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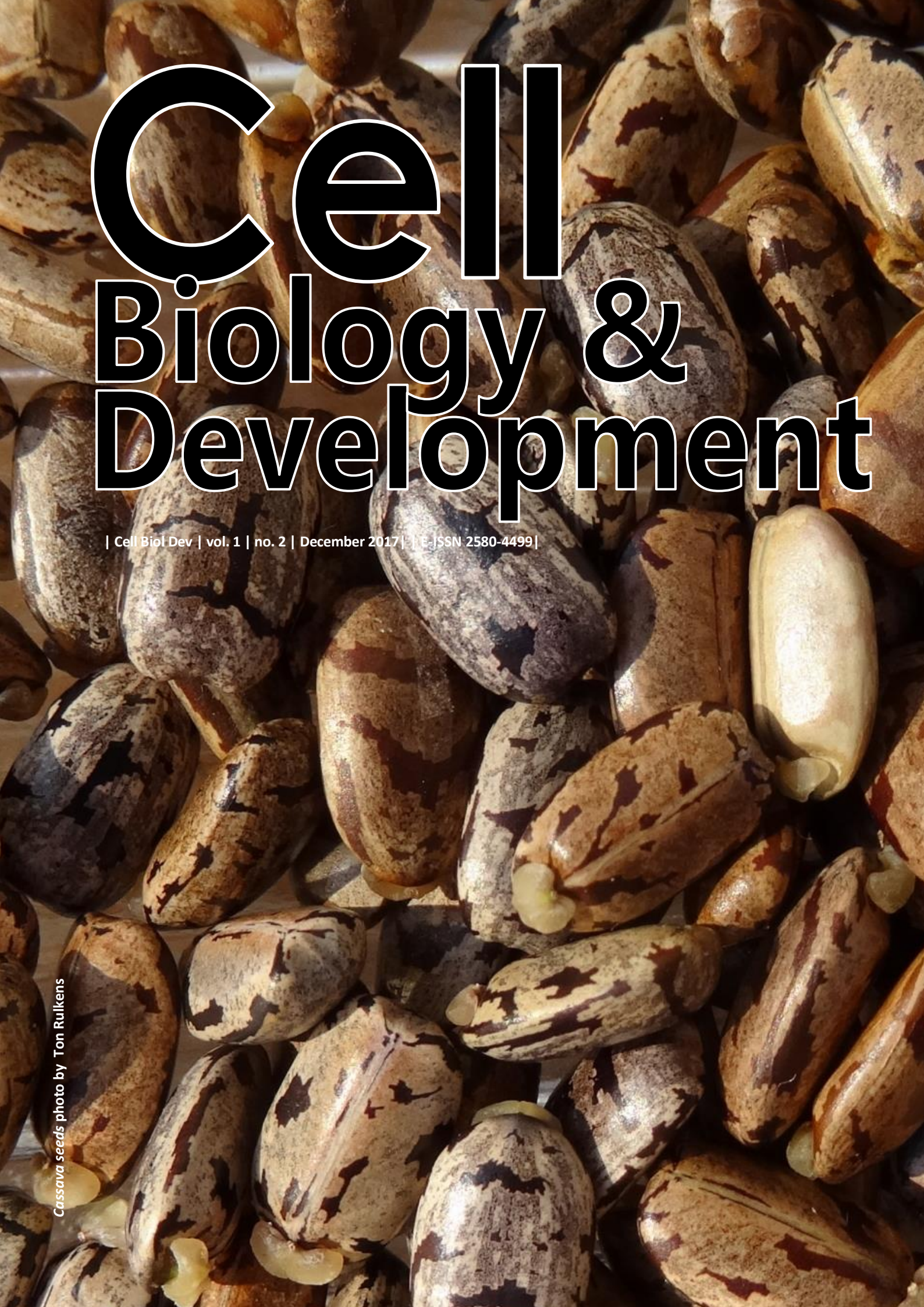

Cassava seeds photo by Ton Rulkens

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Book:

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Chapter in book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds) Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

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:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.) Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

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Genetic potential of cassava biodiversity in Bangka Island, Indonesia

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Manuscript received: 1 May 2017. Revision accepted: 6 September 2017.

Abstract. *Lestari T, Apriyadi R. 2017. Genetic potential of cassava biodiversity in Bangka Island, Indonesia. Cell Biol Dev 1: 41-45.* Cassava is potentially a mixture ingredient of flour in the Bangka's food industry. This study aimed to discover the biodiversity of local cassava in Bangka. This research was conducted in the experimental field of the Faculty of Agriculture, University of Bangka Belitung, Indonesia, from July 2015 to July 2016. The experimental design was randomized block design with 10 local cassavas of Bangka that consisted of upang, sekula, bayel, mentega, kuning, batin, pulut, sutera, rakit, and Selangor. Isozyme analysis was performed using starch gel electrophoresis with horizontal models. Analysis for five Bangka local cassava varieties and one National cassava variety used RAPD group OP A and OP B. The results showed that the phenotypic performance was different on the type of plant, the morphology of leaves, stems, and tubers of local cassava of Bangka. Furthermore, isozyme analysis showed a polymorphic banding pattern, while the eight RAPD primers used did not produce polymorphic. Furthermore, this research showed Bangka local cassava morphologically different based on visual observation. The morphological character of the Bangka local cassava leaf was divided into three shapes of lobe: ellipse (upang, sekula, bayel, mentega, batin, pulut, rakit, Selangor), linear (kuning), and lanceolate (sutera). This research data showed that local cassava's genetic diversity in Bangka is relatively high. Therefore, Bangka local cassava has the genetic potential for plant breeding as a plant propagation material.

Keywords: Isozyme, morphological, PCR, *Manihot esculenta*, primer

INTRODUCTION

Bangka Belitung Province. Indonesia is an archipelago that consists of two big islands, Bangka and Belitung. Bangka Belitung is geographically located between 0°50'-4°10' S and 104°50'-109°30' E. The province of Bangka Belitung Island is divided into land and sea areas, reaching 81,725.14 km^2. The land area is approximately 16,424.14 km^{2,} or 20.10 percent of the total area, and the sea area of approximately 65,301 km^2, or 79.90% of the total area of Bangka Belitung Province.

Cassava is one kind of tuber plant used as food. Cassava ranks five as a world food crop but two for tuber crops after potatoes. The position at number five after rice, wheat, corn, and potatoes. Cassava can grow rapidly. One modification of cassava is mocav, which can be used as a mixture of flour in the food industry. Cassava is a plant with the potency to be developed as the main ingredient for the local food industry in Bangka. Saelim et al. (2008) stated that genetically modified cassava could potentially be developed for food and non-food industries.

Based on BPS data (2015), the productivity of cassava in Indonesia increased from 2011 (20,298 tons ha^{-1}) to 2014 (22,829 tons ha^{-1}). However, although cassava production in Indonesia continued to increase until 2014, Indonesia still imported cassava from other countries. Therefore, the production and productivity of cassava must be improved according to its genetic potential.

The efforts to develop cassava based on industries' need and use cassava as a food ingredient requires plant breeders to produce new varieties with several advantages, including high yield. Enhancement yield potential can be done if genetic diversity resources are available. However, cassava is a plant that can be propagated vegetatively, and the flowering phase needs a specific location only at elevations above 800 m asl. That is the reason that causes cassava has low genetic diversity, especially in Indonesia.

Bangka local cassava needs to be identified to see morphological patterns' biology, and genetic diversity. Morphological characteristics of the plant are closely related to growth rate, adaptation character, and the ability to produce good quality tuber. One effort to determine crop biology and genetic diversity can be made using isozyme analysis. The enzyme system's biology and genetic diversity banding pattern will be known through isozyme analysis.

The molecular markers were an effective technique in genetic analysis and were widely applied in the breeding program. Molecular markers include isozyme and DNA markers, such as the RAPD method (Yunus 2007). Priadi et al. (2009) stated that SSR markers could be routinely used in breeding programs to verify the paternity of interspecific crosses of cassava. This study aimed to discover the diversity of Bangka local cassava as a genetic resource for plant propagation.

MATERIALS AND METHODS

Exploration was conducted in three Bangka Island, Indonesia, including West Bangka District, Bangka District, and South Bangka District. First, the samples were planted, and then morphological character identification of

cassava was observed. The research was conducted in the experiment field of the Faculty of Agriculture, University of Bangka Belitung, and Biology Laboratory of PPSH IPB Bogor, Indonesia, from July 2015 to July 2016.

Planting was done using stem cuttings of cassava. Cassava stems were planted with 100 cm × 100 cm spacing in a 2 m × 5 m plot with a 1 m distance between the plots. The cuttings position was perpendicular or at least 60° from the ground. The cutting depth was 10-15 cm. Fertilizers were given to the hole before planting in the field. Fertilization was done by providing NPK fertilizers composition: N: 100 kg ha^{-1}, P: 30 kg ha^{-1}, K: 50 kg ha^{-1}. The dosage of basic fertilizer (1/3 dose of urea, KCL, and the entire dose of TSP) was given at the planting timer.

Observation of the qualitative character was carried out by scoring 16 characters based on the characterization by Fukuda et al. (2010). The isozyme analysis method used starch gel electrophoresis horizontal models with peroxidase (PER). The genetic analysis applied to five Bangka local cassava varieties derived from the isozyme analysis result, which classified two subgroups based on similarity coefficient. Therefore, four Bangka local cassava varieties were chosen as RAPD samples based on the isozyme analysis result. In addition, new Bangka local varieties, namely 3 Bulan and one national variety (Malang 6), were also used as comparison genotypes. Therefore, there were six genetic analysis samples using RAPD group OP A and OP B (Xue et al. 2010).

Visualization isozyme band form translated into binary data, and the result analysis is based on the presence or absence of the band. Score 1 for the presence band and 0 for the absence band. Then the binary data was converted into a coefficient based on the similarity matrix SM (Simple Matching). Finally, the resemblance value for clustering analysis was calculated using the UPGMA method (Unweighted Pair-Group Method with arithmetic averaging) from NTSYSpc (Numerical Taxonomy System) program version 2.0.

RESULTS AND DISCUSSION

The research results showed that 10 local cassava could be found across three districts (West Bangka, Bangka, and South Bangka). Plant character variations may be influenced by genes as an internal factor and supported by environmental factors as a stimulant to express the characters.

Table 1. Plant type of Bangka local cassava

Clone of cassava	Type of branching	Plant high (cm)	Harvesting (days)
Upang	Tetrachotomy	250	210-230
Sekula	Trichotomy	300	180-215
Bayel	Dichotomy	250	210-235
Mentega	Tetrachotomy	250	150-185
Kuning	Tetrachotomy	250	120-151
Batin	Trichotomy	300	120-151
Pulut	Trichotomy	300	120-155
Sutera	Tetrachotomy	350	210-255
Rakit	Dichotomy	150-250	120-180
Selangor	Trichotomy	300	160-210

The branching type of cassava is divided into 3 types, consist with dichotomy (bayel, rakit), trichotomy (sekula, batin, pulut, Selangor), and tetrachotomy (upang, mentega, kuning, sutera). Plant height averages of 10 local cassava clones Bangka are 150-350 cm. The average harvest age of 10 cassava is 120-230 days (Table 1).

The morphological character of the Bangka local cassava leaf was divided into 3 shapes of the lobe, i.e., ellipse (upang, sekula, bayel, mentega, batin, pulut, rakit, Selangor), linear (kuning) and lanceolate (sutera) (Figure 3; Table 2). Furthermore, the color of young leaves of Bangka local cassava is divided into 3 colors, i.e., light green (upang, sekula, sutera, rakit), brownish-green (bayel, mentega, kuning, batin, Selangor) and greenish brown (pulut). The color of old leaves of Bangka local cassava is divided into 2 color, i.e., green (upang, sekula, bayel, mentega, kuning, pulut, sutera, rakit, Selangor) and old green (batin) (Table 2).

Morphology of Bangka local cassava young stems was divided into four colors, i.e., green (upang, mentega, kuning, sutera), green striped purple (sekula, Selangor), green striped red (bayel, batin, pulut) and light green striped (rakit) (Table 3). The old stem color of Bangka local cassava was dominated by greenish gray (upang, sekula, batin, pulut, and sutera), followed by grey (bayel), brownish green (mentega), light brown (kuning), reddish brown (rakit), and grey (Selangor). Halsey et al. (2008) reported that the risk of gene flow under natural conditions might be limited to a specific subset of wild relatives or conditions due to the natural constraints discussed above.

Morphology of Bangka local cassava tubers divided into two tuber shapes, i.e., conical (upang, sekula, bayel, mentega, kuning, batin, pulut, rakit) and cylindrical (sutera, Selangor). The outside skin color of the Bangka local cassava tubers is divided into five colors, i.e., brown (upang, mentega, kuning, batin, sutera, rakit), reddish brown (sekula), brownish gray (bayel), yellowish gray (pulut) and grayish white (Selangor). Local color of Bangka local cassava tubers are divided into 3 colors, i.e., white (upang, sekula, bayel, batin, pulut, sutera, rakit, selangor), young yellow (mentega), and yellow (kuning) (Table 4). Tubers can potentially be developed as the main ingredient for the local food industry in Bangka. Saelim et al. (2008) stated that genetically modified cassava is an environmentally friendly plant that derives products from food and non-food industries.

Table 2. Leaf morphology of Bangka local cassava

Clone	Shape of lobe	Young leaf color	Old leaf color
Upang	Ellipse	Light green	Green
Sekula	Ellipse	Light green	Green
Bayel	Ellipse	Brownish green	Green
Mentega	Ellipse	Brownish green	Green
Kuning	Linear	Brownish green	Green
Batin	Ellipse	Brownish green	Dark green
Pulut	Ellipse	Greenish brown	Green
Sutera	Lanceolate	Light green	Green
Rakit	Ellipse	Light green	Green
Selangor	Ellipse	Brownish green	Green

Table 3. Stem color of Bangka local cassava

Clone	Young stem	Old stem
Upang	Green	Greenish-gray
Sekula	Green striped purple	Greenish-gray
Bayel	Green striped red	Grey
Mentega	Green	Brownish green
Kuning	Green	Light brown
Batin	Green striped red	Greenish-gray
Pulut	Green striped red	Greenish-gray
Sutera	Green	Greenish-gray
Rakit	Light green striped	Reddish brown
Selangor	Green striped purple	Grey

Table 4. Tuber morphology of Bangka local cassava

Clone	Tuber shape	Color of outside skin	Color of flesh
Upang	Conical	Brown	White
Sekula	Conical	Reddish brown	White
Bayel	Conical	Brownish gray	White
Mentega	Conical	Brown	Light yellow
Kuning	Conical	Brown	Yellow
Batin	Conical	Brown	White
Pulut	Conical	Yellowish gray	White
Sutera	Cylindrical	Brown	White
Rakit	Conical	Brown	White
Selangor	Cylindrical	Grayish white	White

Morphology characters of Bangka local cassava have many differences based on the observed variables such as the shape of the leaf, leaf color, stem color, tuber shape, and color of the tuber. Differences in the characters that occur may cause by a genetic trait that controls the characters' expression and is influenced by the environmental condition. Hurtado et al. (2008) stated that differences in plant character might cause by differences composition of genes possessed by each plant. Environmental condition as a stimulant factor also takes responsibility for the plant's character. Dasumiati et al. (2017) reported the differences in sex types in the flower of *Jatropha curcas* due to their responsiveness to the environment in two different locations.

