seedlings from the variety Sanren, in the planting density of 75 plants/ m^2 was cheaper than the cost of bulb seed, if the price of the bulb seed was IDR 10000 kg^{-1} or higher. In a planting density of 150 plants/ m^2, however, the cost of TSS was cheaper than bulb seed only when the price of bulb seed was more than IDR 15000 kg^{-1}. These results showed that the cost of bulb seed in Central Sulawesi is assumed to be higher than that of TSS. In addition to its lower cost, TSS also produces high yield with uniform bulb size under both highland and lowland conditions. These advantages would enable farmers to shift from the cultivation of shallot from bulb seed to TSS and can help them to grow a disease-free crop in new areas and reduce the production cost by half (Shimeles 2014).

True shallot seed is a new technology in Central Sulawesi. One consideration for developing this new technology is its economic feasibility. Even though shallot crops generated from TSS produce a higher production, farmers need to make sure that the new technology is feasible and profitable. Planting of true shallot seed needs additional treatment since it needs a seedling process similar to that of rice cultivation. The need for this extra work causes a longer production time and is more labor intensive. Also, the financial feasibility of shallot farming needs to be known to see whether it is feasible or not to be cultivated. The feasibility analysis needs to be conducted regarding its high risk of failure, as it is common with horticulture commodities. This study objective was to find out whether shallot farming using TSS is feasible or not, to provide incentives for shallot farmers. The result will serve as evidence to convince farmers to adopt the new technology and to make useful recommendations to develop new technology.

Another critical consideration is the farmer's perception of the new technology or innovation. Innovation can be described as ideas, ways, or objects that are perceived by someone as something new. According to Rogers (1983), the level of adoption of an innovation depends on the adopter's perception of the characteristics of technological innovation. The farmer's understanding of technological innovation requires mental readiness to adopt the technology that is useful and applied through a process of perception. Koster and Basuki (1991) stated that the adoption of a new technology recommended by researchers depends on many interrelated factors. The farmers' decision to adopt a crop cultivation technology is influenced by factors such as socio-cultural, economic, and biophysical aspects, and problems related to these aspects (e.g., capital, know-how, market uncertainty). Their choice is, thus, conditioned by what best fits their situation, thereby reducing risks as far as possible. In general, new technology can be rejected by farmers because the technology itself cannot help farmers to solve their problems. This means that to be adopted by farmers, new technology must be appropriately developed and meet the farmers need.

Since TSS is a new technology in Central Sulawesi, no study has been carried out so far about its feasibility and the farmer's perception of this technology. This research objective was to investigate the feasibility of the use of TSS in shallot farming and to know the farmer perception of TSS based on the three aspects namely technical, economic, and social. Both the TSS feasibility and farmer perception are important to make an effective recommendation to develop new technology.

MATERIALS AND METHODS

The study was conducted in Central Sulawesi Province of Indonesia. Specifically, the study focused on Dolo and Sigi Biromaru sub-district. These study areas were selected purposively, as they are shallot farming centers in Sigi District, Central Sulawesi. Both primary and secondary data were used in the study. The primary data was collected from the shallot farmers. Data were obtained through interviews involving randomly chosen 32 shallot farmers. A structured questionnaire was used to guide the data collection through personal interviews of household heads.

Furthermore, the collected data were tabulated and analyzed based on the research objectives. Secondary data dealt with the socio-economic conditions of the households and the study area. The secondary data was gathered from several sources including annual reports of local governments, the Indonesian Statistical Bureau, Indonesia Ministry of Agriculture, farmer associations and other relevant institutions.

The level of shallot farming income was calculated by calculating the costs incurred in shallot farming or also called production costs. The financial analysis included total revenue, total variable cost, gross margin, and net revenue based on the formula by Hendrayana (2017).

Total Revenue (TR) is the revenue from shallot production that was obtained from the sales of shallot bulbs. The shallot bulbs were sold to the fried shallot industry and in the market for household consumption. Total revenue of each farm was calculated as follows:

$$TR = PY$$

Where:
TR = total revenue from sales of output (shallot)
P = price of shallot per kilogram in Indonesian rupiah
Y = total volume of shallot in kilograms

Total Variable Cost (TVC) is the sum of the total cost of each of input variables used by farmers in shallot production (seed, labor, inorganic fertilizer, organic fertilizer, herbicide, pesticide) and was calculated using the following formula:

$$TVC = \sum_{j=1}^{6} C_j = \sum_{j=1}^{6} P_j X_j$$

Where;
C_j = total cost of each input variable j. j=1, 2 …6. 1-seed, 2-labor, 3-inorganic fertilizer, 4-organic fertilizer, 5-herbicide, 6-pesticide.

P_j= the product of the price (or cost) per unit of input j (P_j) and the total volume of input j (Xj) used in the production of shallot.

Gross margin (GM) is the amount left after deducting from the Total Revenue minus the Total Variable Cost.

GM = TR-TVC

This is supposed to pay for the fixed input used in shallot production, as well as to provide returns to farmers for their labor and management, and for their risk-taking.

Net Revenue (NR) is the amount left after deducting from the Gross Margin the Total Fixed Cost or deducting from Total Revenue the sum of the total variable cost and total fixed cost. This is supposed to provide payment for fixed inputs used in the shallot production, as well as to give returns to farm owners for their labor and management (Kay and Edwards, 1999) and their risk-taking.

NR = GM-TFC or NT = TR-(TVC + TFC)

Fixed costs are the cost associated with the use of fixed assets and management. Fixed costs in this study included rent of the farming land. The study also included interest as one of the cost items and was calculated using the formula:

IOC = AIit

Where;
IOC = Interest on operating capital
Al = Total investment/2 I = interest rate per year (9 percent)
T = length of the crop production period, in month, (4 months)

Feasibility measurement used was the Revenue Cost Ratio (RCR). (RCR is used to indicate the relationship between revenue and cost of any project in monetary terms, while benefit cost ratio (BCR) shows the relationship of cost and benefit. Higher RCR or BCR indicates a higher return from production. Usually, the total cost is used for calculating RCR (Anjum and Barmon 2017).

$$RCR = \frac{TR}{TC}$$

Farmer perception was analyzed using Scoring method (Hendrayana 2014) as follows:

$$Score\ Value = \frac{ni.si}{N}$$

ni = Number of respondents at column i (i = 1,2,3…4)
si = Score of comment in i (i = 1,2,3…4)
N = Total number of the respondent (32)

RESULTS AND DISCUSSION

Characteristics of shallot farmers

Age of the shallot farmer respondents

Farmers in Sigi District were 39 years old on average (Table 1), which is generally a productive age. Farmers in the productive age will accept innovations easier than the older ones. Productive age allows farmers to improve their performance and increase productivity that leads to higher profits. Nurhapsa (2013) found that people have increased the ability to work along with the increase of age, but the ability to work declines at a certain age, as the age influences the maturity and physical ability of the respondents in managing a business.

Educational attainment

Education (formal and non-formal education) can play a vital role in changing the attitude, behavior, and mindset of the farmers. Through education, farmers can get information and new technology innovation, thereby affecting the quality of decision-making. Natawidjaja et al. (2008) reported that formal education level affects the productivity of labor and level of technology adoption. Low education levels can result in low levels of productivity. The higher the education level completed by the farmers, the easier they will understand and accept innovations delivered to them. Shallot farmers have an average of ten years of educational attainment or graduated from Junior High School (Table 1). Under this education level, farmers are expected to be easier to accept new technology.

Shallot farming experience

The average number of years of shallot farming experience was seven years. Nurhapsa (2013) stated that the accumulation of sufficient experience will make it easier for farmers to receive and choose innovation or technology suitable and appropriate for use in farming. With seven years shallot farming experience, farmers are expected to be wise in choosing technology.

