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Tuber diversity of *Dioscorea hispida* from Muna Island, Indonesia, for potential microencapsulated bioherbicide agents

WINDI EGIDYA PUTRI, EVA JOHANNES, SJAFARAENAN♥
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Hasanuddin. Jl. Perintis Kemerdekaan KM. 10, Makassar 90245, South Sulawesi, Indonesia. Tel.: +62-411-586016, Fax.: +62-411-588551, ♥email: enan.gidinnur@gmail.com

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Abstract. *Putri WE, Johannes E, Sjafaraenan. 2025. Tuber diversity of* Dioscorea hispida *from Muna Island, Indonesia, for potential microencapsulated bioherbicide agents. Asian J Agric 9: 174-184.* Dioscoreaceae family tubers scattered across Muna Island have diverse characteristics. This research aims to determine the diversity of *Dioscorea* tubers and their potential as bioherbicides using the microencapsulation method. The study was conducted in 6 villages, namely Tampo, Wakumoro, Oelongko, Lasehao, Napano Kusambi, and Napalakura, by collecting uwi (*Dioscorea alata* L.) and gembili (*Dioscorea esculenta* L.), which are utilized by the local community as staple food to substitute rice, made into chips. Gadung (*Dioscorea hispida* Dennts.) is also used as a natural herbicide. This research uses a Factorial Randomized Block Design (RBD). The treatments were *D.hispida* extract at the concentration of 20%, 30%, and 40%. Other factors were 4 species of weeds, i.e., *Thunbergia alata*, *Centrosema pubescens*, *Phyllanthus amarus*, and *Calopogonium mucunoides*. Every treatment has 4 replications. The data were analyzed using ANOVA. The tuber flesh of *D. alata*. and *D. hispida* was orange-white, while *D. esculenta* was purple-white. Applying a 40% microencapsulated D. hispida tuber extract effectively retard the root length of the weed.

Keywords: Bioherbicide, *Dioscorea hispida, kolope*, microencapsulation, Muna Island, tuber diversity

Abbreviations: ANOVA: Analysis of Variance, SEM: Scanning Electron Microscope

INTRODUCTION

Weeds are currently high enough to cause significant losses to farmers. Weed control can be done chemically or biologically to population levels that are not economically detrimental. The continuous use of herbicides may result in adverse environmental effects, namely the poisoning of non-target organisms, water pollution, soil damage, and poisoning due to herbicide residues in agricultural products. According to Ammar et al. (2023), the alternative to weed control in food crops and plantations is using bioherbicides. Various types of weeds in the plantation, e.g., *Thunbergia alata*, *Centrosema pubescens*, *Phyllanthus amarus*, and *Calopogonium mucunoides,* are broadleaf weeds commonly found in plantation areas. These weeds have different self-defense, which are influenced by various factors (Sahari et al. 2023).

The continuous use of herbicides can negatively impact the environment, poisoning non-target organisms, causing water pollution, soil degradation, and poisoning due to herbicide residues on agricultural products. It raises public awareness of the importance of environmental sustainability, which can increase the demand for environmentally friendly agriculture and safer agricultural products. Therefore, there is a growing need for the use of natural herbicides.

Bioherbicides are natural materials that control weeds because they quickly decompose in the soil without leaving residue. Bioherbicides from tubers can be done by extracting secondary metabolites in liquid form. However, these results are less effective because the extract is easily oxidized, reducing the effectiveness of bioactive compounds. An innovation to overcome this problem is the microencapsulation of secondary metabolites of extracts. Microencapsulation is the process of coating liquid or solid core materials using a special encapsulation to give the core particles the desired physicochemical properties. Allelochemical compounds derived from plants and environmentally friendly are phenolic groups such as alkaloids, tannins, steroids, and other compounds that can be harmful when in contact with germination so that they can inhibit cell division (Kong et al. 2019).

Several species of the *Dioscorea* family are used as staple substitutes for rice. They are low-sugar foods that can be processed into flour, chips, and various products with economic value, specifically from the *Dioscorea alata* L. and *D. esculenta* L. species. Meanwhile, other species of Dioscorea that are not consumed are used as natural herbicides. *Dioscorea hispida* Dennts *is* characterized by creeping tubers, large size, and above-ground growth and might be used as a bioherbicide (Sardar et al. 2022). Its tuber grows wild in lowlands up to an altitude of 50 meters, especially in marginal forests and shrubs, and is cultivated in yards. Besides being used as bioherbicides, tubers from several species of the Dioscoreaceae family can also be used as rice substitutes, such as *D. alata*, *D. esculenta*, *Dioscorea bulbifera*, *Dioscorea nummularia*, *Dioscorea pentaphylla*, and *D. hispida*.

The tuber of *Dioscorea hispida* contains toxins, namely the alkaloid dioscorine (Salehi et al. 2019) and diosgenine (Estiasih et al. 2022). These compounds can be used as bioherbicides to inhibit weed growth; Semwal et al. (2019), the content of alkaloids reaches 71.36%. Alkaloid compounds act as bioherbicides to protect plants from weeds and pathogens. The action of alkaloid compounds involves damaging cell structures through a synergistic mechanism with various active compounds; thus, the activity of the compounds increases in damaging cells (Johannes et al. 2022).

Bioactive compounds maintain their stability during storage by microencapsulation. Bioactive compounds are generally less stable, so they must be protected from physical and chemical degradation due to handling and environmental influences. Microencapsulation generally aims to protect bioactive components, reduce nutrient loss, and transform components from liquid to solid, making them easier to handle and apply in food systems. Microencapsulation using the inclusion complex method with β-cyclodextrin encapsulant has been proven to be the most effective in protecting active ingredients from heat and evaporation (Zabot et al. 2021). Based on the above background, research is needed to determine the diversity and concentration of microencapsulation of *D. hispida* tubers as a bioherbicide agent in controlling broadleaf weeds.