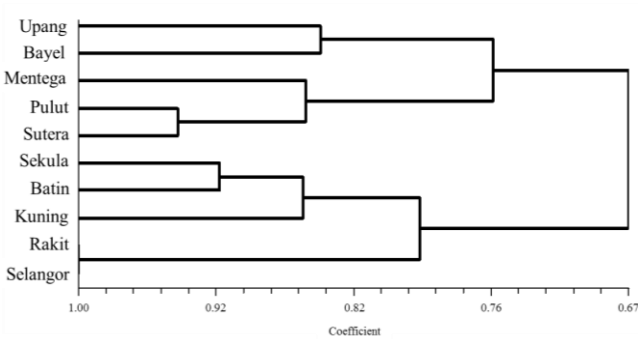

Figure 1. Dendrogram of 10 Bangka cassava using isozyme analysis

Morphological observation using the isoenzyme of 10 Bangka local cassava was given by score based on their band (1) and the absence of band (0), and the score of the group analysis (cluster analysis) was made by using a dendrogram. The similarity coefficient of 0.742. Based on the level of similarity, 10 Bangka local cassava clones can be grouped into two groups. The first group consisted of upang, bayel, mentega, pulut, and sutera. The second group consisted of sekula, batin, kuning, rakit, and Selangor (Figure 1).

Results of the study showed that isozyme analysis on 10 Bangka local cassava samples had variations in polymorphic isozyme banding patterns (Figure 2). Polymorphic isozyme basically could be separated although it was contained in the same organism. Different system enzymes that catalyze a reaction in the cell can be seen through the banding pattern differences by starch gel electrophoresis method after coloring. Hamzah et al. (2009) reported that the mating system of bakau bandul (*Rhizophora mucronata* Lamk) using peroxidase isozyme analysis produces a polymorphic banding pattern.

Isozyme can be used as a genetic trait to study the genetic diversity of an individual in a population, classification of plant species, and identify cultivars hybrid. Utilization of isozyme banding pattern for the benefit of plant biology, including plants' physiology properties, is more reliable because it is governed by a single gene, codominant inheritance, and normal segregation according to the Mendelian ratio. The isozyme technique has proven to be a fast and economical method. Isozyme analysis can also be used in almost all plant tissues. The final choice depends on the availability of plant material and biochemical activity in the plant tissue with a high content of secondary metabolites such as leaves. Isozyme analysis using peroxidase enzyme is previously conducted in analyzing the diversity of cassava to produce two banding pattern peroxidase enzymes that migrate to the positive and negative poles.

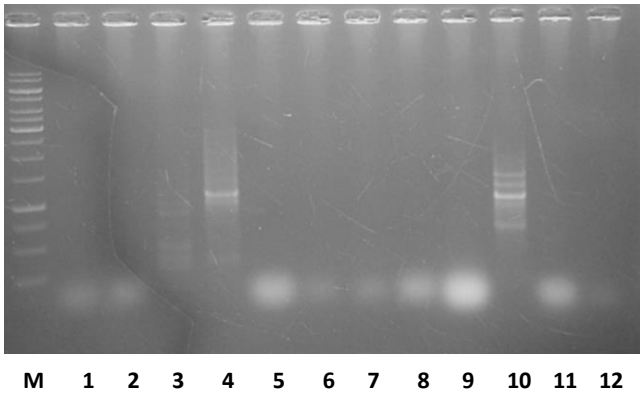

Figure 2. RAPD analysis cassava with primer OP B04 dan OP B06 (118816). Note: 1. Malang 6 variety, 2. Batin, 3. 3 Bulan, 4. Sutera, 5. Mentega, 6. Rakit, 7. Malang 6 variety, 8. Batin, 9. 3 Bulan, 10. Sutera, 11. Mentega, 12. Rakit

The results showed the different performance of qualitative data of five Bangka local cassava varieties and one national variety (Malang 6) on observing morphological characters at five months old plants. Bangka local cassava is morphologically different based on visual observation, but the molecular diversity was unknown. Morphological characters showed the differences between the Bangka local cassava varieties and the comparison. The results showed eight primers used did not produce polymorphic (Figure 2). Hurtado et al. (2013) reported that results SSR markers, while low throughput compared to DArTs, are relatively better at detecting genetic differentiation in cassava germplasm collections. Mezette et al. (2013) reported high genetic diversity among cassava genotypes.

The differences between leaf characters in Figure 3 explain that 8 accessions had similar characters except for kuning and sutera. Kuning accession has a linear lobe shape, while sutera has a Lanceolate lobe shape. On average, most of the leaves had at least seven lobes in 1 leaf except kuning and Selangor, which had eight lobes in one leaf. The environmental condition may affect this character because several leaves in upang, sekula, bayel, mentega, batin, pulut, rakit, and Selangor also have eight lobes in one leaf.

Some characters can be used as a differentiator between local Banka cassava clones. The characteristics were the type of plants and the morphology of leaves, stems, and tubers. Genetic variability in local Bangka cassava clones was based on morphological characters and isoenzyme analysis. The character pattern among the cassava genotypes can be exploited to improve and develop new cassava genotypes (Oduwave et al., 2013; Vieira et al., 2013)

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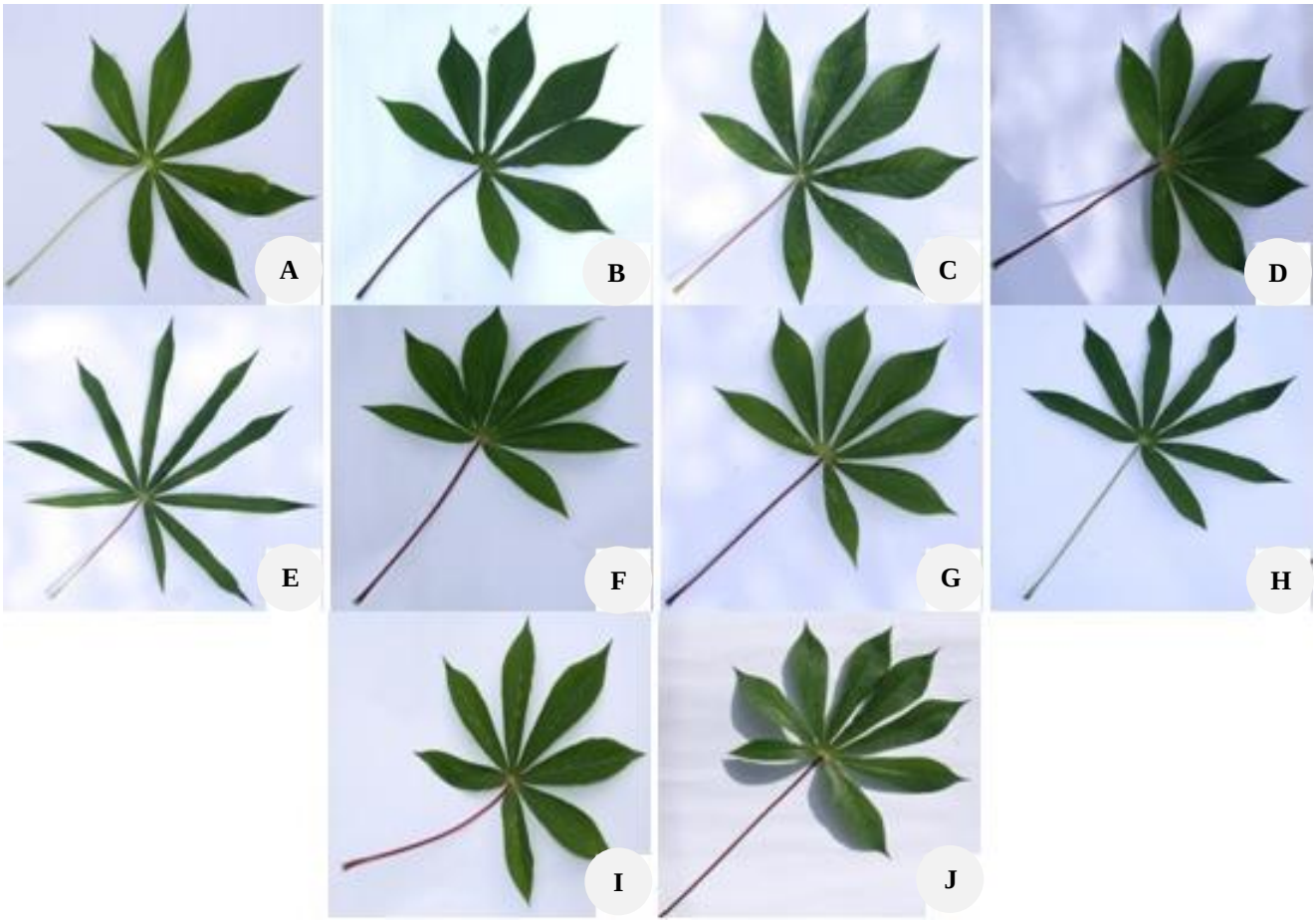

Figure 3. Leaf shape and leaf color of Bangka local cassava. Note: A. Upang, B. Sekula, C. Bayel, D. Mentega, E. Kuning, F. Batin, G. Pulut, H. Sutera, I. Rakit, J. Selangor

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The effect of Urea on epigeic earthworm species (*Eisenia foetida*)

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Abstract. *Long W, Ansari A, Seecharran D. 2017. The effect of Urea on epigeic earthworm species (*Eisenia foetida*). Cell Biol Dev 1: 46-50.* Using chemical fertilizers in intensive agriculture has undoubtedly increased crop production but has adversely affected soil properties over a long period. The effects clearly could be seen in the soil environment and the soil organisms living in that ecosystem. This study was conducted to determine the effects of Urea on epigeic earthworm species (*Eisenia foetida)* in clay soil. A total of five doses of Urea were used i.e 50 mg/kg, 100 mg/kg, 150 mg/kg, 200 mg/kg and 250mg. They were labeled T1, T2, T3, T4, and T5, respectively. A control group was also set up, and the treatment groups were replicated three times. The results indicated that adding Urea affected the soil's chemical properties in treatments inoculated with earthworms (*Eisenia foetida*). The adult earthworm population was 70% in control when compared to different treatment dosage of Urea 50 mg/kg (T1)-85%, 100 mg/kg (T2)-66.67%, 150 mg/kg (T3)- 68.34%, 200 mg/kg (T4)- 86.67% and 250 mg/kg (T5)- 51.67%. The mortality percentage was minimal in treatments T4 and T1, whereas it was maximum in treatment T5 (48%). A 250 mg/kg dose was the most toxic dose of Urea in the soil inoculated with earthworms. The results were significant at p=0.05.

Keywords: Chemical fertilizer, earthworms, physicochemical, mortality, toxic

INTRODUCTION

The chemical fertilizer applied on agricultural land could affect the soil quality and the survival of soil organisms. Earthworms are a major component of soil fauna; the total population is approximately 60% of the macro-organisms in the soil. Earthworms are considered soil quality indicators because they respond and contribute to healthy soils. Their populations depend on several conditions related to soil quality, including pH and organic matter, resistant-waterlogged soil, compact, drought, or excessive sandy (Rani 2016). Agriculture is one of the most important economic activities in Guyana.

Along with other natural resource sectors, agriculture contributed 28% of Guyana's total GDP in 2015 (World Bank 2016). However, many farmers have cultivated their land for rice production for over 15 years; therefore, the lands are thus exhausted and cannot replenish the nutrients needed for high yields efficiently. External agricultural inputs to the soil, such as chemical fertilizer and Urea, have maximized productivity and increased economic returns (Moonilall 2015). Urea is a very common chemical fertilizer used on farms in Guyana. However, researchers have found that nitrogenous fertilizers, such as Urea, are toxic to soil organisms.

Charles Darwin was one of the first people to recognize the value of earthworms in the soil. His research estimated that worm casting alone could provide 1/5 inch of new surface soil per acre of land (Darwin 1881). Earthworms have many important functions in the soil. They can be considered soil quality indicators because they are sensitive to their environment (Iordache and Borza 2010). Earthworms are very important to the soil; tunneling through the soil, drains the water, brings in oxygen, and creates space for plant roots. As worms till the soil, it becomes better aerated and more water absorbent and increases in fertility. Earthworms produce nitrogen, phosphorous, potassium, calcium carbonate, and many micronutrients in a form that all plants can use. They can also help to bring acid soil back to a more neutral pH over time (Ashiya et al., 2015). Their casts contain calcium carbonate (Tiwari 1993). They are very important in soil formation; the presence of earthworms is significant in maintaining the structure and function of the soil, particularly in agroecosystems (Abbirammy and Ross, 2013). The relationship between soil quality, earthworm abundance, and chemical fertilizers has long been the interest of researchers. The use of chemical fertilizers is one such factor. Different fertilizers are used in different doses on crop farms; they all contribute positively or negatively to earthworms.

The study carried out by Edward, and Lofty on the effect of fertilizer on earthworms shows that Urea applied over a long time can increase the earthworm population in the soil where maize is cultivated (Edwards and Lofty 2002). Additional studies agree with this finding that Urea can initially benefit the earthworm population, but the long-term effects are negative (Iordache and Borza 2010). Direct contact with Urea and the earthworm body is lethal even at very small concentrations. Therefore, Urea is classified as highly toxic (Abbirammy and Ross 2013). These studies indicate that nitrogenous fertilizers, such as Urea, can positively and negatively impact the earthworm population. One assumption is that nitrogen allows for faster production, which will cause organic matter to replenish into the soil faster, encouraging more worm

activity. Another assumption is that a small urea concentration is not harmful to the earthworms, but higher concentrations are lethal (Rai et al. 2014).

This research was conducted to determine the effects of Urea, a nitrogenous fertilizer, on earthworms. It was important to determine if there is a significant relationship between the reproduction rate of the earthworms and the use of Urea in the soil. It was also oriented to determine what urea concentration is lethal to the worms in the soil. It would therefore help to regulate Guyana's agricultural policies concerning using Urea on farms. It will also alert the farmers about the effects of Urea on earthworms so that their practices can be adjusted accordingly.

MATERIALS AND METHODS

Earthworm sampling and collection

Earthworms (*Eisenia foetida)* were used in the experiment. The earthworms were collected from a farm in the study area (6.7649° N, 58.0403° W) and identified by a specialist. The hand sorting method collected the adult earthworms from the soil (Valckx et al. 2011). Adult earthworms with a clitellum of relatively homogenous age structure were used for the experiment. The earthworms were collected before setting up the bins and kept in a soil substrate to be used for the test at a temperature of approximately 22±2ºC. The worms were washed with distilled water, and the excess water was removed by placing them on paper preceding their transfer to the bins. The weight of 60 earthworms was recorded, and the average was found to determine the approximate weight of the earthworms in each bin.

The urea materials and treatment preparation

Several recommendations were used to set up the experimental lab conditions (OECD 2015), (Rai et al. 2014), (Iordache and Borza 2010). A Completely randomized block design with three replications and six treatments was used. One control treatment and five treatment groups i.e 50 mg/kg (T1), 100 mg/kg (T2), 150 mg/kg (T3), 200 mg/kg (T4) and 250 mg/kg (T5). The experiment was terminated after sixty days, and the recommended lab analysis was done.