Household size

The average household size of the shallot farmer-respondents was four members. Bigger households have higher living costs, and most farmers do not have other income sources aside from shallot farming. The high consumption cost reduces the farmer’s opportunity to allocate funds for farming inputs as the technology recommends, especially, if the price of input is high. However, the farm size is essential because growing vegetables is labor-intensive farming. Small-scale vegetable farming such as shallot is based on family labor (Beshir and Nishikawa 2012).

Farm size

The small farm remains to be one of the constraints in improving the production capacity of the farms. The average size of shallot farms of the respondents was 0.62 ha (Table 1). Farm size affects innovation adoption as the more the lands are made available, the higher the production output and farmers' income.

In the development of the commodity, extensification of production outside Java, has been limited because of the non-availability of capital. To get a higher income, a larger farm that is balanced by optimum input application to increase the scale of business is needed (Kahn and Maki 1979; Bagi 1982).

Table 1. Characteristics of respondents of 32 shallot farmer-respondents from Sigi District, 2018

Characteristics	Average value
Age (years)	39
Education (years)	10 (Junior High School)
Household size (person)	4
Farming experience (years)	7
Farm size (hectare)	0.62

Table 2. The feasibility of shallot farming using True Shallot Seed (TSS) in Sigi District, Central Sulawesi, 2018

Items	Total (IDR)
Gross Income	
Quantity of onion (kg)	14,900
Selling price (Rp/kg)	15,000
Total Revenue	223,500,000
Cost	
Fixed Cost	
Land cost	3,500,000
Irrigation	150,000
Total fixed cost	3,650,000
Variable Cost	
Seed	12,000,000
Labor	30,085,000
Inorganic fertilizer	2,970,000
Organic fertilizer	4,106,000
Herbicide	2,250,000
Insecticide	14,170,000
Total variable cost	65,581,000
Opportunity Cost of Operating Capital	1,080,400
Total Cost	**71,035,000**
Gross Margin	157,919,000
Net Revenue	152,465,000
Revenue/Cost	3.15
Benefit/Cost	2.15

The feasibility of shallot farming using True Shallot Seed (TSS)

The feasibility of shallot farming using TSS is important to be known as one of considerations for the farmer to adopt the new technology, whether the new technology will be economically feasible or give profit to farmers. The result showed that shallot farming using TSS was feasible and profitable to do.

There are several steps or processes of shallot cultivation namely: land preparation, line-making, planting, fertilizing, managing of pest and plant diseases, weeding/grass clearing, harvesting, and post-harvest. Each cultivation process varies in cost. The farmer’s choice of technology will define the cost. For example, in grass clearing (weeding), either chemical (herbicide spray) or manual method is used. The manual method entails higher labor costs as compared to the chemical/herbicide spray method. Weeding of grasses using herbicides needs only half-day of labor or four hours of work but manual weeding takes one or two days to finish work depending on the farm size. The cost of labor also includes meals and, sometimes, cigarettes. Herbicides are normally applied two times during a growing season; the first is in the land preparation stage and the second is one month after planting. Labor cost for harvesting includes the cost of uprooting, transporting from field to house or storage place, cleaning, and binding. Harvesting is counted by man-days or by the number of plant lines. In either two ways, conversion to the man-days system was practiced.

Since labor is needed in many shallot cultivation processes, the labor expense is included as a significant cost sector in shallot farming. In TSS farming, additional labor is only required for seedling preparation. The seedling cost was around ten man-days or Rp. 650000 (one man-days equals Rp. 65000). The additional seedling cost distinguishes TSS from the bulbs shallot farming. Meanwhile, the bulb seeds are planted directly in the farming land after the dormancy break during storage. The performance of shallot farming using True Shallot Seed was described in Table 2.

Based on Table 2, shallot farming using TSS was profitable and feasible to be cultivated with the Revenue and Cost Ratio of 3.15. Shallot farming using TSS is still new in Indonesia while in other countries TSS technology has already been widely used for shallot or onion. Shallot farming using TSS is profitable as proved by Hewavitharane et al. (2011) who found that onion farming using TSS had an RCR of 2.01. As the risk of horticulture farming is higher compared to cultivation of other commodities, the high RC ratio of this commodity is a crucial consideration. The high risk of more significant loss in horticulture is mere because of the high production cost. Another factor affecting the profitability is the price of shallot. At the time of study, the price of shallot was Rp. 15000 on average, which is good enough to support farmers' income and profits. However, the shallot price is not stable as with that of other commodities. This high fluctuated shallot price is affected by the availability of shallot in the field. The fluctuating price of shallot profoundly affects the shallot farming business using seeds from bulbs, since the cost for seeds from bulbs will increase when the price of bulbs is high. This will increase the cost of shallot farming, and consequently, the farmers will not be able to cultivate their land as they have not enough capital to afford the farming expenses.

Farmer’s perception on True Shallot Seed (TSS) technology

Farmers' perception of technical, social, and economic aspects of TSS development was assessed based on the reasons stated by farmers. Perception was measured using a Likert scale. The decision of farmers to apply technology is mainly determined by internal factors within the farmer, including the attitude and purpose in conducting farming. The attitude of farmers, in this case, is very dependent on the characteristics of the farmers, which includes socioeconomic characteristics, and aspects related TSS technology, i.e., technical, economic, and social aspects. Generally, the goal of farmers in carrying out their farming business is to increase the family income. The low level of technology adoption by farmers is influenced by many factors, including the lack of capital, high input prices, and low output prices (Sugandi dan Astuti 2012).

Table 3. Farmer perception on True Shallot Seed, 32 farmer-respondents from Sigi District, 2018

Aspects	Total score
Technical aspects	
Application of TSS technology	2.563
Access to technology information	2.250
Access to production inputs	2.188
Economic aspects	
Decrease of production cost	3.031
The suitability between the cost of technology change and profitability	3.125
Marketing of product	3.094
Social aspects	
Farmer's knowledge	2.094
The liveliness of farmer group	2.813
The support of public services	2.781

In general, farmers are interested in using TSS. Among all aspects considered, the economic aspect had the highest value of tendency or positive attitude from farmers (Table 3). On the economic aspect, the statement that the benefits of using TSS are commensurate with the cost of changing technology had the highest value. Based on what was seen in the implemented demonstrations, the farmers assessed that the costs incurred to change the conventional bulb seed technology into the TSS are commensurate with the benefits earned so that it is worth trying.

From the marketing side, there was no problem with the bulbs produced from TSS. Physically, there was no difference between bulbs from TSS and those from bulb seed. The bulb size and shape of the two seed sources were the same; therefore, it is acceptable by the market. Bulbs from TSS can be divided to have a size that fits the farmers and market preferences. Meanwhile, the color of shallot will depend on the variety. Another consideration in the economic aspect that received a positive response from the respondents was the decrease in production costs, especially the price of TSS that was quite more affordable as compared to that of seeds from bulbs. The lower price of TSS saved up to 40% of seed production cost expensed by the farmers, considering that the price of seeds is costly, around Rp. 25000 to Rp. 50000 per kg. Seeds from bulbs will require additional cost for transportation and storage.

The present study results are like those of Brink and Basuki (2012), who stated that the introduction of true shallot seed could be an option to improve competitiveness of Indonesian shallot production, and the recently developed true shallot seed cultivars are of high yielding, short duration, and good quality for the local market, which has increased the feasibility of true shallot seed production. Alfu et al. (2013) also indicated that there are many shallot cultivars in Indonesia with a varying degree of seed production potency, morphological traits, and yield, where such variations also indicate high genetic variations for the flowering date and resistance to pests and pathogens. The existence of these high-yielding shallot varieties with additional superior traits will reduce the risk of yield loss in shallot farming.

One of the social aspects that received positive responses was the liveliness of the farmer group. Farmer groups are a means of gaining support in the form of knowledge or skills as well as physical support from the government because, through these groups, the farmers' activities are accommodated. However, farmers' knowledge of TSS technology is still low, which necessitates extensive dissemination of technology to increase the farmer's understanding of the technology, which then allows them to be interested in adopting the technology. Supporting infrastructure facilities are also considered by farmers in adopting new technologies, including locally available production facilities.

From the technical aspect, farmers still have a limitation in TSS cultivation technology. Therefore, extensive dissemination of the TSS is urgently required. Furthermore, as a new thing, farmers even do not know the places to get production facilities, including TSS seeds. Currently, TSS technology is still at the level of Central Sulawesi Assessment Institute of Agricultural Technology (AIAT) researchers; thus, this technology needs to be more expanded, especially to the extension agents who can guide farmers directly and continuously in using this technology.

In conclusion, shallot cultivation using TSS was found profitable in the study area. It was economically beneficial and feasible to be managed with an R/C of 3.15. Perception of farmers in Sigi Regency on TSS was positive regarding three aspects, namely economic, social, and technical. Furthermore, TSS products can be accepted by the market. From a social aspect, infrastructure facilities and the existence of farmer groups supported the development of TSS while the knowledge of farmers about TSS application needs to be improved. The study results necessitate more extensive dissemination to improve the knowledge of farmers and extension workers about TSS through demonstration plots in the farming level.

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Effect of inoculation of two Aztobacter and nitrogen fertilizer on of peppermint (*Mentha piperita*) essential oil

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Abstract. *Bidgoli RD. 2019. Effect of inoculation of two Aztobacter and nitrogen fertilizer on of peppermint (*Mentha piperita*) essential oil. Asian J Agric 3: 22-25.* This study was conducted to study changes of percentage and yield of essential oil of peppermint (*Mentha piperita*) as a split factorial in a randomized complete block design (RCBD) with three replications in 2016-2017. The experimental treatments were nitrogen at four levels 0, 50, 100 and 150 kg/ha as the main factor and two strains of PGPR **(***Azotobacter chroococcum* MZ11, MZ26) in two states of (use and non-use) was considered as subfactors. Results showed that use of these two *A. chroococcum* strains has led to an increase in the percentage and yield of peppermint essential oil more than all Nitrogen fertilizer treatments. Also, the highest peppermint essential oil percentage (0.73%) was obtained in the triple interaction of Nitrogen (100 kg/ha) *A. chroococcum* MZ11 and *A. chroococcum* MZ26. The highest essential oil yield (91.65 kg/ha) was observed under the influence of the triple interaction of Nitrogen (100 kg/ha), *A. chroococcum* MZ11 and *A. chroococcum* MZ26.

Keywords: *Aztobacter chroococcum*, essential oil, fertilizer, peppermint, plant yield

INTRODUCTION

This study was carried out to investigate the effect of biofertilizers on the essential oil of peppermint in 2018. Peppermint (*Mentha piperita* L.), which belongs to the family Lamiaceae, is a hybrid species obtained from the confluence of *Mentha spicata* and *Mentha aquatica* species (Foster 1996; Peirce 1999). This plant species is a perennial plant, with a height of 50 to 60 cm, a quadrangular stalk that is usually purple in color and smooth (Govedarica et al. 2003). The global peppermint essential oil production is about 8,000 tons/year. The main compounds of peppermint essential oil are menthol (29%), menthon (20-30%), and methyl acetate (1 to 3%). Extraction of peppermint essential oil from the aerial parts of the plant at the beginning of the flowering stage is usually by steam distillation method. About 30 to 70% of its essential oil is menthol and esters of menthol, and the rest are more than 40 other compounds (Afzal 2010). Other compounds found in the peppermint essential oil include flavonoids (12%), polymerized polyphenols (19%), carotene, tocopherol betaine and choline (Murrray 1995). Currently, peppermint is used for the treatment of irritable bowel syndrome (IBS), inflammatory bowel disease (Crohn and ulcerative colitis), gallbladder inflammation, biliary system defects and liver problems (Blumenthal 1998; Fleming 1998).

Peppermint is a long day plant (LDP), and its planting in long day conditions leads to increase in its production and yield. (Okan et al. 2004). Singh and Chatterjee (1989) stated that nitrogen increases the vegetative growth in the plant and leaf area index, number of sub-branches and flowering branches, for example in *Mentha sativa***,** with use of 150 kg/ha nitrogen obtained the highest of desired traits. Anvar et al. (2005) found that the application of 100 kg/ha nitrogen fertilizer increased the number of branches, number of leave pigments and the dry matter per unit and yield of essential oils. Masto et al. (2006) studied the effect of micronutrients and planting density on essential oil content and essential oil yield of the peppermint plant, and reported that the solution spraying of microelements produced the highest essential oil yield.

Valad Abadi et al. (2008) reported on the effects of different levels of drought stress and nitrogen on the *Calendula officinalis* L. and showed the effect of nitrogen on the percentage essential oil yield was significant at (1% level). Akbarinia et al. (2012) evaluated effect of fertilizer on the percentage and yield of *Trachyspermum ammi* essential oil. They stated with increasing nitrogen and phosphorus, grain yield increased up to 90 and 60 kg/ha, respectively. Chemical fertilizers had no effect on essential oil percentage and 60 kg/ha nitrogen, 40 kg/ha phosphorus and 25 tons of manure per hectare and 60 kg/ha nitrogen with 15 tons manure per hectare produced the highest grain and essential oil yield. Imam et al. (2014) reported that the use of nitrogen fertilizers increases the size, longevity, and freshness of leaves and shoots in the plant.

Also, Balyan and Sobti, (1990) reported that application rate of 80 kg/ha nitrogen resulted in the accumulation and highest increase of dry matter in basil. Indiscriminate use of chemical fertilizers, most especially, nitrogen, coupled with lack of organic fertilizers in recent years, has resulted in significant reductions in the amount of organic matter in Iranian agricultural soils (Malekooti 2018). Green fertilizers are plants that are modified to improve the physical, chemical, and biological properties of soils, and to supply essential nutrients for optimum plant growth in successive growing seasons (Cherr et al. 2016). In fact, increased plant growth by inoculation of Azotobacter, has been attributed to the hormones produced by these bacteria and root growth (Zaied et al. 2003). The use of green fertilizers in addition to nitrogen fertilizers causes the nutrients to be readily available for crop production during the growing season and achieves more performance compared to the sole application of chemical fertilizers (Aktar et al. 1993; Paramanic et al. 2014).

MATERIALS AND METHODS

Plant cultivation

This study was conducted in a split factorial randomized complete block design (RCBD) with 3 replications from fall 2016 to spring 2017. The nitrogen factor of urea source at four levels, 0, 50, 100, 150 kg/ha as the main factor and two types of Azotobacter (*Azotobacter chroococcum* MZ11, MZ26) in two states (use and non-use) as sub-factor were considered.

Rooted branches with lengths of 8 to 10 cm were cut from 2- to 3-year-old plants at 3 leaf stage and cultivated in 12 plots (4 treatments with 3 replications) of 25 m^2. The selected plants were same in terms of size and height and based on the main and subfactors. The nitrogen at four levels 0, 50, 100 and 150 kg/ha were the main factors and other treatments were divided into 4 groups $N_{0:}$ (MZ11, MZ26), $N_{50:}$ (MZ11, MZ26),), $N_{100:}$ (MZ11, MZ26)), $N_{150:}$ (MZ11, MZ26). The nitrogen treatments herein were applied as done previously to investigate the interaction of the treatments. Planting was done in early January and due to the high sensitivity of *Mentha piperita* to drought stress in the region, irrigation was carried out every 4 days. Also, weed control was done manually owing to the experimental design adopted and the medicinal properties of the peppermint plant, from the beginning of the vegetative stage to the end, weeding was carried out continuously. There was no incidence of pest invasion, hence chemical pest control was not carried out. This was achieved because of the scent emitted by the plant. Physical and chemical properties of soil are presented in Table 1.