The fertilizer used in the experiment was granular Urea. It was purchased from a local pet store. Non-transparent plastic bins with a holding capacity of 12 kg and a diameter of 25 cm were used on collected. The bins were thoroughly washed, punctured to allow infiltration, and finally appropriately labeled. Eighteen bins were used for the experiment (6 treatments * 3 replication=18 bins).

The clay soil was used in the experiment, collected from the study area, dried excess water, chipped to an appropriate size, and removed any excess debris. The pH, TDS, organic carbon, nitrogen, phosphorus, and potassium were taken. A substrate of 5 kg of soil was added to each bin. Dried filtered cattle dung was also collected from a local farmer. 2.5 g of cattle dung was added to each of the bins. The soil and cattle substrate was thoroughly mixed and dampened with water. After two weeks, the soil amendment paddy shell was added to the mixture-top to loosen the soil and keep it moist. Twenty adult Red Earthworms were placed on the surface of the soil and cattle dung mixture, any worm that did not burrow into the soil after 15 minutes was then replaced with a healthy worm. The bins were then randomly distributed on a level table, and a thin mesh was placed over it to prevent the worms from escaping from the bins.

The bins were carefully observed for 6 days before the first application of Urea was made. Then, the Urea was dissolved in 100 mL of water and applied to the surface by spraying. According to the custom for such experiments, the bins were left open for one hour afterward. The bins were carefully observed during this time to ensure no worms escaped. After this period, Urea will be carefully weighed out and added to the bins based on the amount of urea calculation. Next, to calculate the amount of Urea needed for one treatment, dried clay soil was placed in a one-meter squared area to a height of ten centimeters, and the soil was then weighed. An estimate of the amount of soil to a depth of ten centimeters in one hectare was then made. Finally, the amount of Urea needed for the experiment was calculated using this estimate and the standard range of Urea used by crop farmers in Guyana, which is 65 kg-180 kg/hectare.

Urea was added to the containers three times; the first application was 50% of the dose of Urea. The next application was made three weeks after the first treatment, and the final application was made three weeks after the second. 25% of Urea was added both times. The dried and filtered garden cattle manure was used as food for the earthworms. The initial application of 2.5g of cattle dung was added to each bin. Next, a 10g was added two weeks later, and the last application was after four weeks with an additional 50g to each container. The bins were carefully observed to see if any further food application was needed, but this was not necessary. The worms' activity was carefully observed. The mortality and number of juveniles were recorded every 15 days. The hand sorting technique counted the number of adults and the number of juveniles in each bin. After the 60^{th} day, the number of juveniles was counted twice for accuracy.

The experiment was terminated after 60 days. The live adult worm was then flushed with distilled water and weighed using a digital scale. The earthworms were then dried in an oven at a constant temperature of 45 to 50ºC for 5 days. The dried worms were crushed, and the amount of Nitrogen, Phosphorous, and Potassium in the dried powdered worms was determined using the methods listed below. In addition, 60 questionnaires were prepared and distributed among different crop farmers in Foulis East Coast Berbice. That is because much of Guyana's agricultural products come from this area. The questionnaires were self-administered, and farmers were selected based on availability.

Soil analysis

Several tests were done to determine the pH of the soil, the EC, bulk density, NPK, and organic carbon. NPK test was also done on the worm powder. The soil analysis

methods (Homer (2003) were used for the lab analyses. The soil pH gives a good indication of the major chemical reactions in the soil. The pH can affect several things, including organic and inorganic matter, clay material, and other compounds. The pH of the soil varies from 4-9. The tests were done at the Biology Lab at the University of Guyana. The pH and TDS were measured using a pH tester and TDS meter (Homer 2003). The organic carbon was measured at the Guyana Sugar Inc. Central Laboratory, LBI compound. Organic Carbon by Titrimetry (Walkley and Black Method) was used. Total Kjeldahl Nitrogen in Acid Digests by Spectrophotometry was then done. Next, potassium (K) in Kjeldahl Digests by Flame Photometry was used. This test was also carried out at the Guyana Sugar Inc. Central Laboratory, LBI compound. K was measured using the flame photometer (Homer 2003).

RESULTS AND DISCUSSION

Results

In the present research, adult earthworm mortality was considered the endpoint. In addition, the weight change of adult earthworms was also considered to determine the sub-lethal effects of Urea on the earthworms. The final objective was to determine if there was a significant effect on the reproduction of earthworms exposed to Urea. It was also important to consider the conditions the earthworms had no access to and the other factors that could have affected the mortality of the earthworms. The results obtained are summarized in this section.

Table 1 reflects the chemical properties of soil in different treatments. There was an increase in pH, TDS, NPK, and organic carbon after introducing earthworms to the soil (Control). Adding Urea (T1 to T5) does not affect phosphorous, potassium, and OC. Nitrogen increase was observed from T1 to T5 due to an increase in the doses of Urea. pH decreased from T1 to T5, which could be attributed to the nitrification of ammonium-N in Urea that is converted to nitrate, releasing H^+ ions and causing acidity in the soil. The fluctuation in TDS was also recorded due to changes in urea dosage in different treatments.

The first urea application was made six days after the bins were set up and nine days after the first worm check. The results indicate that the worms were already affected by the Urea. The highest loss at this time was in the control group, as shown in (Table 2); this was because the soil was waterlogged. In the bins treated with Urea, the highest number of death was eight in T2; only one died in T4 and four in T5. It shows that the initial 50% of Urea had a minor effect on the worms in clay soil. The second application of Urea, 25%, was made two days before the second check for the number of live worms at 30 days. Although the number of live worms in T2 and T5 decreased rapidly, the other groups treated with Urea did not have such a significant loss. The last application of Urea was made after the third check on the 45th day of the experiment; it is noteworthy that there was no significant difference in the number of live worms in each bin from the previous check at this time. The final fertilizer application of 25% was made one week before the last check on the 60th day. A drastic decrease in the number of earthworms in T5 was observed (Table 2). The treatment groups with lower Urea did not see such a significant number of deaths. In the Urea dose of 250 mg/kg, the earthworms also weakened and ruptured the epidermis, as seen in some indicated in (Figure 1).

The treatment groups had the following dose of Urea, 50 mg/kg, 100 mg/kg, 150 mg/kg, 200 mg/kg and 250 mg/kg. There was a variation in the mortality of the earthworms. The control group had a mortality of 30% at the end of 60 days. Partially this was because one of the replicates was waterlogged; after this was corrected by adding the soil amendment, the number of deaths decreased. In T1 and T4, the mortality was very low, reaching over 15% and 13% after 60 days. The mortality was higher in T2 and T3, reaching 33% and 32% in both treatment groups. The highest mortality was in T5, where mortality among the test subjects reached 48% (Table 3).

Table 1. The variation in the soil chemical properties before and after urea treatments

Treatment	pH	TDS (ppm)	Total N (%)	Total P (%)	Total K (%)	Total OC (%)
Initial sample	6.3	301.00	6.10	0.60	0.01	0.08
Control	8.4	413.67	6.50	0.65	0.02	0.12
T1 (50 mg/kg)	8.2	322.33	6.57	0.64	0.02	0.12
T2 (100 mg/kg)	8.0	282.67	6.69	0.62	0.02	0.10
T3 (150 mg/kg)	7.9	310.33	6.97	0.68	0.02	0.12
T4 (200 mg/kg)	7.8	337.33	7.14	0.67	0.02	0.11
T5 (250 mg/kg)	7.8	282.20	11.76	0.70	0.02	0.12

Table 2. The number of live adult earthworms under the control and experimental set up during 60 days

Treatment	1 day	15 days	30 days	45 days	60 days
Control	60	51	47	46	42
T1 (50 mg/kg)	60	54	52	52	51
T2 (100 mg/kg)	60	52	46	42	40
T3 (150 mg/kg)	60	55	54	51	41
T4 (200 mg/kg)	60	59	54	54	52
T5 (250 mg/kg)	60	56	44	44	31

Table 3. The percentage mortality of adult earthworms under the control and experimental set up during 60 days

Treatment	15 days (%)	30 days (%)	45 days (%)	60 days (%)
Control	15	22	23	30
T1	10	13	13	15
T2	13	23	30	33
T3	8	10	15	32
T4	2	10	10	13
T5	7	27	27	48

Figure 1. Fragmented body of the dead earthworms under 250 mg/kg dose of Urea after 60days

Table 4. The weight of the earthworms before and after being exposed to Urea and the control group

Treatment	Number of live earthworms	Initial weight (g)	Final weight (g)	Average weight of one earthworm (g)
Control	42	38.53	51.78	1.23
T1	51	38.53	55.02	1.08
T2	40	38.53	48.93	1.22
T3	41	38.53	44.17	1.08
T4	52	38.53	42.64	0.82
T5	31	38.53	33.32	1.07

Table 5. The number of juveniles under the control and experimental set up during 60 days

Treatment	15 days	30 days	45 days	60 days
Control	7	36	28	78
T1	12	10	39	44
T2	11	23	20	49
T3	5	9	26	57
T4	15	12	13	55
T5	3	45	13	26

In all treatment groups except T4, the final weight of an individual earthworm was higher than the initial weight. It should be noted that the initial weight is an average weight achieved from weighing 60 earthworms and taking the mean, and the final weight is the weight of the live earthworms at the end of the experiment. The number of earthworms, therefore, varied from each group. The highest weight of a single worm at the end of 60 days was in the control group, which had 1.23g. The earthworms that weighed the least were in T4, which had 0.82g (Table 4).

The first application of Urea was six days after the bins were set up; nine days after the first worm check was done, the results indicated a small number of juveniles in each bin. The second urea application was made two days before the second check at 30 days. At that time, the highest number of juveniles was in the treatment group T5. The treatment groups with a lower dose of Urea did not significantly increase the number of juveniles compared to the first check. However, the number of worms in the control group also increased. At the third check, the number of juveniles in each group decreased approximately a week after the second dose of Urea was applied. The treatment group T5 had the highest mortality at this time. At the final check, the number of juveniles in each group increased; the highest number was seen in the control group, while T5 had the least number of juveniles (Table 5).

As indicated in Table 6, there is a significant difference between the control and treatment groups with Urea when measuring the endpoint, mortality. As the amount of Urea increased, the mortality of earthworms also increased. There was no significant effect on the juveniles; therefore, the urea concentration added to the soil did not affect the mortality of the juveniles.

Table 6. Summary of the ANOVA test showing the significant relationship of the data collected on mortality and reproduction ANOVA Two-way (p=0.05)

Population characteristics	Treatments	Time interval (days)
Al	0.0045	9.90E-07
Survival percentage of Adult worms	0.0044	9.90E-07
Number of Juveniles after 60 Days	0.6316*	4.20E-04

Note: * Not significant

Discussion

Urea is one of the most common chemical fertilizers used on crop farms in Guyana. The recommended Urea for rice production in Guyana is 36 kg/ha, but the actual amount used is far higher at 96 kg/ha (Moonilall 2015). The amount of Urea recommended for cash crops varies, but this varies depending on the crop type. The misuse of Urea on farms is toxic to soil organisms, including earthworms which are vital to the soil. Earthworms maintain the soil's chemical and physical properties (Rai et al., 2014). Therefore, healthy agricultural soils possess a high concentration of earthworms (Iordache and Borza 2010).

The results obtained from this experiment reveal many interesting facts. Five different concentrations i.e 50 mg/kg, 100 mg/kg, 150 mg/kg, 200 mg/kg, 250 mg/kg and one control group was set up with replicates of three. The experiment shows that the mortality of earthworms with a dose of 250 mg/kg was the highest, reaching 48%, as shown in (Table 2). The bodies of the earthworms in this treatment were ruptured, and the live worms were weakened. They

were not very active and took a long time to burrow into the soil. This observation resonated with what was found by (Rai et al. 2014; Rani 2016). There were also deaths in the other treatments with Urea; T2 and T3 had over 30% mortality, as shown in (Table 3). The treatment with a low concentration of Urea, T1, had low mortality of adult earthworms, and a large number of castings was observed (Bhattacharya and Kumar Sahu 2014).

The LOEC (Lowest Observed Effect Concentration) was T4 since it had the lowest mortality of 33%, as shown in (Table 3). The worms in these bins, however, remained at the bottom, and it was observed that a lower number of castings were in these bins compared to other treatment groups. In addition, this group had the earthworms with the lowest weight. The study by (Iordache and Borza 2010) shows similar results in clay soil; there was no significant effect on earthworm mortality at a moderate concentration; however, there were sub-lethal effects such as loss in weight. The weight in all other groups increased, similar to the results by (Rai et al. 2014). There could be two theories for this; first, the bodies of the earthworms exposed to Urea were swollen, so the weight increased. The other theory was that the earthworms were healthy and gained weight (Rai et al. 2014). From observations, the earthworms in the control group were much healthier than those exposed to Urea. There was no significant change in morphology observed in the treatment groups.

The experiment also shows that Urea affects the reproduction of adult earthworms and the mortality of juveniles. After the juveniles were exposed to Urea, their number decreased. The treatment groups with Urea had significantly fewer juveniles than the control group (Table 5). That shows that Urea at any concentration affects juveniles. The treatment with a dose of 250 mg/kg of Urea had the least amount of juveniles. The earthworms were also small, indicating that they were newly hatched earthworms, and some of them counted before they died. The study carried out by Bhattacharya and Kumar Sahu (2014) had similar results for the mortality of juveniles. It was also observed that the growth of the juveniles was slow; this could be a direct result of their exposure to Urea.

In conclusion, it was determined that Urea at a low concentration is safe for earthworms to survive in clay soil. At a high concentration, the effects of Urea on earthworms are observed by high mortality, negative effects on reproduction as well as the changes in the activity of the earthworms in the soil. It was observed that Urea affects the juveniles at any dose. The mortality of juveniles is proportional to the concentration of Urea. The growth rate of Juveniles is also affected by exposure to Urea. Earthworms burrow into the lower regions of the soil to avoid exposure to Urea.

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Allelopathic effects of mesquite (*Prosopis juliflora*) aqueous extracts on seeds germination and seedlings growth of alfalfa, sesame and sorghum

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Abstract. *Omer HHM, Mohammed IS. 2017. Allelopathic effects mesquite (Prosopis juliflora) aqueous extracts on seeds germination and seedling growth of alfalfa, sesame and sorghum. Cell Biol Dev 1: 51-54.* Mesquite plant [*Prosopis juliflora* (Swartz) DC] is an invasive tree or shrub native to South America. Unfortunately, the plant threatens biodiversity and agriculture due to deliberate distribution within Sudan. Furthermore, this plant has an allopathic potential that may be caused by falling leaves, fruits, root exudates, or plant leachates. These allelochemicals may inhibit the germination and growth of agricultural crops. This study was conducted in the Laboratory of Plant Pathology, College of Agricultural Studies, Sudan University of Science and Technology, Khartoum, in January-February 2016. The aim of this study was to elucidate the potential of allopathic effects of aqueous extract of different parts of mesquite plant, i.e., leaves, fruits, bark, and roots, on germination and early seedlings growth of alfalfa, sesame, and sorghum. The results indicated that the aqueous extracts of different parts of the mesquite plant significantly ($P \leq 0.05$) inhibited the seeds' germination and reduced the early growth of the seedlings. These suggest that the inhibitory substance (s) were widely distributed in mesquite plants but to varying extents. Moreover, fruits and leaves extracts were more pronounced and consistent than bark and roots. That could be attributed to the mesquite fruits and leaves aqueous extracts containing more water-soluble allelochemicals than roots and bark. They gave 0.0% germination in alfalfa and sesame and 47.6-86.7% in sorghum, respectively, compared to control. At the same time, the length of hypocotyl and radical was reduced to 0.0 cm and up to 2.7 cm depending on the efficacy of extract and the response of the test crop. Thus, it is recommended to study the nature of inhibitors to determine whether allelopathic is the cause of the extraordinary success of mesquite on the flat plains of agricultural land in Sudan.

Keywords: Allelopathic, aqueous extracts, mesquite, *Prosopis juliflora*

INTRODUCTION

Common mesquite *Prosopis juliflora* (Swartz) DC of the family Leguminosae is an invasive, evergreen, and multi-purpose leguminous tree or shrub (Babiker 2006). The plant, native to semi-arid areas of the West Indies, Mexico, Central America, and northern South America, was introduced to Sudan in 1917's (Brown and Massey 1929; Pasiecznik 2001; Felker et al. 2003). The plant is known to be well adapted to harsh environmental conditions of many arid zones. At its center of origin, the shrub has played an important social role. In addition to its role in combating desertification and supply of high-value mechanical wood products, firewood and charcoal mesquite provides shelters, animal feed, and food for humans in areas where protein intake is very low and under adverse conditions of drought and famines (Ibrahim 1989). Unfortunately, in Sudan, where mesquite was introduced in 1917 from South Africa and Egypt (Brown and Massey 1929), and due to underutilization of the plant, mismanagement, and its deliberate distribution within the country, the plant became a threat to agriculture and biodiversity **(**Babiker 2006).

The ground vegetation under the plant's canopy indicates that it has some allelopathic potential which might have been caused either by falling leaves, plant leachates, or root exudates. Consequently, releasing allelochemicals into the soil inhibits seed germination and the establishment of agricultural crops and vegetation (Rice 1974). The mesquite plant is an invasive species which widespread in many countries. Shankhla et al. (1965) reported the inhibitory effect of *Prosopis juliflora* aqueous extracts on the growth of some plants. Many researchers reported similar results (Noor et al. 1995; Warrag 1995; Al-Humaid and Warrag 1997; Nakano et al. 2001).

Allelopathy is the ability of plants to inhibit or stimulate the growth of other plants in the environment by exuding chemicals. Hans Molisch first introduced the concept of allelopathy to describe both the beneficial and the detrimental chemical interactions of plants and microorganisms (Molisch 1937; Narwal and Jain 1994). Since then, the term 'allelopathy' has undergone several changes. First, it has been defined as any direct or indirect harmful or beneficial effects of one plant on another through the production of chemical compounds that it releases into the environment (Rice 1979).

The present study aimed to elucidate the Allelopathic potential of different parts aqueous extract of *P. juliflora* plant in respect of their *in vitro* effect on germination and growth of some field crops with the following objectives: (i) To investigate the effect of the aqueous extracts of different parts of mesquite plant on germination of seeds of some field crops, and (ii) To study the inhibitory effect of

different mesquite plant parts extracts on early growth of seedlings hypocotyl and radical length.

MATERIAL AND METHODS

Experimental site

The research was conducted in the Laboratory of Plant Pathology, Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology, Shambat, Khartoum, in January-February 2016.

Collection of mesquite plant and crop seeds samples

Different parts of the mesquite plant, i.e., fruits, leaves, barks, and roots, were collected from trees growing on the campus of the College of Agricultural Studies, Shambat. The parts collected were cleaned from dust and strange material by hand, washed with distilled water, surface sterilized with 1% sodium hypo chloride, thoroughly washed in sterilized water and dried under shade at ambient temperature (25-30c), ground and powdered separately to obtain fine powder for extraction and kept till use.

The central grain market obtained healthy, uniform grains of sorghum, sesame, and alfalfa. Before germination, the grains were surface sterilized with 1% sodium hypochlorite for 20 minutes, then rinsed with distilled water several times to remove excess of the chemical.

Preparation of aqueous extract of different plant parts

Aqueous extracts of each plant part were prepared as Okigbo (2006) recommended. The obtained fine powder from different parts of mesquite was weighted (500 gm), added to it 1000 ml sterilized distilled water, and then placed in a shaker for 24 hrs. The extracts were filtered using Whatman No. 1 filter paper, and the filtrate was kept in the refrigerator to serve as stock solutions.

Bioassay

The seed samples were alfalfa, sesame, and sorghum germinated by being platted on filter papers (dia. 9.0 cm), placed in 9.0 cm sterilized plastic Petri-dishes, and then moistened with the respective test extract (four test extracts). Twenty-five seeds were plated from each sample. A total of four seed samples per crop, with three replications, were used. Treatments were arranged in a CRD (completely randomized design) with three replications and kept in the dark place for seed germination. Distilled water was used as a control. Five mL of the respective test extract was added to each petri-dish every other day until the end of the experiment after four days.

Measurement and statistical analysis

The seeds of each crop were examined for germination and early growth of seedlings upon the emergence of radical and hypocotyls after four days. Seeds were considered germinated upon radical emergence. Germination is measured based on the number of germinated seeds in each treatment and expressed as the percentage of the total number of treated seeds. Seedlings were then retrieved, and radicle and hypocotyl lengths were measured using a millimeter ruler and recorded. The seeds' germination percentages and seedlings' growth were employed as measures for allelopathic activity.

The data were analyzed by ANOVA (analysis of variance) using MStat software. Means were separated for significance using Duncan's Multiple Range Test (DMRT) ($P \leq 0.05$).

RESULTS AND DISCUSSION

Effect of mesquite plant aqueous extracts on the germination percentages

The results of the effect of aqueous extracts from different parts of *P. juliflora* on the final germination percentages of seeds of various test crops after four days from sawing are presented in Table 1 and Figure 1. Generally, the results showed that aqueous extracts of different parts of mesquite screened invariably and significantly inhibited the seeds germination of the test crops compared to control (100%). The inhibitory effect resulted in germination percentages ranging from 0.0% to 93.3%.

Among different parts of mesquite extracts, that of fruits and leaves reduced significantly (0.05) and consistently germinated all seeds of test crops. They gave 0.0% germination in alfalfa and sesame fruits extracts and 46.7% and 70.7% in sorghum; respectively; followed in descending order by bark extract, which gave 46.7%, 86.7%, and 64.0% and roots extract 80.0%, 93.3% and 86.7% germination in seeds of alfalfa, sesame, and sorghum; respectively. The roots extract exhibited the lowest inhibitory effect on the germination of seeds of all crops. Moreover, the suppressing effect of fruit extract was more pronounced on seeds of all crops than on other parts of mesquite. However, among crops, the germination of sorghum seeds was the least affected by the different extracts.

Effect of aqueous extracts of mesquite plant on the growth of hypocotyl and radical of alfalfa

The effect of aqueous extracts from different parts of *P. juliflora* on the growth of hypocotyl and radical of test crops four days after sawing is presented in Tables 2, 3, and 4. The data revealed that the extracts of different parts of the mesquite plant screened exhibited considerable differences in their inhibitory effect on the early growth of the test crops' hypocotyl and radicle of seedlings compared to control. The inhibitory effect ranged from 0.0% cm to 2.7 cm in length.

Among different parts of mesquite extracts, fruits and leaves expressed similar consistency in reducing significantly ($P \leq 0.05$) the growth of hypocotyl and radical of seedlings of the test crops compared to control. The length of the hypocotyl and radical was reduced to 0.0% cm in alfalfa, and sesame ranged from 0.10% cm for radical to 1.033 cm in hypocotyls of sorghum. The reduction effect given by bark and roots extracts on both

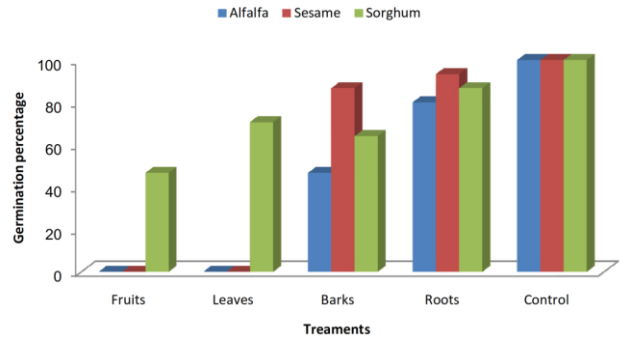

Figure 1. Effect of aqueous extracts from different parts of *mesquite plant* on the germination of seeds of various crops

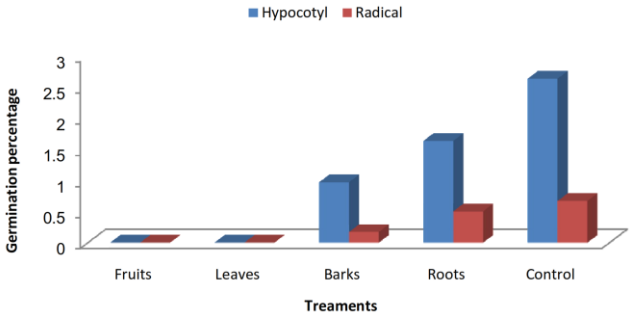

Figure 2. Effect of aqueous extracts from different parts of mesquite plant on the mean seedlings hypocotyl and radicle length (cm) of alfalfa

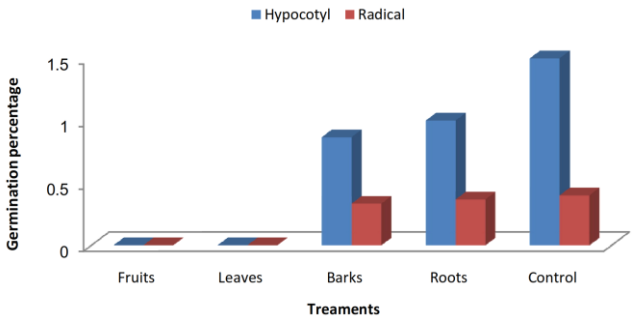

Figure 3. Effect of aqueous extracts of different mesquite plant parts on the mean seedlings hypocotyl and roots radical length (cm) of sesame.

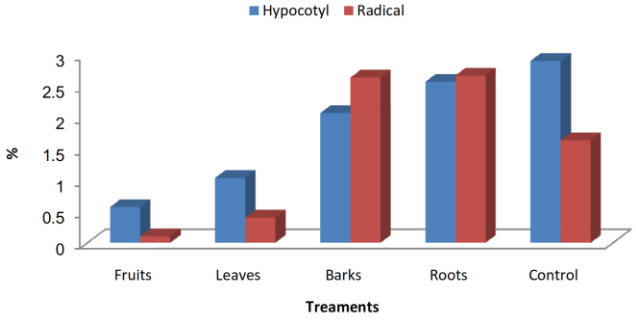

Figure 4. The effect of aqueous extracts of different mesquite plant parts on the mean seedlings' hypocotyl and radical length (cm) of sorghum crop

growth parameters was less in all seedlings of the test crops. Furthermore, the root extract exhibited the lowest inhibitory effect on the growth of hypocotyl and radicle of test crops. Moreover, the suppressing effect of fruit extract was more pronounced on the seedlings' growth of all crops. However, among crops, the growth of the sorghum seedlings was the least affected by the different extracts.

Discussion

The presence of biochemical inhibitors associated with some weeds, shrubs, or tree parts is widespread in the plant kingdom (Hedge and Miller 1990). Many trees are reported to have phytotoxins (Akram et al. 1990; May and Ash 1990; Chou and Lee 1991; Ferguson 1991); Kil and Yun (1992). For example, Chou and Yang (1982) showed that leachates of the bamboo, *Phyllostachys edulis* (Carr.), contain significant amounts of allelopathic compounds that can suppress the growth of undergrowth weeds. However, the water-soluble allelopathic substances released by the woody plants that participate in such interactions have been identified as phenolic compounds, flavonoids, and alkaloids distributed in woody species (Chou 1989; Harborne 1989). However, the ecological significance of phytotoxins in old field succession and other natural communities has attracted the attention of many workers (Mizutani 1989; May and Ash 1990; Choesin and Boerner 1991).

This study investigated the effect of aqueous extracts from different parts of P. juliflora on the final germination percentages of seeds and the early growth of seedlings of various test crops. The data revealed that extracts of different parts of the mesquite plant screened significantly inhibited the seeds germination of the test crops compared to control, with considerable differences among crops.

Moreover, the effect of fruit and leaf extracts was found to be more pronounced than that of bark and root. This highly significant inhibitory effect of fruits and leaves extracts could be attributed to the fact that the mesquite fruits and leaves aqueous extracts contain water-soluble allelochemicals than that of roots and bark; hence, the inhibitory effect was more. These results confirm that of Sazada et al. (2009), who reported similar results on wheat seeds, and Chellamuthu et al. (1977), who reported that the *P. juliflora* significantly reduced the germination percentage of gram and sorghum.

In this regard, Chou (1989) reported that the allelopathic metabolites leached out from woody plants often suppress the growth of undergrowth species sharing the same habitat. The results obtained in this study are also in line with Akram et al. (1990) and Kil and Yun (1992). They reported that the allelopathic effects generally produce germination inhibition and seedlings' early growth. Moreover, Macias et al. (1992) reported that although allelochemicals' specific mode of action was not investigated, many other studies demonstrated inhibition occurring through limiting cell division, respiration, photosynthesis, or disrupting membrane regulation. Accordingly, the presence of allelochemical activity in some parts of mesquite explains the possibility of the role of allelopathy in the phenomenal success of *P. juliflora* as

an invader. These results also suggested that the inhibitory substance (s) were widely distributed in mesquite plants but to varying extents.

The data also demonstrated that the extracts of different parts of the mesquite plant screened inhibited the early growth of seedlings as measured by hypocotyl and radical length with considerable differences among crops. However, the inhibitory effect of root extract on growth parameters was found to be the least. That could be attributed to active allelochemicals' continuous release and leaching during crop growth. However, this mere presence of a suppressing effect does not prove that allelopathy does occur under natural conditions. Similar results were reported by Mehar et al. (1995). They demonstrated that mesquite root extract has the least effect on germination and early seedlings growth of various cultivars of *Zea mays* and *Triticum aesitvum.*

To conclude, this present study proved that the different parts of mesquite plant extracts invariably significantly reduced seed germination and early growth of all test seeds crops seedlings, indicating the presence of Allelopathic potential in *P. juliflora.* Furthermore, there are considerable differences in the inhibitory effect of the mesquite parts screened. Likewise, the seeds crops tested responded differently to the suppressing effect of mesquite parts extracts.