Table 1. Physical and chemical properties of farm soil

Soil property	Value
Cu (ppm)	1.60
Mg (ppm)	24.80
Mn (ppm)	9.88
P (ppm)	12.50
K (ppm)	255.00
N (ppm)	0.08
$CaCO_3$ (ppm)	6.00
Texture	Loam
Sand (%)	38.00
Silt (%)	35.00
Clay (%)	27.00

After harvest operations, the yield of fresh weight was determined immediately and to determine the yield of dry weight, samples were dried at room temperature (25ºC) and in shade for 10 days and then weighed. In order to determine the percentage and yield of *Mentha piperita* essential oil obtained from the different treatments, the plant samples were dried and weighed at room temperature and in shade. The samples were subjected to laboratory analyses, wherein essential oil was obtained by the water distillation method. The Clevenger device was used to calculate the essential oil percentage dehydration by dry Sodium sulfate (Na_2SO_4). The essential oil yield was obtained from the multiplication of the essential oil percentage in the biological function divided to 100. Data were analyzed by MSTAT-C software, and mean comparison was carried with Duncan's method at 1% level of significance.

RESULTS AND DISCUSSION

Essential oil percentage

The results presented in Table 2 show that the individual effect of nitrogen on essential oil percentage of *Mentha piperita* was significant at 5% level and two *A. chroococcum* strains were significant at 1% level. According to Table 3 and the results of the independent effect of the treatments, the lowest and highest essential oil percentages were obtained from the control nitrogen (mean = 0.15%), and the *A. chroococcum* MZ26 treatment (mean = 0.44%), respectively. Omid Beigi (1995) and Anvar et al. (2005) reported that the application of 100 kg of nitrogen would increase the yield of the branches, the number of leaves, pigment of leaves and increase of dry matter yield per unit area and yield of essential oil. Also, Bist et al. (2000) found that with addition of nitrogen fertilizer to soil, percentage, and some components of *Anethum graveolens* essential oil increased.

These observations could be attributed to the soil's ability to retain more moisture because of improved soil structure upon the incorporation of the PGPR treatments. This ultimately resulted in the increased biological yield of the peppermint plant and its essential oil yield. On the other hand, the increase in essential oil from the application of different fertilizer treatments could be due to the readily availability of nutrients, such as nitrogen and phosphorus, for the formation of ATP and NADPH, which serve in the pathway to the formation of terpenoids and isoprenoids in the essential oils (Loomis and Corteau 1972).

The interactions of nitrogen fertilizer with incorporated *A. chroococcum* MZ11 and *A. chroococcum* MZ26 on the percentage of essential oil were significant at 5% and 1% levels respectively, however, their triple effect was not significant (Table2). In the double interaction of Nitrogen and this two *A. chroococcum,* results showed the highest percentage of essential oil was obtained from 150 kg/ha nitrogen in combination with incorporated *A. chroococcum* MZ11 (0.62%), and the lowest essential oil percentage, obtained from the interaction of these two *A. chroococcum* (MZ11 and MZ26)as 0.16% (Table 4). In the essential oil yield, the highest and lowest values were obtained in these treatments too in a similar study, Valad Abadi et al. (2008) investigated the effects of different levels of nitrogen on *Calendula officinalis* and concluded that nitrogen increased the percentage and yield of its seed oil.

The interaction of *A. chroococcum* MZ11 and *A. chroococcum* MZ26 on the percentage of essential oil was significant at 5% level and presented in Table 2. According to Table 3, *A. chroococcum* MZ26 increased the essential oil percentage more than *A. chroococcum* MZ26, but in terms of essential oil yield, MZ26 was more effective than MZ11. The triple interaction of nitrogen, and *A. chroococcum* MZ11 and *A. chroococcum* MZ26 on the percentage of essential oil were significant ($p < 5\%$) (Table 2).

In the triple interaction of Nitrogen, *A. chroococcum* MZ11 and *A. chroococcum* MZ26 the highest percentage of essential oil of 0.48% was obtained from the treatment with 150 kg/ha nitrogen and the two *A. chroococcum* strains, and the lowest percentage with mean of 0.38% and the lowest from the 50 kg/ha nitrogen and the two *A. chroococcum* strains with a mean value of 0.38% (Table 5). Like the study by Akbarinia et al. (2012), fertilizer application resulted in an increase in essential oil content and essential oil yield in *Trachyspermum ammi*. They also stated that application of nitrogen and phosphorus up to 60 and 90 kg/ha, respectively resulted in increased grain yield size, longevity of leaves and branching, and the freshness of leaves in the plant.

Essential oil yield

The individual effect of nitrogen on essential oil yield of *Mentha piperita* was significant ($p < 5\%$) (Table 2). In this regard, the highest yield was obtained from the *A. chroococcum* strains (55.80 kg/ha), and the lowest was related from the control of nitrogen (25.23 kg/ha) (Table 3). According to the results, the increase in the use of nitrogen from zero to 150 kg/ha increased the essential oil percentage and essential oil yield (Table 4). These results are a clear indication of the role of nitrogen in increasing the vegetative growth in plants, hence, increase in total dry matter yield per unit area. A close analysis of the results revealed that the triple interaction of nitrogen, *A. chroococcum* MZ11, and *A. chroococcum* MZ26 had the most significant effect on the percentage and yield of essential oil, which implies that the combined effect of these treatments has an enormous effect compared to their individual and cross-linking effects.

The individual effects of *A. chroococcum* MZ11 and *A. chroococcum* MZ26 on essential oil yield of *Mentha piperita* were significant at 1% level. (Table 2). The interactions of nitrogen fertilizer and *A. chroococcum* MZ11 and *A. chroococcum* MZ26 on the yield of essential oil were significant at 5% and 1%, respectively (Table 2). According to the results, it is obvious that the *A. chroococcum* MZ11 and *A. chroococcum* MZ26 complemented the role of the nitrogen fertilizer to effectively meet the plant requirement. Based on the results of Table 4, the combination of these two *A. chroococcum* strains with nitrogen fertilizer have a greater effect on the combination of these two *A. chroococcum* strains together. The combined effect of *A. chroococcum* MZ11 and *A. chroococcum* MZ26 on the yield of essential oil was significant at 5% (Table 2). In this regard the highest yield of essential oil obtained 91.65 kg/ha and the lowest yield, being 52.62 kg/ha (Table 5). The triple interaction of nitrogen and two *A. chroococcum strains* on the yield of essential oil were also significant at 1% (Table 2).

Table 2. Analysis of variance of Nitrogen, *A. chroococcum* MZ11 and *A. chroococcum* MZ26 on the percentage and yield of essential oil of *Mentha piperita*

SOV	df	MS	
		Essential oil percentage	Essential oil yield
Replication	2	1.280^{ns}	6325648.333^{ns}
N	3	182.025*	3325416.226*
Error	6	20.335	358469.875
MZ11	1	2225.036**	72136548.215**
MZ11*MZ26	3	48.387*	2569823.7012**
MZ26	1	523.081**	13269587.658**
N*MZ11	3	11.685*	2569875.325**
N*MZ26	1	52.431**	22564.548*
N* MZ26*MZ11	3	83.152*	203269.559*
Error	24	25.325	1352648.562
CV (%)	-	13.23	8.2

Table 3. Comparison of the average effect of nitrogen, *A. chroococcum* MZ11 and *A. chroococcum* MZ26 on the percentage and yield of essential oil of *Mentha piperita*

Treatment	Essential oil percentage	Essential oil yield (kg/ha)
Nitrogen (kg/ha)		
0	0.15d	20.23b
50	0.20c	27.53d
100	0.22b	30.45d
150	0.35a	45.60c
MZ11	0.43a	55.80b
MZ26	0.44a	49.50a

Table 4. Comparison of the average double interaction of nitrogen, *A. chroococcum* MZ11 and *A. chroococcum* MZ26 on the Percentage and yield of essential oil of *Mentha piperita*

Treatment	PGPR	Essential oil percentage	Essential oil yield (kg/ha)
Nitrogen (kg/ha)	MZ11		
0		0.25d	33.70c
50		0.30c	42.58c
100		0.39b	53.38b
150		0.62a	80.30a
Nitrogen (kg/ha)	MZ26		
0		0.22b	27.26d
50		0.28a	38.05ab
100		0.34a	44.33b
150		0.41a	55.90a
MZ11	MZ26	0.16c	21.38c

Table 5. Comparison of the average triple interaction of Nitrogen, *A. chroococcum* MZ11 and *A. chroococcum* MZ26 on the percentage and yield of essential oil of *Mentha piperita*

Average		Treatment		
Essential oil yield (kg/ha)	**Essential oil percentage**	**MZ26**	**MZ11**	**Nitrogen (kg/ha)**
0.15i	20.23i	Non-use	Non-use	0
0.44gh	49.50gh	Use		
0.43ef	55.80ef	Non-use	Use	
0.16cd	21.38c	Use		
0.20i	27.53i	Non-use	Non-use	50
0.28h	38.05h	Use		
0.30fg	42.58fg	Non-use	Use	
52.62de	0.38de	Use		
0.22i	36.45i	Non-use	Non-use	100
0.34h	44.33gh	Use		
0.39cde	53.38cd	Non-use	Use	
65.09b	0.73b	Use		
0.35b	45.60b	Non-use	Non-use	150
0.41gh	55.90i	Use		
0.62a	80.30a	Non-use	Use	
91.65c	0.48c	Use		

Similarly, Banchio et al. (2009) reported that the application of biological fertilizers to basil, was very effective in increasing the biomass and yield of essential oils. Thus, the positive effects of biofertilizers such as incorporation of Aztobacter on improving the nutritional conditions in plants have been proven in this study.

According to the results obtained in this study, the application of different Aztobacter both singularly and in combination resulted in the increase in the yield of essential oil of peppermint. Thus, the incorporation of *A. chroococcum* MZ11 and *A. chroococcum* MZ26, especially by small-holder farmers will serve as a good alternative to application of chemical fertilizers. Generally, the use of crop techniques and environmental factors in the cultivation of crops to increase the amount of active ingredients is a very interesting and important subject matter, for example, the use of legumes as green fertilizers, nitrogen will be released gradually over a long period of time to enhance nitrogen absorption by plants during successive growing periods.

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Effect of container and potting media on raising quality seedlings of *Acacia auriculiformis* in the nursery

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Abstract. *Islam MDA, Rahman MDA, Hossain MK. 2019. Effect of container and potting media on raising quality seedlings of* Acacia auriculiformis *in the nursery. Asian J Agric 3: 26-32.* This study elucidates the effect of container and potting media on raising quality seedlings of *Acacia auriculiformis* in the nursery of the Institute of Forestry and Environmental Sciences, University of Chittagong to find out a suitable container and potting media for raising large scale quality seedlings. The seedlings were evaluated by testing five containers and seven potting media treatments for eight months. A Completely Randomized Block Design (CRBD) was adopted for the study with three replications for each treatment. The Analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) was tested for the analysis to explore the possible treatment variations. However, the study reveals nodulation with growth parameters; shoot and root dry biomass production and quality index were highest in 20 cm × 15 cm size polybags whereas the highest root length and shoot-root ratio was observed in 15 cm × 10 cm and 15 cm × 13 cm size polybag respectively. Considering the potting media, highest nodulation, growth parameters, shoot dry and fresh weight, shoot- root ratio and biomass were found in combination of soil + cow dung + phosphorus (0.16 g/polybag). Highest root length, root fresh and dry weight, and quality index were found in the combination of soil + cow dung (3:1). Therefore, it is recommended that containers of 20 cm × 15 cm size polybag and with a potting media of soil + cow dung + phosphorus (3 parts soil, 1 parts cow dung + 0.16 g/polybag) combination produce quality *A. auriculiformis* seedlings in the nursery.

Keywords: Akashmoni, Bangladesh, quality index, nodulation, shoot-root ratio

INTRODUCTION

Demand for different land uses and continual deforestation are responsible for decreasing natural forestland. As a result, continuous supply of wood from natural forests is becoming very difficult for different purposes (Asif et al. 2017). Plantations of fast-growing species must be established as a compensation package for the declining supply from natural forests (Sharma et al. 2011).

Akashmoni (*Acacia auriculiformis* A. Cunn. ex Benth.) is an evergreen, exotic, heavily branched, forked bole species, mostly planted on roadsides and railway embankments, parks and gardens because of its ornamental and shade bearer qualities in Bangladesh (Das and Alam 2001; Hossain et al. 2009; Girijashankar 2011; Islam et al. 2013). It is a multipurpose tree species and is considered one of the most promising plantation species because of its ability to survive on a wide range of degraded environmental conditions (Alam et al. 1991; Das and Alam 2001; Jahan et al. 2008). Globally, in good soil conditions, the species can reach a height up to 35 m (Orchard and Wilson 2001). Where in consequence, transmission poles and posts can be made from rounded timber (Sattar et al. 1999). Considering the growth, short rotation, non-palatability to grazing animals, nowadays, *A. auriculiformis* is also preferred for afforestation, reforestation, and agroforestry purposes in Bangladesh (Hossain et al. 1994; Uddin et al. 2007; Azad et al. 2011). Good quality durable heartwood of *Acacia* is suitable for being used to create furniture and for other constructional purposes (Pinyopusarerk 1990). In addition, *Acacia* wood is ideal for fuelwood, charcoal making, and it has also been proved as a good pulpwood species (CABI 2013; Islam et al. 2013). To fulfill the high demand, many organizations are producing akashmoni seedlings in nurseries in Bangladesh to supply those in the plantation programs (Khan et al. 2014). However, the productivity of the plantations is not up to the mark. The main reason for lower productivity includes declining soil fertility and suppression of growth due to competing of seedlings especially in the nursery (Hulikatti and Madiwalar 2011).

Nursery establishment is the first and foremost obvious task in raising a successful plantation. Direct seeding results in wastage of improved seeds while planting in the nursery including losses and possible mortalities (Adu-Berko et al. 2011; Adu-Yeboah et al. 2015). In current forest nursery practices, container size of all dimensional features like, volume, height, diameter, and shape with different pre-sowing treatment are required to get good germination, required and desired quantity of seedlings in the nursery (Annapurna et al. 2004; Farhadi et al. 2013; Mozumder et al. 2018).

A lot of studies have been conducted to determine enhanced germination, growth performance and survivability of seedlings using different pre-sowing treatments (Napier 1987; Palani et al. 1995; Alamgir and Hossain 2005; Iqbal et al. 2007; Khan et al. 2014; Mridha et al. 2016) and container (Bharathia 1999; Natarajn 1999;

Annapurna et al. 2004; Biradar et al. 2014) of different species. But a detailed experiment on both (container and potting media) aspects of *A. auriculiformis* has not been conducted yet. Therefore, the present study was aimed to evaluate the suitable shape and size of containers and potting media to ensure production of quality seedlings of *Acacia auriculiformis* in nursery.