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Growth and ursolic acid content of pearl grass (*Hedyotis corymbosa*) on variations in water availability

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Abstract. *Anam K, Mudyantini, Rakhmawati R. 2017. Growth and ursolic acid content of pearl grass (*Hedyotis corymbosa*) on variations in water availability. Cell Biol Dev 1: 55-62.* The purpose of this study is to determine how different water sources affect the growth and levels of ursolic acid in pearl grass (*Hedyotis corymbosa* (L.) Lam.). A factorial, completely randomized design (CRD) with a single factor of five replications each was used for the experiment. Water stress conditions were created by providing different levels of water availability in the growing media, namely 40, 60, 80, and 100% field capacity (control). The plants were given the treatment for 8 weeks. Plant growth, including the number of leaves, fresh and dry weight, and plant ursolic acid content, were all measured in this study. The data were analyzed using analysis of variance (ANOVA), and the DMRT test at the 5% test level was used to determine the significant difference between treatments. The results showed that the water availability treatment significantly affected the number of leaves, fresh weight, and dry weight, but it did not affect the ursolic acid content in plants. The treatment with the lowest water availability (40% KL) produced low yields on growth parameters and ursolic acid levels. Ursolic acid levels were lowest under drought stress conditions (40% KL), while they were highest under excess water conditions (100% KL).

Keywords: Growth, pearl grass, ursolic acid, water availability

INTRODUCTION

The desire to reconnect with nature is increasingly dominating the community at this time. Synthetic treatment is deemed too costly and has serious side effects. Furthermore, the monetary crisis that has gripped Indonesia since mid-1997 has caused the price of medicines to skyrocket, rendering them unaffordable to the general public (Yuliani 2001). Traditional medicine is generally considered to be safer than modern medicine. This is because traditional medicine has fewer side effects than modern medicine (Sari 2006). The growing use of traditional medicines necessitates the development of more genuine traditional medicines in terms of health, economic potential, and community welfare (Yuliani 2001).

As one of the mega biodiversity countries, Indonesia is well-known for its medicinal plant reserves. Around 9,600 of the approximately 30,000 plant species in Indonesia have medicinal properties. Some 283 were identified as important medicinal plants for the traditional medicine industry (Kusuma and Zaky 2005; Sukandar 2006). The Indonesian people have long known and used medicinal plants to treat health issues. Knowledge of medicinal plants has been passed down from generation to generation based on experience and skills (Sari 2006). The benefits of using traditional medicines that are felt directly by the community are the ease with which they can be obtained, and the raw materials can be planted in their yards, inexpensive, and mixed themselves (Zein 2005).

Pearl grass (*Hedyotis corymbosa* (L.) Lam.) is one of the plants with medicinal potential. *H. corymbosa* is a plant that grows in moist soil on the sides of roads, yards, and ditch edges. The grass grows shady and scattered, is rather weak, grows 15-50 cm tall, and has many branches. The stem is angular, the leaves are opposite each other, the leaf stalk is short/almost sitting, the leaf length is 2-5 cm, the tip is pointed, and there is one leaf bone in the center. Short hairs cover the tips of the leaves. Flowers emerge from the leaf axils in the form of 2-5 compound flowers, with flower stalks (mother) that are hard like wire and 5-10 mm long. The fruit has been constructed, and the ends have been cracked (Ipteknet 2005).

Ursolic acid is one of the secondary metabolites found in *H. corymbosa*. This compound has anti-tumor (Yamaguchi et al. 2008), anti-inflammatory (Baricevic et al. 2001), hepatoprotective, anti-ulcer, anti-hyperlipidemic, and anti-microbial properties (Pendleton 2009). Hsu (1998) investigated three *H. corymbosa* compounds: ursolic, oleanolic, and geniposidic acids. As a result, ursolic acid and oleanolic acid can inhibit hep-2B cell growth and subcutaneous tumor growth.

Hedyotis corymbosa is commonly used in traditional medicine to treat cervical, stomach, breast, rectum, nasopharynx, fibrosarcoma, and lymphosarcoma cancers (Ipteknet, 2005). However, this plant has not been widely cultivated. Because this plant is frequently neglected and considered a weed, its population is declining because it is removed when the yard is cleaned. As a result, studies to assess the growth of this plant are required, given its high potential as a medicinal plant.

The growth and ursolic acid content of *H. corymbosa* have received little attention. When a plant is stressed, its

growth and secondary metabolite content increase. Water availability is an important environmental factor influencing plant secondary metabolite growth and content.

The purpose of this research is to determine the growth and ursolic acid content of *H. corymbosa* plants under various water availability conditions. Optimally treatment can be used in the field to boost productivity and secondary metabolite content.

MATERIALS AND METHODS

Materials

The main materials used are pearl grass seeds (*Hedyotis corymbosa* (L.) Lam.). of uniform age.

Research design

This study used a completely randomized design (CRD), with each treatment with 5 replications. The treatment of water availability (A) is as follows:

A_{100} = control (100% field capacity)
A_{80} = 80% field capacity
A_{60} = 60% field capacity
A_{40} = 40% field capacity

Procedure

Preparation

The seedling was carried out in perforated plastic tubs. The media used was a combination of soil and manure with a ratio of 2:1. In the media, an indentation is made 1-1.5 cm deep, then the seeds are inserted into it and covered with media. Watering is done 2 times every day. After the seeds grow about 4-5 cm, they are transferred in polybags with media like during seed nursery. Plants are grown in polybags for 2 weeks.

Determination of field capacity

The drained planting media mixture is weighed one kilogram in a perforated polybag at the bottom. The polybag was then watered until the water stopped dripping from the bottom hole, allowing the volume of water used for watering and its field capacity to be calculated. The following formula is used to calculate field capacity:

KL= (Weight of soil + polybag + water) - (Weight of soil + polybag) (Patoni 2000).

Treatment:

Plants were treated with various variations of water availability, including 100, 80, 60, and 40% of field capacity, by watering every 2 days.

Cultivation:

The cultivation time was 2 months at the Green House of the Faculty of Agriculture.

Growth observation:

The number of leaves: The number of leaves that appear was observed every 1 week until harvest. *Fresh weight*: Fresh weight of plants was weighed after the plants were treated with different water availability for 2 months. *Dry weight*: The plants were dried in an oven at 60ºC until dry and weighed.

Extraction

After being treated, *H. corymbosa* plants are dried in an oven to produce dry Simplicia, then powdered with a blender and sieved with a specific size sieve to produce dry powder, then macerated with methanol and allowed to stand for 24 hours. Maceration was performed three times. The maceration results were then filtered and dried to obtain methanol extract.

Thin layer chromatography

For comparison, each extract was spotted on the same TLC plate as standard ursolic acid. The mobile phase of petroleum ether: ethyl acetate (4:1 v/v), was then used to elute the sample (Srinivasan et al. 2008). The cerium (IV) sulfate reagent was used to detect the presence of organic compounds, while Lieberman-Burchard (LB) reagents were used to detect the presence of ursolic acid.

Determination of ursolic acid levels

The UV-vis spectrophotometer method was used, and the following procedures were followed:

Preparation of a standard ursolic acid solution standard curve: A concentration series is created by dissolving 10 mg of standard ursolic acid in 50 mL of sulfuric acid. The absorbance of each solution was measured with a wavelength of 310 nm to create a standard curve (Murav'ev et al. 1972). The linear regression line equation, generally formulated as $y = bx + a$, is searched using the standard solution content data as the x-axis and the absorbance value as the y-axis.

Ursolic acid concentration determination in the sample: Take 5 mg of the methanol extract sample and dissolve it in 10 mL of sulfuric acid at a series of different levels. A UV-vis spectrophotometer set to 310 nm was used to measure the absorbance of each sample solution. Five replications are performed.

Level calculation

The ursolic acid content of the sample is calculated by entering the absorbance value of the sample into the equation $y = bx + a$ obtained from the standard curve. Then the results of the ursolic acid content are converted into units of mg/g dry weight using the formula:

$$R = \frac{S \times V}{B}$$

Where; R: ursolic acid levels (mg/g); S: ursolic acid levels of spectrophotometric result sample (mg/L) and B: powder weight (g) (Hary 1998).

Data analysis

Quantitative data on the number of leaves, fresh weight, and dry weight of plants and levels of ursolic acid were analyzed by Anova; if there was a significant difference between treatments, then DMRT was carried out at test level 5%.

RESULTS AND DISCUSSION

Growth

Growth is defined as an increase in plant material. The whole process chemically changes these raw materials and adds them to the plant (Goldsworthy and Fisher 1992). According to Sitompul and Guritno (1995), growth is a process in plant life that causes changes in plant size and determines plant yields.

Three events occur as part of the growth and development process: (i) cell division, in which one adult cell divides into two separate cells; (ii) cell enlargement, in which one or both daughter cells increase in volume; and (iii) cell differentiation, in which cells that have already reached the volume become specialized. Cell division does not increase size; rather, it is the products of cell division that grow and cause growth. The expansion of plant body size is a real result of an increase in the size of cell parts caused by cell growth (Sitompul and Guritno 1995). The growth parameters observed in this study were the plant's number of leaves, fresh weight, and dry weight.

Number of leaves

Because leaves are sensitive to environmental changes, they are one of the parameters that can be observed. Because leaves are photosynthetic organs, they play a critical role in plant growth. Plant leaves, in general, are the site of carbohydrate synthesis. As a result, leaf observation is critical as a growth indicator and as supporting data to explain the growth process that occurs (Sitompul and Guritno 1995).

The analysis of variance results revealed that treatments with water availability of 40, 60, 80, and 100% field capacity (KL) significantly affected the number of leaves of *H. corymbosa* plants. Table 1 shows data on the average number of leaves on *H. corymbosa* plants with varying water availability over two months.

Figure 1 shows that the 100% KL water availability treatment had the most leaves, while the 40% KL treatment had the fewest. As can be seen, water availability of 100% KL is more optimal in increasing the number of leaves than water availability of 40%, 60%, and 80% KL. Figure 1 depicts a graph of the average number of *H. corymbosa* leaves for two months with varying water availability.

The highest number of leaves (409.2 strands) was found in 100% KL water availability. Photosynthesis is generally used for the growth of photosynthesis-active organs in conditions of abundant water. The fewest number of leaves discovered at 40% KL water availability was 180.4 strands. This is due to a lack of water availability in the soil, which causes plants to be thirsty and interferes with plant growth. Lack of water can reduce the photosynthesis rate, owing to stomata guard cells' turgidity. When there is a lack of water, the turgidity of the guard cells decreases, causing the stomata to close (Lakitan 1995).

Figure 2 depicts a graph of the average number of leaves on *H. corymbosa* plants every week for two months with varying water availability. Figure 2 depicts the total number of leaves in each treatment. From the first week to the end of the treatment, the number of leaves on *H. corymbosa* plants increased every week. Water stress decreases the water potential in plant cells. The decrease in water potential influences changes in plant hormone concentrations, particularly the hormone ABA (abscisic acid). When there is a lack of water, the ABA content of the leaves increases before the stomata close. The accumulation of ABA stimulates the flow of K+ ions, which causes water to exit the guard cells, lowering turgor pressure and causing stomatal closure. Closing these stomata prevents CO_2 uptake, which is required for carbohydrate synthesis. Closure of stomata, on the other hand, is beneficial because it can reduce the transpiration rate, thereby reducing plant water loss.

Table 1. The average number of *H. corymbosa* leaves for 2 months with different water availability

Treatment	Water availability (%)			
	40	60	80	100
The average number of leaves (strand)	180.4[a]	278[b]	307.6[b]	409.2[c]

Notes: The numbers followed by the same letter were not significantly different at the 5% DMRT test level

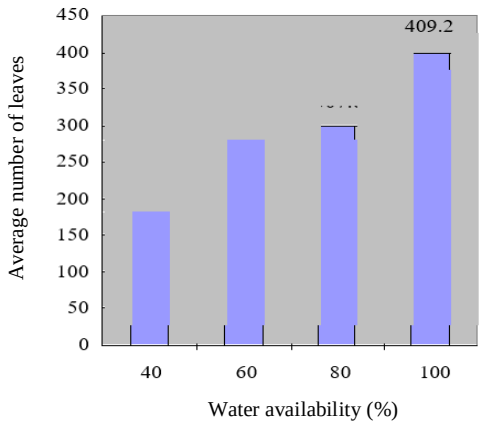

Figure 1. The average number of *H. corymbosa* leaves for 2 months with different water availability

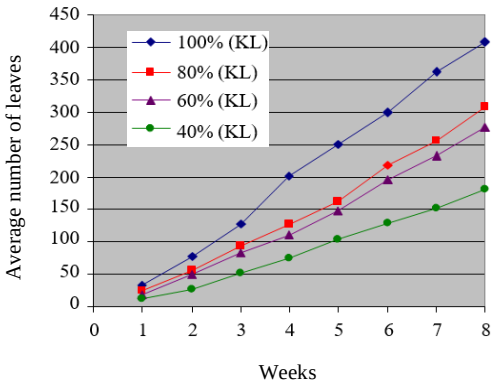

Figure 2. The average number of leaves of *H. corymbosa* plants every week for 2 months with different water availability

Plant growth is linked to cell division and enlargement. Auxin is a growth hormone that encourages cell growth and elongation (cell enlargement). Auxin is transported from cell to cell polarly in the stem, particularly in the basipetal. This auxin transport requires energy, which will be hampered if ATP synthesis is inhibited or there is a lack of oxygen (Anggarwulan and Solichatun 2001). When there is a lack of water, the photosynthesis rate slows, inhibiting ATP synthesis. This ATP synthesis inhibition will prevent auxin distribution, thereby inhibiting cell elongation and elongation. Plant growth will be slowed if cell elongation and elongation are inhibited.

Cell division can also cause a reduction in the number of leaves in plants (Abdalla and El-Khoshiban 2007). Cytokinins are hormones that promote cell division. Cytokinin levels will generally decrease during water stress (Pospisilova et al. 2000). Low cytokinin levels can inhibit cell division, resulting in a reduction in the number of leaves. According to Nautiyal et al. (1994), the number of leaves in three plant species, *Eucalyptus hybrid* (*E. camaldulensis* Dehnh. *x E. tereticornis* Sm.), *Casuarina equisetifolia* L., and *Melia azedarach* L., decreased as drought stress increased. Greitner et al. (1994) discovered that drought stress reduced the number of leaves on *Populus tremuloides* Michx.

Fresh weight

Plant biomass (weight) is the most commonly used metric for describing and studying plant growth. This is because the estimated plant weight is relatively easy to measure and represents an integration of almost all previous events encountered by the plant (Sitompul and Guritno 1995). The fresh weight of the plant reflects the plant's metabolic activity, and its value is influenced by the water content of the tissue, nutrients, and metabolic products. Weight gain is frequently determined by harvesting the entire plant or the desired part and quickly weighing it before too much water has evaporated from the material; this is known as fresh mass (Salisbury and Ross 1995). Table 2 displays the average fresh weight of *H. corymbosa* plants over two months with varying water availability.

Table 2 shows that differences in water availability result in significantly different fresh weights of plants. The highest average value of fresh plant weight was found at 100% KL, and the lowest was found at 40% KL. According to the findings of this study, lower levels of water availability result in lower plant fresh weight, while higher levels of water availability result in higher plant fresh weight.

The 40% KL water availability treatment had the lowest fresh weight was 1.25 g. This is due to a lack of water availability in the soil, which causes plants to be dehydrated, interfering with plant metabolism. According to Wilkinson (1994), a lack of water directly affects plant vegetative growth. Turgor stress governs this process in plants. Turgidity tension loss can halt cell growth (multiplication and enlargement), resulting in stunted plant growth. Figure 3 depicts a graph of the average fresh weight of *H. corymbosa* plants over two months with varying water availability.