MATERIALS AND METHODS

Study site

The study was conducted from April 2017 to December 2017 in the nursery of Institute of Forestry and Environmental Sciences, University of Chittagong (IFESCU), Bangladesh. It lies approximately at the interaction of 91°50´E longitude and 22°30´N latitude (Figure 1) (Hossain et al. 2005). The altitude of this area is 14 m to 87 m above from the mean sea level (Mridha et al. 2016). The nursery site enjoys tropical monsoon climate characterized by hot, humid summer and cool, dry winter. The average annual rainfall of this area is about 2500-3000 mm, which mostly takes place between June and September. There is a mean monthly maximum temperature of 29.75°C and a monthly minimum of 21.24°C. The highest temperature usually occurs in May as 32.60°C, and minimum in January as 14.10°C (Peel et al. 2007).

Seed collection and experimental design

Seeds of *A. auriculiformis* were collected from Seed Production Areas (SPAs) of Institute of Forestry and Environmental Sciences, University of Chittagong, Chittagong, Bangladesh. The soils used in the experiment were collected from the forest floor of the University campus. To determine suitable container for *A. auriculiformis* seedling, the soil was sieved well (<3 mm sieve) and mixed with dried cow dung in a ratio of 3:1. For another experiment soil, cow dung, sand, sawdust, and phosphorus were used where polybags (15 cm × 13 cm) were filled with different combinations of mixture. To facilitate aeration and proper drainage, holes were made in the polybag by punching before filling with prepared mixture of soils. A Completely Randomized Block Design (CRBD) was adopted for the study with three replications for each treatment. There were five treatments of different containers and seven treatments of different mixtures used for the experiment. The treatments and containers type and size and their combination are given in Table 1 and Table 2.

Seed treatment and sowing

Seeds of *A. auriculiformis* were treated by soaking in hot water for 30 seconds, followed by being placed in cold water overnight before sowing in the pre-filled polybags and root trainer. Seeds were sown in the polybags and root trainers filled with growing media. Two seeds were sown in each polybag and root trainer directly. Seeds were dibbed to 0.5 cm under the soil by pressing them with thumb. Afterward, they were covered with a thin layer of soil.

Assessment of physiological growth performance

Three seedlings from each treatment were randomly selected and uprooted carefully during harvesting seedlings. All three seedlings were used to assess nodule number and to estimate growth. After taking records of shoot length, root length, collar diameter, fresh weight of shoots and roots separately, then oven-dried at 70ºC for 24 hours. Afterward, the dry weight of shoots and roots was taken. Average height of the seedlings for each treatment was also recorded. All the data were recorded monthly from the three months till to the eight months.

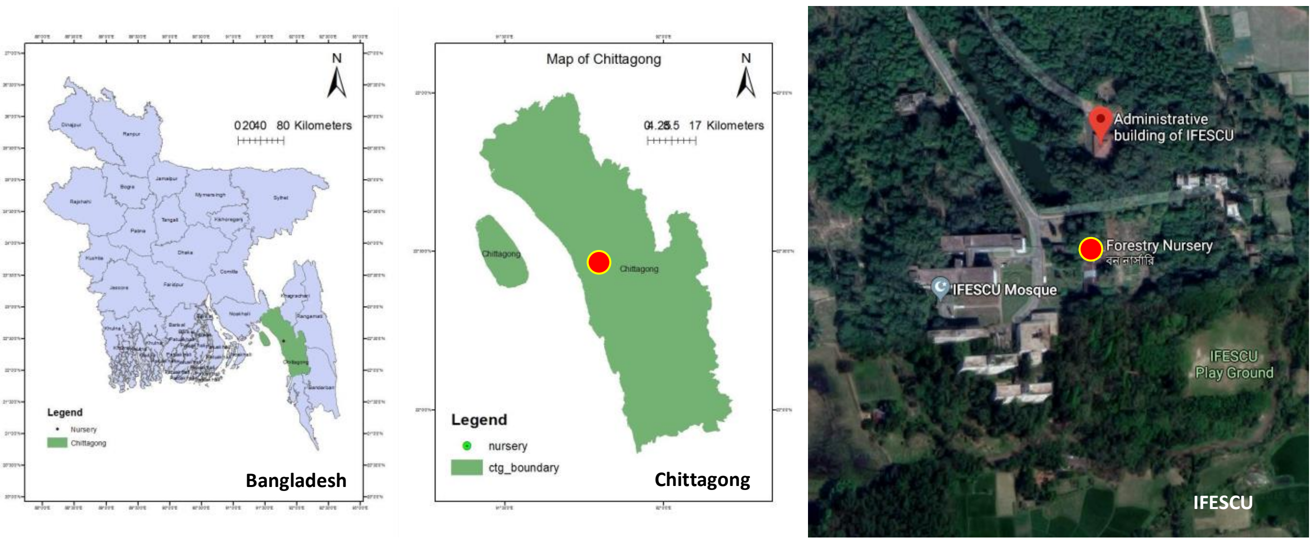

Figure 1. Map shows the location of the nursery of Institute of Forestry and Environmental Sciences, University of Chittagong (IFESCU) in Bangladesh where the experiment was conducted (Hathazari Upazila 2018; Google Maps 2019).

Table 1. Treatments and container type and size used to carry out the experiment.

Treat-ments	Containers type and size	No. of replication	No. of seedlings
T_1	13 cm × 10 cm (polybag)	3	60
T_2	15 cm × 10 cm (polybag)	3	60
T_3	15 cm × 13 cm (polybag)	3	60
T_4	20 cm × 15 cm (polybag)	3	60
T_5	Root trainer (20 cm × 5 cm)	3	60

Table 2. Treatments and their combination used to carry out the experiment.

Treat-ments	Combination	No. of replication	No. of seedlings
T_1	Sand only	3	60
T_2	Soil only	3	60
T_3	(Soil: Cowdung = 3:1)	3	60
T_4	Sawdust only	3	60
T_5	Soil + Cowdung + Phosphorus (0.16 g/polybag) @ 120 kg/ha	3	60
T_6	Soil + Phosphorus (0.16 g/polybag) @ 120 kg/ha	3	60
T_7	(Soil: Cowdung = 4:1)	3	60

Collection of root and shoot samples

During harvesting, soil around the seedling was loosened using hand softly, and all fine and coarse roots were collected carefully from the ground. To avoid damage, collected roots with adhered soil were immersed in water, in a clean white bowl, to allow soil particles to separate away. Water was changed several times for a complete wash.

Shoot-root ratio

Shoot-root ratio is the value obtained by dividing shoot (leaf and stem) with the root.

Quality index

The quality index (QI) as developed by Dickson et al. (1960) to quantify seedlings morphology was calculated as follows:

$$QI = \frac{\text{Total seedlings dry weight (g)}}{\frac{\text{Shoot height (cm)}}{\text{Collar diameter (mm)}} + \frac{\text{Shoot dry weight (g)}}{\text{Root dry weight (g)}}}$$

Statistical analysis

Recorded data related to seed germination and seedling growth attributes were analyzed statistically by using computer software SPSS ver.20.00. The Analysis of variance (ANOVA) and Duncan's Multiple Range test (DMRT) were tested for the analysis to explore the possible treatment variations.

RESULTS AND DISCUSSION

Physical parameters of the seedlings, e. g. height was recorded monthly from the age of three months and continued to eight months. At the end of 8-month average height, shoot length, root length, collar diameter, nodule number, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight and shoot-root ratio, biomass, and quality index of the seedlings of *A. auriculiformis* were measured and calculated.