A lack of water will cause disruptions in cell metabolism, including photosynthesis. Photosynthesis will be hampered if the main ingredient, water, is only available in limited quantities, resulting in lower photosynthesis results. Photosynthate produced will also be hampered in its circulation to all parts of the plant, reducing plant weight (Harjadi and Yahya 1988). Drought stress reduced the wet and dry weight of sesame (*Sesamum indicum* L.) plants, according to Fazeli et al. (2006).

Dry weight

According to Lakitan (1995), plant dry weight reflects the accumulation of organic compounds successfully synthesized by plants from inorganic compounds, particularly water and CO_2. The dry weight of the plant is obtained by drying the fresh weight of the plant to remove the water content in the plant; however, the fresh weight of the plant does not always determine the dry weight of the plant. The Table 3 shows the average dry weight of *H. corymbosa* plants after two months of different water availability.

Table 2. The average fresh weight of *H. corymbosa* plants for 2 months with different water availability

Treatment	Water availability (%)			
	40	60	80	100
The average fresh weight (g)	1.25[a]	3.76[b]	4.47[b]	6.34[c]

Notes: The numbers followed by the same letter are not significantly different at the 5% DMRT test level

Table 3. The average dry weight of *H. corymbosa* plants for 2 months with different water availability

Treatment	Water availability (%)			
	40	60	80	100
The average dry weight (g)	0.26[a]	1.19[b]	1.59[b]	2.29[c]

Notes: the numbers followed by the same letter are not significantly different at the 5% DMRT test level

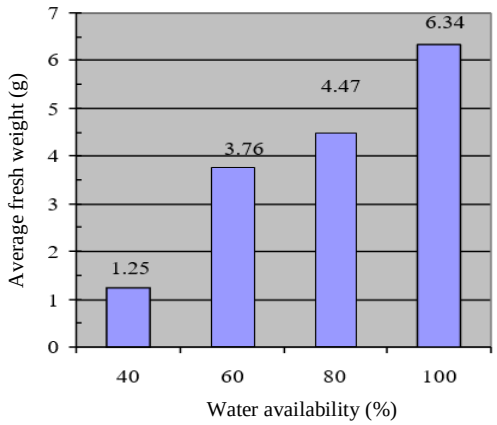

Figure 3. The average fresh weight of *H. corymbosa* plants for 2 months with different water availability

The analysis of variance revealed that treatments with water availability of 40, 60, 80, and 100% KL significantly affected the dry weight of *H. corymbosa* plants. The highest dry weight was found at 100% KL water availability and the lowest at 40% KL water availability.

The 40% KL water availability treatment had the lowest plant dry weight was 0.26 g. (Figure 4). Water scarcity can cause stomatal closure, reducing CO_2 uptake and resulting in stunted growth and reduced dry weight (Gardner et al. 1991). Water affected dry weight, according to Fitter and Hay (1998). This is related to the photosynthesis process. Photosynthesis accounts for the majority of plant dry weight. Figure 4 depicts the average dry weight of *H. corymbosa* plants over two months with varying water availability.

When there is a lack of water, the concentration of ABA hormone in plants increases, the increase in ABA hormone concentration will cause stomata to close, reducing CO_2 uptake for photosynthesis and, as a result, the rate of photosynthesis will decrease. According to Gardner et al. (1991), the reduced rate of photosynthesis caused by water stress occurs because the leaves formed under these conditions are inhibited by cell enlargement, resulting in smaller leaves than plants growing under normal conditions. This entails decreasing light absorption, thereby decreasing photosynthesis ability. The reduced rate of photosynthesis reduces the synthesis of body structures and food reserves, reducing dry weight. Drought stress reduced leaf height, length, dry weight, and leaf area in *Cymbopogon nardus* (L.) Rendle and *C. pendulus* (Nees ex Steud.) W.Watson, according to Sangwan et al. (1994). According to Solichatun et al. (2005), low water availability reduces the dry weight of Javanese ginseng (*Talinum paniculatum* (Jacq.) Gaertn.) plants.

Ursolic acid compounds

One of the chemical compounds found in *H. corymbosa* plants is ursolic acid. This compound is a pentacyclic triterpenoid compound found naturally in most herbaceous and fruiting plants (Pendleton 2009). These compounds have anti-tumor (Hah et al. 1992; Yamaguchi et al. 2008) and anti-inflammatory (Baricevic et al. 2001) effects, as well as hepatoprotective, anti-ulcer, anti-microbial, anti-hyperlipidemic, and anti-virus properties (Pendleton 2009).

Detection of ursolic acid compounds

Extraction is the first step in obtaining a compound from a sample or material. Before extraction, the harvested plants were dried in a 60°C oven and blended to obtain a dry powder. Extraction is the process of transferring or withdrawing the mass of the active substance in the cell; the filtered fluid will penetrate the cell wall and enter the active substance-containing cell cavity, causing the active substance to dissolve. Organic solvent extraction methods such as maceration, percolation, and soxhletation are used, as are water extraction methods such as infusion, decocted, and stem distillation (Silva et al. 1998).

The maceration extraction method was used in this study. Maceration is a straightforward extraction method. Maceration is derived from the Latin word macerase, which means to soften. To soften the cell structure, Simplicia powder with the desired fineness can be immersed in the liquid filter, and substances easily soluble in the liquid filter will be pulled out of the cell (Ansel 1989). Methanol can be used to extract ursolic acid compounds, according to Hamzah and Lajis (1998), so this study uses methanol to extract ursolic acid compounds. The dry Simplicia powder was macerated in methanol for 24 hours, occasionally stirring. The stirring process allows the liquid to penetrate the cell wall and filter out the compounds. The extraction process was repeated three times before filtering the juice and residue. The extracted extract was dried to produce a thick methanol extract.

Ursolic acid compounds were detected in a methanol extract of the *H. corymbosa* plant. TLC was used to detect the presence of these compounds, with petroleum ether as the mobile phase: ethyl acetate (4: 1 v/v) (Srinivasan et al. 2008), and standard ursolic acid as a comparison. Spray detection was also performed using cerium (IV) sulfate and Liebermann-Burchard (LB). The color reagent cerium (IV) sulfate generally is used to determine the presence of organic compounds; if organic compounds are present, the color of the spots will change. The LB color reagent is used to detect the presence of triterpenoid compounds; if triterpenoid compounds are present, the spots will turn green to blue (Harborne 1987).

Figure 5 depicts the chromatogram of the *H. corymbosa* plant methanol extract. The detection of the chromatogram with UV_{254} in Figure 5 shows the presence of attenuation, which is indicated by the presence of several dark spots. The presence of dark spots indicates the presence of a compound. UV_{366} detection revealed three fluorescent spots, one reddish (Rf 0.65) and two purple (Rf 0.13 and 0.41). This indicates that the compound has a long conjugated double bond and can fluoresce when exposed to long-wave UV irradiation. Because the chemical structure of these compounds did not contain conjugated double bonds, detection with UV_{254} and UV_{366} revealed no spots of ursolic acid compounds.

Chromatograms sprayed with LB color reagent yielded bluish spots with an Rf value of 0.35 on standard ursolic acid compounds and the same Rf value on each plant extract sample (Figure 5). It can be concluded that ursolic acid compounds are present in the methanol extract of the *H. corymbosa* plant.

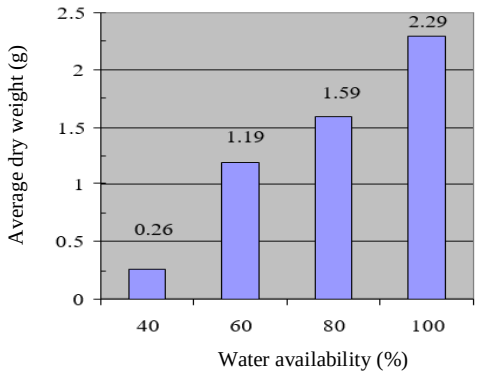

Figure 4. The average dry weight of *H. corymbosa* plants for 2 months with different water availability

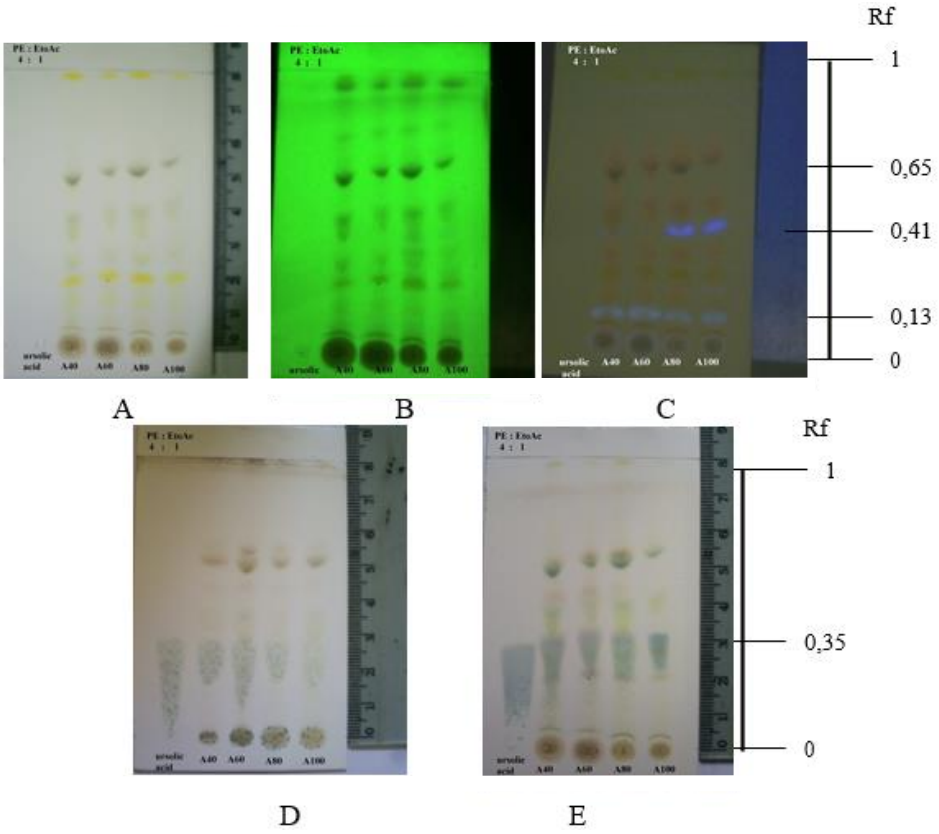

Figure 5. Chromatogram of *H. corymbosa* methanol extract with standard ursolic acid as a comparison with the detection of (A) visible light, (B) UV_{254}, (C) UV_{366}, (D) cerium (IV) sulfate, and (E) liebermann burchard Stationary phase: silica gel GF_{254}. Mobile phase: petroleum ether: ethyl acetate (4 : 1 v/v)

Ursolic acid compound levels

The analysis of variance revealed that treatments with water availability of 40, 60, 80, and 100% KL had no significant effect on ursolic acid compound levels in *H. corymbosa* plants. Table 4 shows the average levels of ursolic acid compounds in *H. corymbosa* plants after being given different water availability for two months.

The highest concentrations of ursolic acid compounds were found in 100% KL water availability and the lowest in 40% KL water availability. The lowest ursolic acid compound levels were found at 40% KL water availability, which was 37.96 mg/g dry weight (Figure 6). This occurs because the plants are deprived of water in these conditions. Because the turgidity of stomata guard cells decreases when there is a lack of water, photosynthesis can be slowed. As a result, the stomata close (Lakitan 1995). Closure of stomata in most species due to a lack of water in the leaves reduces the rate of CO_2 absorption and, as a result, the rate of photosynthesis (Goldsworthy and Fisher 1995). Figure 6 depicts a graph of the average levels of ursolic acid compounds in *H. corymbosa* plants over two months with varying water availability.

The slower rate of photosynthesis results in lower photosynthetic yields, which reduces the formation of ursolic acid compounds. Ursolic acid compounds are created through the glycolysis process, in which glucose molecules produced by photosynthesis are converted into pyruvate and acetyl-CoA.

Table 4. Average levels of ursolic acid compounds in *H. corymbosa* plants after 2 months with different water availability

Treatment	Water availability (%)			
	40	60	80	100
Average levels of ursolic acid (mg)	37.96	43.30	48.45	75.34

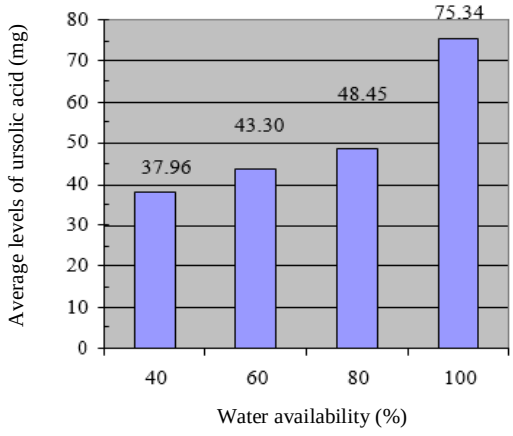

Figure 6. Average levels of the ursolic acid compound in *H. corymbosa* plants for 2 months with different water availability

Glycolysis, the first stage of carbon metabolism, is a series of reactions that convert hexose sugars (usually glucose) into pyruvic acid. Generally, glycolysis is divided into two stages: I A series of reactions that convert various forms of glucose and fructose from carbohydrate reserves to fructose-1,6-bisphosphate. (ii) Fructose-1,6-bisphosphate (FBP) is converted further into pyruvate (Anggarwulan and Solichatun 2001).

In addition, the pyruvate produced by glycolysis will be converted into acetyl-CoA via an oxidative decarboxylation process. Acetyl-CoA is converted into mevalonic acid, a precursor in the formation of ursolic acid compounds; however, if the raw material for the formation of mevalonic acid, namely glucose, is reduced, the formation of ursolic acid compounds is inhibited.

Furthermore, the concentration of the hormone ABA in leaves and fruit increased under water stress conditions. ABA is a 15-carbon sesquiterpene synthesized in chloroplasts and other plastids via the mevalonate pathway. The first reactions in the synthesis of ABA are identical to those of other isoprenoids like gibberellins, sterols, and carotenoids. A small amount of ABA is produced in chloroplasts by the breakdown of violaxanthin, a xanthophyll carotenoid that is converted to ABA during their metabolism (Anggarwulan and Solichatun 2001).

Mevalonic acid is the starting point for both ABA and ursolic acid. MVA kinase phosphorylates mevalonic acid to form mevalonic acid-5-phosphoric acid (MVAP), which is then phosphorylated by MVAP kinase to form mevalonic acid-5-diphosphate (MVAPP). Decarboxylation of MVAPP produces isopentenyl diphosphate (IPP), a precursor compound in forming various terpenoid compounds. Terpenoids are formed when IPP and its isomer dimethyl allyl diphosphate (DMAPP) combine to form larger molecules (Taiz and Zeiger 1998; Croteau et al. 2000). Geranyl transferase catalyzes the reaction of IPP and DMAPP to form geranyl pyrophosphate and farnesylpyrophosphate (Ngan 2005). Farnesyl-pyrophosphate is a precursor of sesquiterpenes (ABA), formed when two molecules of farnesylpyrophosphate combine to form squalene, a precursor of triterpenes (ursolic acid). Plants accumulate ABA when there is a water shortage. Mevalonic acid was possibly used to synthesize ABA, thereby inhibiting the production of ursolic acid.

Based on the research, it is clear that: (i) variations in water availability have a significant effect on the growth of *H. corymbosa* plants. The greater the availability of water, the greater the rate of growth. (ii) Variations in water availability did not affect ursolic acid compound levels in *H. corymbosa* plants. The highest level, 75.34 mg/g dry weight, was obtained at 100% KL water availability.

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Effect of type of seedling media and duration of synthetic auxin immersion on germination and initial growth of papaya (*Carica papaya*) seedlings

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Abstract. *Mulati A, Supriyono, Wiryowidodo W. 2017. Effect of type of seedling media and duration of synthetic auxin immersion on germination and initial growth of papaya (*Carica papaya*) seedlings. Cell Biol Dev 1: 64-70.* Papaya (*Carica papaya* L.) is a well-known fruit plant with high economic value. The uniformity of germination and initial growth must be considered to get normal mature plants. Using appropriate seedling media and synthetic auxin can support the germination and initial growth of papaya seedlings. This study aimed to determine the effect of the type of seedling media and the duration of immersion of synthetic auxin, and the combination that had a positive effect on germination and initial growth of papaya seedlings. This research was conducted in Badranrejo, Kemiri, Mojosongo, Boyolali, and the Laboratory of the Faculty of Agriculture, Sebelas Maret University, Surakarta, from April to July 2009. This study used a Completely Randomized Design (CRD) with two treatment factors and three replications. The first factor was the type of seedling media: soil, soil + farmer-produced cow manure (1:1), soil + self-produced cow manure (1:1), and soil + farmer-produced cow manure + self-produced cow manure (1:1:1). The second factor was the immersion time in synthetic auxin: 0 hours, 1 hour, 2 hours and 3 hours. Data were analyzed by analysis of variance and if there was a significant difference, proceed with DMRT 5%. The results showed that the interaction between seedling media and immersion time in synthetic auxin did not occur. Therefore, adding cow manure as a medium is unnecessary, especially for papaya seed germination. Synthetic auxin immersion from 1 to 3 hours did not increase all variables of germination and initial growth of papaya seedlings.

Keywords: Auxin, *Carica papaya*, papaya, seedling media

INTRODUCTION

Papaya (*Carica papaya* L.) is a well-known fruit plant with high economic value. Therefore, the plant is suitable for planting in the tropics and subtropics (da Silva et al. 2007). The flesh of the plant is soft with red or yellow color. It tastes sweet and refreshing because it contains a lot of water. The nutritional value of this fruit is quite high because it contains a lot of provitamin A, vitamin C, and calcium minerals (Kalie 1983).

According to Kalie (2003), papaya plant propagation can be done by grafting, layering, or seeds. However, grafting propagation is rarely done by farmers or seed breeders because it requires plants for rootstock in large quantities. Propagation by grafting has also not been widely applied considering the relatively difficult implementation; therefore, seed propagation is the easiest alternative to propagate this fruit plant. Seeds can be planted directly in the garden, nursery, or polybag. However, in this seed propagation, the germination time is often not the same, so the plants are not grown simultaneously.

Root formation is a very important initial factor in germination. Seeds with roots will have the ability to grow better. One of the factors that can affect the formation of roots is the seedling medium. Seedling media is a place to germinate seeds. Seedling media for germination must meet the requirements, including crumb structure, namely a balanced ratio of micro and macro pores so that it does not inhibit root growth and can bind water and nutrients needed for plant growth. Several types of media can be used as a germination medium. Each type of media has different characteristics, so it is necessary to look for it to get a suitable growing medium for a plant. Not many papaya seed growers in Indonesia, especially in Boyolali, know the right seedling media for the germination and growth of papaya seeds. So it is because there is not much information about the right media for germination and growth of papaya seeds.

The success of germination is not only influenced by the seedling media but also by external stimuli that stimulate roots, for example, by giving growth regulators. Growth regulators are organic compounds that are not nutrients, which in small amounts can support, inhibit, and can change plant physiological processes (Abidin 1994). For example, according to Koesriningroem and Setyati (1979), one growth regulator type is auxin, which can stimulate cell elongation. Auxin initiates cell elongation by influencing the relaxation or flexibility of the cell wall. As a result, cell elongation will cause stem and root elongation. Auxins are produced naturally by plants, such as IAA (indoleacetic acid) and IBA (indolebutyric acid). In contrast, auxins produced by companies are called

synthetic auxins, such as NAA (naphthalene acetic acid) and 2,4 D (2.4 dichlorophenoxyacetic acids).

One type of synthetic auxin sold in the market is atonic. Atonic is a trademark and contains growth regulators that can stimulate root growth and accelerate seed germination. However, this atonic is only effective during immersion. According to Danusastro (1973), the method of giving growth regulators can be immersion, spraying, smearing, and others. Fresh seeds can be soaked in a solution of vitamin B1 or a solution of growth regulators for 30 minutes, while for dry seeds, the minimum immersion time is 2 hours.

The aims of this study were: (i) to determine the interaction between the type of seedling medium and the duration of immersion in a synthetic auxin in its effect on germination and growth of papaya seedlings; (ii) to determine the seedling media that gives the best effect on the uniformity of germination and growth of papaya seedlings; (iii) to determine the duration of immersion in a synthetic auxin which gives the best effect on the uniformity of germination and growth of papaya seedlings.

MATERIALS AND METHODS

Place and time of research

The research was conducted in April-July 2009 at Badranrejo, Kemiri, Mojosongo, Boyolali with an altitude of 228 m above sea level and in the Laboratory of the Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia.

Materials and Tools

The research materials were plant seeds from local Papaya Boyolali (a Thai variety that has been adapted for a long time), soil, farmer-produced cow manure (purchased directly from farmers), and self-produced cow manure (fermented pure cow vesicles mixed with EM-4 and molasses). The research tools were polybag, ruler, scale, oven, label paper, masonry trowel, and stationery.

Research design

The study was arranged based on a completely randomized design (CRD) with 2 treatment factors. Factor I: type of seedling media (S1 = soil, S2 = soil: farmer-produced cow manure (1:1), S3 = soil: self-produced cow manure (1:1), S4 = soil: farmer-produced cow manure: self-produced cow manure (1:1:1). Factor II: immersion time (A1 = without immersion in atonic, A2 = immersion in atonic for 1 hour with a concentration of 1 ml/L, A3 = immersion in atonic for 2 hours with a concentration of 1 ml/L, A4 = immersion in atonic for 3 hours with a concentration of 1 ml/L. Based on these two factors, 16 treatment combinations were obtained. Each treatment combination contained 5 populations, and each combination was repeated 3 times so that there were 240 polybags as experimental units.

Research implementation

Media preparation

Prepare the soil, farmer-produced cow manure, and self-produced cow manure. Fill the plastic bag with the media according to the treatment. Farmer-produced cow manure was obtained from farmers directly by buying, and self-produced cow manure was obtained by fermenting pure cow feces mixed with EM-4 and molasses.

Seed preparation

The seeds came from fruit that was old or ripe on the tree. The fruit was split; the seeds were taken and located in the center of the fruit. Seeds were cleaned from the thin layer using kitchen ash, washed, and dried under the sun for 2 days.

Preparation and immersion in atonic

One (1) mL of atonic was diluted with distilled water until the volume reached 1 L and then shaken until homogeneous. After that, the dried seeds were immersed in the atonic solution according to the treatment.

Seeding

After being immersed in an atonic, seeds were sown in polybags filled with seedling media according to treatment. Then, the seedling media was perforated as deep as 1 cm, the seeds (2 seeds each) were inserted, and the holes filled with seeds were covered with a little seedling media.

Upkeeping

Plant upkeep included watering, weeding, and controlling pests and disease-causing agents. Watering was done every day (morning or evening). Weeding was carried out on weeds growing around the plant. Controlling of pests and disease-causing agents was carried out.

Observation variable

Germination

Germination rate. Count the number of normal germination on the fourteenth day after the seeds germinated, then calculate the germination rate percentage. The germination rate can be calculated using the formula:

Germination Rate (GR) = (number of normal germination on day 14/total number of seeds) x 100%

Germination capability. Count the number of normal germinations on the twenty-first day after the seeds germinated, then calculate the germination percentage. Germination capability can be calculated using the formula:

Germination capability (GC) = (number of normal sprouts on day 21/total number of seeds) x 100 %

Germination uniformity. Germination uniformity was measured by calculating the addition of the highest percentage of seeds that grew normally compared to the previous day.

Growth

Root length (cm). Measure the root length from the base to the tip of the root. Take measurements at the end of the study.

Stem circumference (cm). Calculate the circumference of the stem by wrapping a thread around the seedling. Measurements were made approximately 5 cm from the root neck of the seedling.

Plant height (cm). Measure the height of the seedling from the root neck to the growing point. Perform once-a-week observation on plant height (cm) after the seeds were one week old until the end of the study.

Number of leaves (strand). Count the number of leaves that have opened completely. Observations once a week after the seeds were 1 week after germination until the end of the study.

Leaf area (cm^2). Calculate leaf area using the gravimetric method at harvest. The calculation is as follows:

Leaf area = Wr/Wt x Lk

Where:
Wr : Replica paperweight
Wt : Total paper weight (3.5 g)
LK : Total paper area (710.64 cm^2)

Total seedling fresh weight (g). Weigh the fresh weight of the seeds, including the roots, stems, and leaves, at the end of the study.

Total seedling dry weight (g). Weigh the seeds' dry weight, including the roots, stems, and leaves, after drying in an oven at a temperature of 60-70^oC for ± 24 hours until the weight is constant.

Data analysis

Analysis of Variance (Anova) analyzed observational data, and if there was a significant difference, it was continued with Duncan's Multiple Distance Test (DMRT) at a 95% confidence level.

RESULTS AND DISCUSSION

Germination rate

Germination rate is a measure of seed vigor which states the number of days required for the emergence of the radicle/plumule (Mugnisjah and Setiawan, 1990).

The analysis of variance showed no interaction between the type of seedling media and the immersion time of synthetic auxin on the germination rate. However, the type of seedling media had a very significant effect on the rate of germination of papaya seeds. At the same time, the immersion time of synthetic auxin did not significantly affect the papaya seeds' germination rate.

Table 1 (DMRT 5%) shows that soil media has the best effect on papaya seeds' germination rate. It is presumably because the soil moisture is maintained so that there is a critical point of germination. If there is a critical point of germination, seeds will be hydrolyzed. Seed germination begins with the absorption of water. In absorption, water imbibition will cause the seed coat to soften. By softening the seed coat, the elements needed for seed germination can enter the seed easily.

Germination power

Germination is the percentage of the number of seeds that grow normally in a predetermined period. According to Mugnisjah and Setiawan (1990), germination is a seed viability measure that predicts seeds' potential viability.

The results of the analysis of variance showed that there was no interaction between the type of seedling media and the immersion time of synthetic auxin on germination. The seedling media had a very significant effect on the germination of papaya seeds, while the immersion time of synthetic auxin did not significantly affect the germination of papaya seeds.

Table 2 (DMRT 5%) shows that the soil medium had the best effect on the germination of papaya seeds. Presumably, the seeds used were physiologically ripe, where the moisture content decreased rapidly to about 20%. Under these conditions, the seeds had maximum dry weight, growth, and germination (Kamil 1979). Therefore, high germination indicates the high viability of the seed. In addition, high germination will save on using seeds and costs incurred for purchasing seeds.

Physiologically ripe seeds can be obtained from fruit that has reached a maturity level of 99% or is commonly referred to as ripe. It can be seen visually from the color of the fruit, which has become reddish yellow in almost all parts of the fruit (Kamil 1979).

Table 1. The average germination rate in the treatment of various types of media for seedlings of papaya seeds 10 WAP

Seedling media	Average (%)
S1:soil	0.26 a
S2:soil:farmer-produced cow manure (1:1)	0.03 b
S3:soil:farmer-produced cow manure (1:1)	0.17 a
S4:soil:farmer-produced cow manure:self-produced cow manure (1:1:1)	0.04 b

Note: Numbers followed by the same letter show no significant difference in DMRT 5%

Table 2. Average germination of various types of seedling media on papaya seeds at 10 WAP

Seedling media	Average (%)
S1:soil	0.32 a
S2:soil:farmer-produced cow manure (1:1)	0.07 b
S3:soil:farmer-produced cow manure (1:1)	0.28 a
S4:soil:farmer-produced cow manure:self-produced cow manure (1:1:1)	0.08 b

Note: Numbers followed by the same letter show no significant difference in DMRT 5%

Germination uniformity

The uniformity of germination in a seed depends on the vigor of a seed. Vigor can be interpreted as the ability of seeds to grow normally in suboptimal environmental conditions (Sutopo 2002). Therefore, with high uniformity, it is expected to produce normal mature plants so that production can be optimal.

The analysis of variance showed no interaction between the type of seedling media and the immersion time of synthetic auxin on the uniformity of papaya seed germination. The type of seedling media and the duration of immersion in synthetic auxin did not significantly affect the uniformity of papaya seed germination.

Figure 1 shows that treatment with soil media with seeds soaked in synthetic auxin for 3 hours provided the best germination uniformity compared to other treatments. The germination uniformity depends on vigor. Vigor is synonymous with germination rate. If the germination rate is high, the vigor is also high. It can be seen that the variable rate of germination of soil media gives the best effect compared to other media. High seed vigor usually lasts a long time for storage, is resistant to pests and disease-causing agents, grows quickly and evenly, and can produce normal mature plants that produce well in a suboptimal growing environment. High seed vigor can achieve high production levels (Sutopo 2002).

Root length

Roots are an integral part of the plant and have the same important function as the top of the plant. For example, in the process of photosynthesis, the upper part of the plant, in the form of a canopy, functions to absorb CO^2 to carry out the photosynthesis process, while the lower part, in the form of roots, functions to absorb water and nutrients (Sitompul and Guritno 1995).

The analysis of variance showed no interaction between the type of seedling media and the immersion time of synthetic auxin on the root length of papaya seedlings. However, the seedling media had a very significant effect on the root length of papaya seeds. At the same time, the duration of immersion in synthetic auxin did not significantly affect the root length of papaya seeds.

Root length is one parameter that indicates a plant can grow well. Long roots indicate that the plant is growing actively because plant roots grow elongated, looking for water and nutrients. In addition, long roots indicate that the plant's growing medium is less fertile. Table 3 shows that soil media has the best effect on the root length of papaya seedlings compared to other seedling media. It is suspected that the soil used has little nutrient content, per Sutejo's (2002) statement that the organic matter content in the regosol soil is low, causing plant roots to grow lengthwise looking for water and nutrients for water photosynthetic activity. Per Wahyudi (2009), root length growth is influenced by the availability of little nutrients, causing the roots to elongate in search of nutrients. In addition, Gardner et al. (1991) stated that roots that penetrate deep into the soil might grow into unexploited soil layers, which generally have low mineral content.