Height of the seedlings grown in different containers

Initial height (cm) growth was taken after three months of sowing the seeds. Treatment T_4 showed highest height (37 cm) at the age of three months followed by treatment T_3. However, T_4 showed the average maximum height growth starting from three months till the end of the experiment, whereas it reached an average height of 136.2 cm at the end of the experiment followed by treatment T_3 (129.6 cm) and T_2 (117.3 cm) (Figure 2).

Height of the seedlings grown in different potting media

For potting media used in the nursery, the study revealed the difference between the average height of the seedlings with the respective treatment at the age of 8 months (Figure 3). At the age of 3 months, Treatment T_5 attained maximum height (27.2 cm) followed by T_6 (26.5 cm). However, T_5 showed the average maximum height (128.9 cm) from the beginning of the measurement to till the end of the experiment followed by the treatment T_6 (115 cm) (Figure 3).

Morphological growth parameters of the seedlings grown in different containers

The 8 months old seedlings grown in different containers showed highest shoot length **(**137.5 cm) in T_4 followed by T_3 (125.2 cm), they were significantly ($P<0.05$) different from others where the lowest shoot length (81.1 cm) was found in T_5 (Table 3). Considering the growth of root length, highest root length was also recorded in T_4 (38.1 cm) followed by T_2 treatment (29.6 cm). However, similar trends of results were observed for collar diameter and nodule number count whereas the highest collar diameter was found in treatment T_4 (9.5 mm) followed by T_3 (7.3 mm) and maximum nodule number (71) was observed in the treatment T_4 followed by T_3 (55 mm).

Effects of containers on fresh and dry matter production of the seedlings

Fresh and dry matter production, e.g., shoot fresh weight, shoot dry weight, root fresh weight and root dry weight of 8 months old *A. auriculiformis* seedlings are shown in Table 4. Maximum shoot fresh weight (56.16 g) was found in treatment T_4 followed by T_3 (36.83 g) which is significantly different from other treatments except for T_3. Besides, maximum root fresh weight (12.66 g) was found in treatment T_4 followed by T_2 (6.83 g). In the case of dry weight, shoot dry weight was maximum (26.10 g) in T_4 followed by T_3 (16.38 g) whereas maximum root dry weight (6.80 g) was found also in T_4 followed by T_2 (3.43 g) and T_3 (3.28 g).

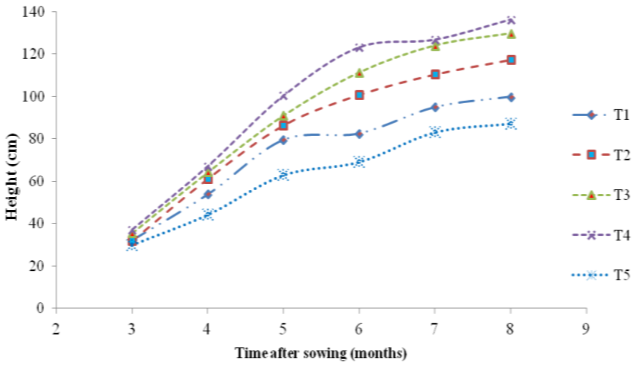

Figure 2. Height of *A. auriculiformis* seedlings at the age of 3 to 8 months grown in different containers in the nursery.

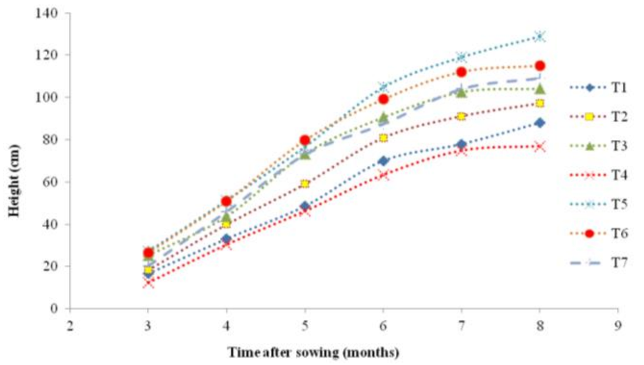

Figure 3. Height of *A. auriculiformis* seedlings at the age of 3 to 8 months grown in different potting media in the nursery.

Effects of container on shoot-root ratio, biomass (g) and quality index

The highest shoot-root ratio was found in treatment T_3 (5.05) followed by T_2 (4.10), this was significantly different (P<0.05) from other treatments. Biomass production of 8 months old *A. auriculiformis* seedlings were highest (32.90 g) in T_4 treatment followed by T_3 (19.67 g). Similarly, the highest value of quality index was found in treatment T_4 (1.81) followed by T_3 (0.89) and it was significantly different from other treatments (Table 4).

Morphological growth parameters of the seedlings grown in different potting media

For 8 months old seedlings, shoot length was highest (133.8 cm) in T_5 followed by T_6 (121.6 cm) and is significantly different (P<0.05) with other treatments (Table 5). Considering the root length, highest root length was recorded in T_3 (40.1 cm) followed by T_5 treatment (39 cm). In addition, the highest collar diameter was recorded in T_3 treatment (7.9 mm). However, T_6 (7.4 mm) and T_5 (7.3 mm) showed promising growth performance for collar diameter where the least was recorded in T_4 (5.1 mm). Similarly, maximum nodulation was observed in T_5 (68) followed by T_3 (61) and T_6 (55) respectively (Table 5).

Effect of potting media on fresh and dry weight (g) of shoot and root of the seedling

Maximum shoot fresh weight (45.16 g) was recorded in T_5 followed by T_3 (37.16 g) (Table 6) while root fresh weight was maximum (9.16 g) in T_3 followed by T_5 (8.83 g). An almost similar trend of results found for shoot dry weight production and highest value (19.22 g) was found in T_5 treatment followed by T_3 (16.87 g). However, maximum root dry weight (4.72 g) was found in T_3 treatment followed by T_5 (4.64 g).

Table 3. Effect of containers on shoots and root length, collar diameter and nodule number of 8 months old *A. auriculiformis* seedlings.

Treatments	Shoot length (cm)	Root length (cm)	Collar diameter (mm)	Nodule number
T_1	93.4^{bc}*	26.4^{b}	6.4^{b}	37^{c}
T_2	117.9^{ab}	29.6^{b}	7.0^{b}	45^{bc}
T_3	125.2^{a}	26.2^{b}	7.3^{b}	55^{b}
T_4	137.5^{a}	38.1^{a}	9.5^{a}	71^{a}
T_5	81.1^{c}	24.4^{b}	6.0^{b}	42^{bc}

*Means followed by the same letter (s) in the same column do not vary significantly at P<0.05, according to Duncan's Multiple Range Test (DMRT).

Table 4. Effect of containers on fresh and dry weight of shoot and root, shoot- root ratio, biomass (g) and quality index of 8 months old *A. auriculiformis* seedlings.

Treatment name	Fresh weight (g)		Dry weight (g)		Biomass (g)	Shoot: root	Quality index
	Shoot	Root	shoot	Root			
T_1	23.50b*	5.16b	10.80b	2.78b	13.58b	3.87b	0.73b
T_2	31.83b	6.83b	14.39b	3.43b	17.82b	4.10b	0.86b
T_3	36.83ab	6.33b	16.38b	3.28b	19.67b	5.05a	0.89b
T_4	56.16a	12.66a	26.10a	6.80a	32.90a	3.80b	1.81a
T_5	17.33b	4.83b	7.34b	2.62b	9.97b	2.78c	0.61b

*Means followed by the same letter (s) in the same column do not vary significantly at P<0.05, according to Duncan's Multiple Range Test (DMRT)

Effect of potting media on shoot-root ratio, biomass (g) and quality index

In the case of 8 month old seedlings, highest shoot-root ratio was found in T_5 (4.05) followed by T_6 (3.93). But this was not significantly different with other treatments (Table 6). Similarly, biomass production was highest (23.86 g) in T_5 followed by T_3 (21.59 g) and maximum (1.16) quality index was found in T_3 treatment followed by T_5 (1.06).

Discussion

Successful germination and raising seedlings are mandatory steps for conservation and enlargement of plant communities (de Melo et al. 2015). A vital ingredient for the success of plantation programs is the availability of adequate supplies of quality seedlings. However, the present study indicates that the growth parameters (shoot and root length, collar diameter, fresh and dry weight of shoot and root, shoot root ratio, quality index and biomass) and nodule number of seedlings recorded from different combinations of container and potting media treatments in *A. auriculiformis* varied significantly compared to control.

Considering the container type and size, the present study revealed that the average maximum height of *A. auriculiformis* seedlings from 3 months was found in T_4 treatment of 20 cm × 15 cm polybags. Seedlings at the age of 3 months attained a height of 37 cm and 136.2 cm at the age of 8 months. Similarly, the study revealed longest shoot length (137.5 cm), root length (38.1 cm) and collar diameter (9.5 mm) in T_4 treatment. Venkatesh et al. (2002) reported collar diameter of 5 months old *Acacia nilotica* seedlings was maximum (7 mm) in 25 × 15 cm polybag and not supported by present study. Maximum nodule number (70) was also found in 20 cm × 15 cm size polybag. This is because T_4 treatment contains much-growing media which supplies more nutrients to the seedlings and the results support the findings of Hossain et al. (2009).

In case of dry matter production, maximum shoot weight (26.10 g) and root weight (6.80 g) were also recorded in the same treatment (T_4). However, the result also coincided with Venkatesh et al. (2002) who reported highest shoot and root dry weight of 5 months old *Acacia nilotica* seedlings were 6.68 g and 3.42 g in 25 × 15 cm polybags. Moreover, it was also found that if polybag size increased then the value of fresh and dry matter also increased.

The shoot-root ratio was highest (5.05) in treatment T_3 (15 cm × 13 cm size polybag). The biomass 32.9 g and quality index 1.81 of the seedlings was highest in T_4 and are significantly different from other treatments whereas Annapurna et al. (2004) reported the highest biomass and quality index was 3.08 g and 0.37 of 6 months old sandalwood seedlings in root trainer (black) which partially supported the present study.

In the study, except shoot-root ratio, all the parameters showed best performance in 20 cm × 15 cm size polybag. However, several researchers found suitable container sizes for species, such as 30 cm × 20 cm for Cocoa (Keshavachandran and Larson 1985), 30 cm × 13 cm for *Santalum album* (Karivaradharaaju et al. 1999), 26 cm × 12.6 cm for *Azadirachta indica* (Bharathia 1999) and 25 cm × 15 cm for *Albizia lebbek* (Natarajn 1999).

The seedlings raised on good media will ensure better establishment and growth when planted in the main field. The ultimate advantage of good potting mixture is good drainage, water holding capacity and thereby, it gives excellent disease-free growth of the seedlings (Noble 1993). In the case of a balanced potting mixture, the present study indicated that the average maximum height of *A. auriculiformis* seedlings from 3 months till the end of the experiment was found in treatment T_5, soil + cow dung + phosphorous (0.16 g/polybag) mixture. In treatment T_5, at the age of 3 months, highest height was 27.2 cm and at the age of 8 months, it was 128.9 cm. Ramesh (2007) reported highest seedling height (36.36 cm) of 4 months old *Pongamia pinnata* in Black soil + Black sand + Vermicompost mixture where the media was different from present study.

Table 5. Effect of potting media on shoot and root length, collar diameter and nodule number of 8 months old *A. auriculiformis* seedlings.

Treat-ments	Shoot length (cm)	Root length (cm)	Collar diameter (mm)	Nodule number
T_1	88.8c*	36a	6.9ab	47ab
T_2	92.6bc	37.7a	5.7bc	33b
T_3	118.7ab	40.1a	7.9a	61a
T_4	66.6c	32.6a	5.1c	37b
T_5	133.8a	39a	7.3ab	68a
T_6	121.6ab	32a	7.4a	55ab
T_7	109.6ab	26.2a	6.4abc	48ab

Note: *Means followed by the same letter (s) in the same column do not vary significantly at P<0.05, according to Duncan's Multiple Range Test (DMRT)

Table 6. Effect of potting media on fresh weight and dry weight of shoot and root of 8 months old *Acacia auriculiformis* seedlings

Treatment name	Fresh weight (g)		Dry weight (g)		Biomass (g)	Shoot: root	Quality index
	Shoot	Root	shoot	Root			
T_1	26ab*	7ab	11.71abc	3.63ab	15.35abc	3.12a*	0.96a
T_2	18.83b	4.83ab	8.36bc	2.87ab	11.23bc	2.94a	0.59a
T_3	37.16ab	9.16a	16.87ab	4.72a	21.59ab	3.56a	1.16a
T_4	15.16b	3.33b	6.54c	1.93b	8.47c	3.38a	0.56a
T_5	45.16a	8.83a	19.22a	4.64a	23.86a	4.05a	1.06a
T_6	32.66ab	7.16ab	15.07abc	3.77ab	18.84abc	3.93a	0.93a
T_7	24.83ab	6.83ab	12.45abc	3.92ab	16.37abc	3.11a	0.81a

Note: *Means followed by the same letter (s) in the same column do not vary significantly at P<0.05, according to Duncan's Multiple Range Test (DMRT).

More shoot length (133.8 cm), nodule number (68), shoot fresh weight (45.16 g), shoot dry weight (19.22 g), shoot- root ratio (4.05) and biomass (23.86 g) was found in T_5, soil +cowdung + phosphorus (0.16 g/polybag) mixture. Hossain et al. (2009) found highest shoot length (79.7 cm), shoot fresh weight (24.85 g), shoot dry weight (24.85 g) and nodule number (46) in soil and residential sludge combination 2:1 of 3 months old *A. auriculiformis.* Uddin et al. (2007) reported highest shoot length (57.67 cm) and nodule number (56.67) was found in 80-day old, fertilized seedling of *A. auriculiformis.* The present result shows the similarity with their findings. Similarly, Uddin et al. (2012) found a positive correlation between seedling growth and the different doses of organic fertilization. So, it is evident that phosphorus enhances the seedlings growth as well as seedling quality in the nursery.

In other cases, more root length (40.1 cm), collar diameter (7.9 mm), root fresh weight (9.16 g), root dry weight (4.72 g) and quality index (1.16) was found in T_3 (soil: cow dung = 3:1). Uddin (2007) also reported highest root length (28.33 cm) and collar diameter (7 mm) was found in 80-day old, fertilized seedling of *A. auriculiformis*. Belen (1987) reported phosphorus fertilization increased the total P, Mg content and P uptake, where the study agreed with the present results.

In conclusion, availability of planting stock in proper time with adequate quantity and proper quality is a challenge for plantation establishment. To overcome the problems of poor-quality seedlings, the study of containers and effect of mixture growing on seedlings in the nursery is of great importance. Quality seedlings can ensure better survival and successful establishment of plantations, acclimation to variable of environment and reduce rotation by increased yield. Therefore, the study revealed maximum growth parameters including nodule formation was highest in 20 cm × 15 cm polybag than other types and size of container and containing soil + cow dung + phosphorus (0.16 g/polybag) mixtures than other potting media. However, the people of our country are hardly conscious of the impact of container and potting media on quality seedling raising programs in nursery. So, there is a need to have further investigation of established plantations raised with quality seedlings of *A. auriculiformis* by using the present containers and potting media.

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