Table 3. Average root length in the treatment of various types of seedling media on papaya seedlings at 10 WAP

Seedling media	Average (%)
S1:soil	19.93 a
S2:soil:farmer-produced cow manure (1:1)	10.15 bc
S3:soil:farmer-produced cow manure (1:1)	17.63 ab
S4:soil:farmer-produced cow manure:self-produced cow manure (1:1:1)	5.83 c

Note: Numbers followed by the same letter show no significant difference in DMRT 5%

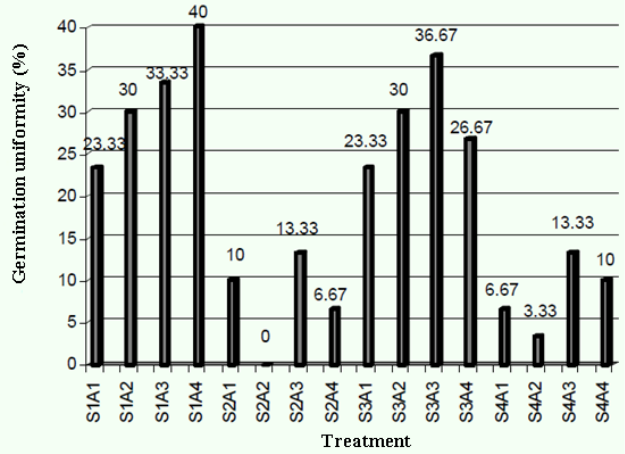

Figure 1. Germination uniformity of papaya seedlings at 10 WAP

Stem circumference

Stem circumference will show the robustness of the seedling so that it shows the ability of the seedling to support the canopy above it. Ashari (1995) added that the stem supports the growth of leaves, flowers, and fruit and is a storage place for food, water, and minerals.

The results of the analysis of variance showed that there was no interaction between the type of seedling media and the immersion time of synthetic auxin on the stem circumference of papaya seedlings. The type of seedling media and the duration of immersion in synthetic auxin did not significantly affect the increase in stem circumference of papaya seedlings.

The size of the stem circumference indicates the growth process as a result of cell enlargement and differentiation. It is influenced by the absorption of water (H_2O) and nutrients from the soil by plants to form plant tissues and organs. In addition, it is also influenced by the photosynthesis process, which will result in the accumulation of photosynthesis in plant organs (Mardani 2008).

Based on Figure 2, it can be seen that the mixed media of soil and self-produced cow manure and synthetic auxin soaking for 3 hours had a good effect on the stem circumference of papaya seedlings. With this media, stem circumference from week to week increased compared to other media. It is suspected that the mixed media of soil and self-produced cow manure is a porous medium, so the water absorption is good, and the water needed for the

photosynthesis process can be fulfilled. According to Lakitan (2004), a plant's photosynthesis rate is limited by the availability of water. Therefore, lack of water can inhibit the rate of photosynthesis, especially its effect on the turgidity of stomata guard cells. If there is a lack of water, the turgidity of guard cells will decrease, causing the stomata to close. The closing of the stomata will inhibit the absorption of CO_2, which is needed for carbohydrate synthesis.

Plant height

Plant height is a plant size that is often observed either as a growth indicator used to measure environmental effects or the treatment applied and is the easiest to see (Sitompul and Guritno 1995).

The results of the analysis of variance showed that there was no interaction between the type of seedling media and the immersion time of synthetic auxin on the growth of papaya seedling height. The type of seedling media and the duration of immersion of synthetic auxin did not significantly affect the increase in height growth of papaya seedlings. It is presumably due to the unpreparedness of the nutrients in the seedling medium for plants, even though the amount is high. Sutejo (2002) stated that although N, P, and K are in the soil, not all of them are ready to be absorbed by plants.

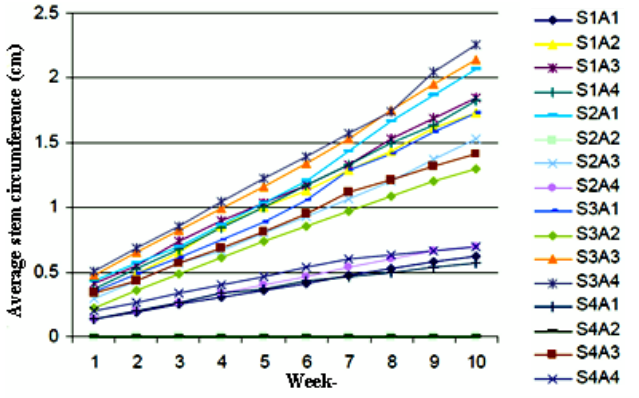

Figure 2. Stem circumference of papaya seedlings at 10 WAP

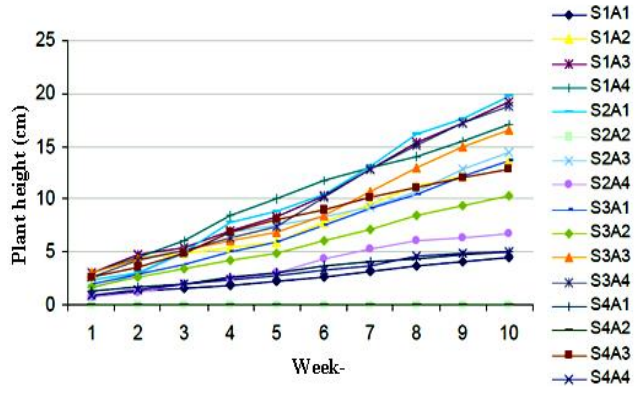

Figure 3. Papaya seedling height at 10 MST

Figure 3 shows that the soil media treatment mixed with farmer-produced cow manure and without immersion in atonic resulted in the highest plant height compared to other treatments. Therefore, the soil media mixed with farmer-produced cow manure is suspected of containing a high total N (see appendix). In the presence of high N, plant growth can increase. Furthermore, Sutejo (2002) states that nitrogen is the main nutrient for plant growth, which is generally required for the formation or growth of vegetative parts of plants, such as leaves, stems, and roots.

The use of synthetic auxin growth regulators in papaya nurseries aims to stimulate seedling growth so that the seeds produced are fast and uniform in growth so that they are always ready for planting on time and can produce normal and high-producing mature plants even in suboptimal growing conditions. One thing to be aware of is that growth substances do not always have a good effect on plant growth, but sometimes they inhibit plant growth. Somantri and Evizal (1987) cit. Murni (1994) suggested that the growth stimulant sodium nitrophenol was less effective because it was not much different from that without a growth stimulant.

Number of leaves

Leaves are generally the site of carbohydrate synthesis for plants. Therefore, leaf observation is necessary as an indicator of growth and as supporting data to explain the growth process. The number of leaves is one of the variables that can be used to measure plant growth other than plant height (Sitompul and Guritno 1995).

The analysis of variance showed no interaction between the type of seedling media and the duration of immersion of synthetic auxin on the number of leaves. However, the type of seedling media had a very significant effect on increasing the number of papaya seed leaves. In contrast, the duration of immersion in synthetic auxin did not affect increasing the number of papaya seed leaves.

Table 4 shows that soil media has the best effect on the number of papaya seedling leaves compared to other seedling media due to the medium total N content in soil media, namely 0.35%. Sufficient N content can affect the formation of plant vegetative parts, in this case, the leaves. According to Gardner et al. (1991), if the plant lacks N, the growth process will be disrupted, the plant will be stunted, the plant leaves will turn yellow and then fall off, and the dry weight of the crop will decrease. The yellowed leaves then fall off, reducing the number of leaves present.

Table 4. The average number of leaves in the treatment of various types of media for seedlings on papaya seedlings at 10 WAP

Seedling media	Average (%)
S1:soil	8.72 a
S2:soil:farmer-produced cow manure (1:1)	3.75 b
S3:soil:farmer-produced cow manure (1:1)	8.22 a
S4:soil:farmer-produced cow manure:self-produced cow manure (1:1:1)	2.12 b

Note: Numbers followed by the same letter show no significant difference in DMRT 5%

The content of N can affect the formation of vegetative parts of plants, but it can also be influenced by aeration that occurs in the soil media. Although the soil used as the media is regosol soil, it is suspected that the regosol soil used has better aeration than other media. Good aeration will facilitate the absorption of nutrients.

Leaf area

Leaf area is the main parameter concerning the function of leaves as light receivers and photosynthetic tools. Therefore, leaf area largely determines the rate of photosynthesis per unit plant. In other words, information about plant photosynthesis can be obtained (Sitompul and Guritno 1995).

The analysis of variance showed no interaction between the type of seedling media and the duration of immersion of synthetic auxin in the leaf area of papaya seedlings. The type of seedling media and the duration of immersion in synthetic auxin did not significantly affect the leaf area of papaya seedlings.

In Figure 4, it can be seen that papaya seedlings treated with a mixture of soil and farmer-produced cow manure and without synthetic auxin immersion were able to produce the highest leaf area compared to other treatments. In the mixed media of soil and farmer-produced cow manure, the N content is high, namely 0.70%. High N content can affect the leaf area of a plant. Sallah et al. (1998) cit Sulandjari (2008) stated that applying N can increase leaf area and photosynthetic activity. It is supported by Humphries and Wheeler (1963) cit. Gardner et al. (1991) stated that N fertilization significantly affected leaf expansion, especially on leaf width and area. Suppose there is more available N content than other elements. In that case, the carbon skeleton converted into protein as a protoplasm component will run faster to produce more protein (Sutedjo 2002). Humphries and Wheeler (1963) cit. Gardner et al. (1991) stated that N deficiency can also cause a reduction in leaf area due to the aging of lower leaves. It can be seen that the leaf area of papaya seeds with soil media alone has a lower leaf area than the other treatments. It is because the soil media used had moderate N content, so it was not sufficient for the vegetative growth of papaya seedlings.

Total seedling fresh weight

Growth is an activity that processes substrate inputs to produce growth products. The yield of growth products can be measured simply by the weight gain of the whole plant or plant parts, including the harvested part and other parameters (Sitompul and Guritno 1995). Salisbury and Ross (1995) added that the plant's fresh weight showed the plant's metabolic activity, and the fresh weight's value was influenced by tissue moisture content, nutrients, and metabolic products.

The analysis of variance showed no interaction between the type of seedling media and the duration of immersion of synthetic auxin on the total fresh weight of papaya seeds. The type of seedling media and the duration of immersion in synthetic auxin did not significantly affect the total fresh weight of papaya seeds.

Based on Figure 5, it can be seen that the mixed media of soil and farmer-produced cow manure could have the best effect on the total fresh weight of seedlings. The total fresh weight of seedlings is influenced by the size of the leaf area of a plant and by the accumulation of other parts of the plant, such as roots and stems, as well as the water content present in each part of the plant. As the leaf area increases, the leaf weight produced will also increase.

Therefore, high leaf weight will affect the total fresh weight of the plant. In the observation of leaf area, the largest leaf area was produced in a mixture of soil and farmer-produced cow manure, causing the total fresh weight of seedlings on the same medium to be high. Besides being influenced by leaf area, the total fresh weight of seedlings was also influenced by the availability of N in the media used. According to Harjadi (1991), the presence of N in the media could be absorbed by plant roots which could be utilized in the division and development of cells by plant tissues. It resulted in the formation of large vacuoles that can hold large amounts of water, thereby increasing the fresh weight of the plant.

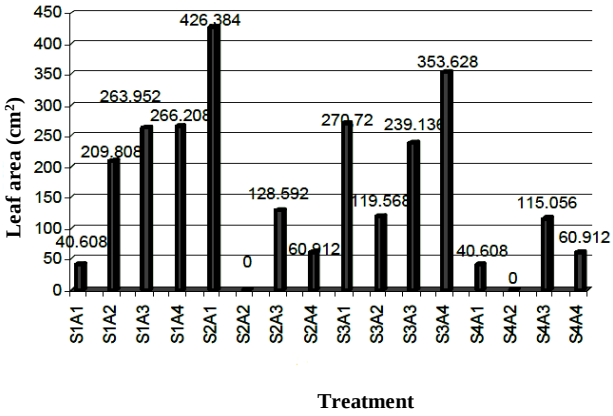

Figure 4. Papaya seedling leaf area at 10 WAP

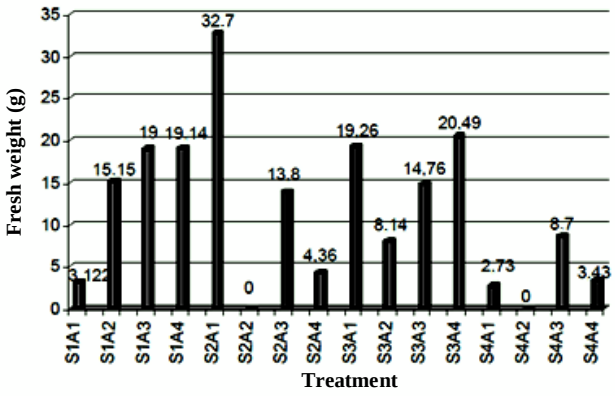

Figure 5. The total fresh weight of papaya seeds at 10 WAP

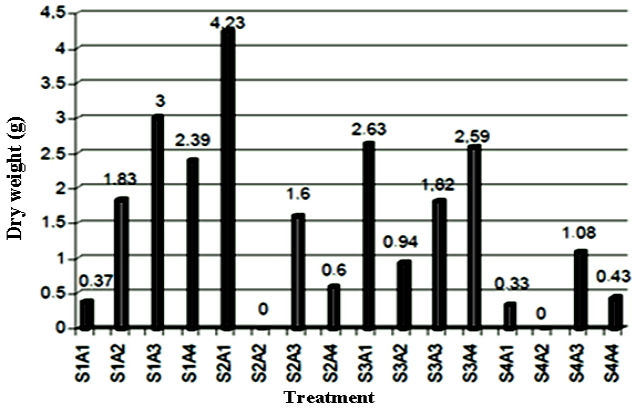

Figure 6. The total seedling dry weight of papaya at 10 WAP

Total seedling dry weight

The total dry weight of the plant shows the amount of organic matter accumulated or stored in the plant. Plant dry weight results in a balance between CO_2 uptake (photosynthesis) and excretion (respiration). Gardner et al. (1991) and Utama (1999) cit. Suryaningsih (2006) added that dry weight reflects the nutritional status of plants because dry weight depends on the rate of photosynthesis and plant respiration.

The analysis of variance showed no interaction between the type of seedling medium and the duration of immersion of synthetic auxin on the total dry weight of papaya seeds. The type of seedling media and the duration of immersion in synthetic auxin did not significantly affect the dry weight of the whole papaya seeds.

Figure 6 shows that the highest total dry weight of seeds was produced by a mixture of soil and farmer-produced cow manure. Dry weight is closely related to the fresh weight of a plant. If the fresh weight is high, the dry weight is also high. The increase influenced the dry weight of the plant in the leaf area of the plant. It is in line with the statement of Pujiasmanto (2001) that an increase in leaf area will increase the material obtained. Harjadi (1991) adds that the availability of nutrients absorbed by plants can stimulate the formation of carbohydrates, fats, and proteins through photosynthesis. Protein synthesis will result in an increase in the size of plant cells and the accumulation of carbohydrates in the form of dry weight that cannot be reversed.

In conclusion, based on the research, it can be seen that: (i) the interaction between the types of seedling media and the duration of immersion in synthetic auxin did not occur in all observed variables; (ii) the expected uniformity of germination through all combinations of treatments such as seedling media and synthetic auxin was not achieved; (iii) the use of soil media with the addition of farmer-produced cow manure and self-produced cow manure has not succeeded in increasing all variables of germination and early growth of papaya seedlings; (iv) synthetic auxin immersion for up to 3 hours did not increase all variables of germination and early growth of papaya seedlings.

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