MATERIALS AND METHODS

Research location and sampling

The sampling locations were several villages in Muna Island, Southeast Sulawesi Province, Indonesia, i.e. Tampo (122°41'19.424"E, 4°37'36.028"S), Wakumoro (122°30'41.806"E, 5°416.523"S), Oelongko (122°28'14.546"E, 5°7'50.719"S), Lasehao (122°36'35.661"E, 4°38'50.535"S), Napano Kusambi (122°32'0.217"E, 5°0'36.589"S), Napalakura (122°39'25.285"E, 4°38'10.904"S) (Figure 1). The research was conducted at the Botany Laboratory, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar Indonesia. The extraction and preparation of microencapsulation were done at the Phytochemistry Laboratory, Faculty of Pharmacy. The bioherbicide application on weed was conducted in the plantation fields of Tampo Village, Napabalano District, Muna Regency. Interviews with *D. hispida* tuber farmers were done to obtain additional information about the tuber.

Experimental design

This study is an experimental quantitative research using a Randomized Block Design (RBD) with 3 treatments of each extract microencapsulation concentration, namely control, 20%, 30%, and 40%, with 4 replications. Tests were carried out on plantation land. Data were analyzed using ANOVA and continued with variance analysis and the Least Significant Difference (LSD) test at the 5% level. The materials used in this study were *D. hispida* tubers obtained from Muna Island, *T. alata* weed, *C. pubescens, C. mucunoides*, and *P. amarus.*

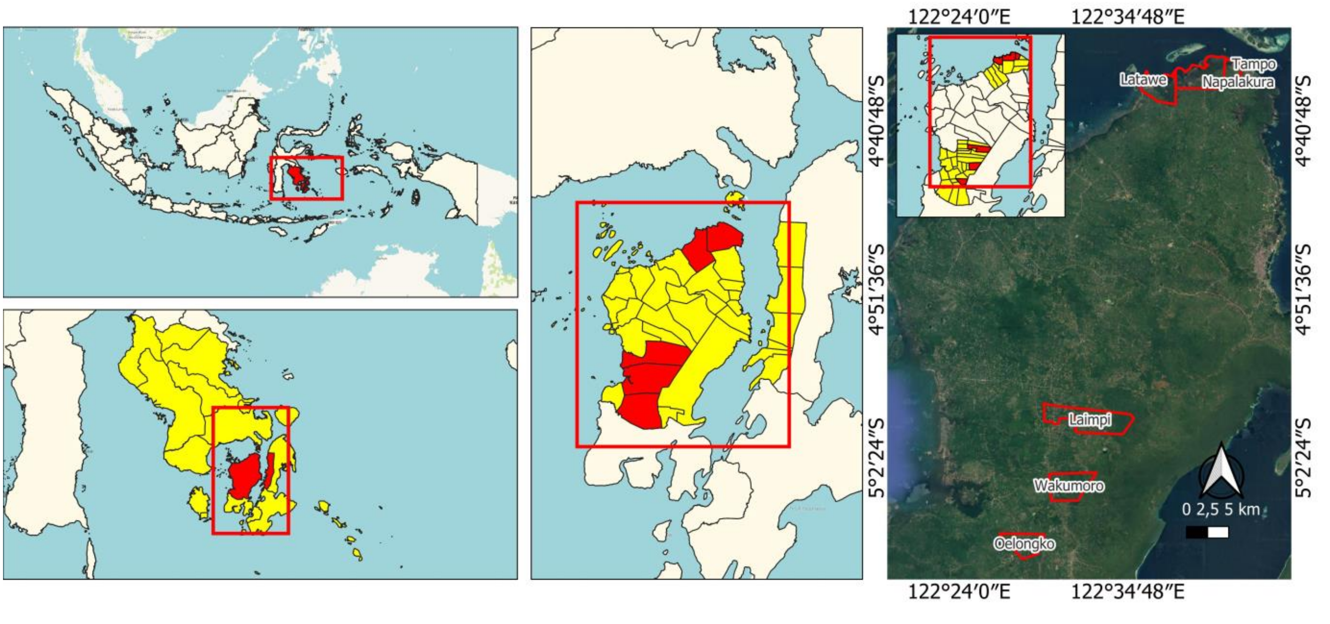

Figure 1. Sampling locations of *Dioscorea* tuber are in Tampo, Wakumoro, Oelongko, Lasehao, Napano Kusambi, Napalakura Villages of Muna Island, Southeast Sulawesi Province, Indonesia

Phenotypic character identification of *Dioscorea* tuber

The identification of phenotypic characters of *Dioscorea* tubers refers to Suleman et al. (2021). The parameters identified are morphometric characters such as habitus, tuber, tuber flesh, stem shape, leaves, roots, and other properties. It is known that the closeness between *Dioscorea* species is based on genetic material that can affect certain phenotypic characters. Taxa below the type can be identified by comparing differences in morphological characters, i.e., the morphological character of the tuber (Davis and Heywood 1973). The observed morphological characters are the shape, the size (length and diameter), the branching, the roots, the location of the root growth, the surface of the tuber skin, the color of the skin inside the tuber, the color of the tuber flesh, and the presence or absence of sap contained in the tuber.

Extraction of *Dioscorea hispida* tuber extract

The tuber of *Dioscorea hispida* was cleaned, sliced thinly, and dried. The dried gadung (*D. hispida*) tubers are ground and then mixed with distilled water at predetermined concentrations, namely 20% (50 g/250 mL), 30% (75 g/250 mL), and 40% (100 g/250 mL), followed by incubation for 3 days at room temperature. Once a day, the bottle is opened to allow the gas inside to escape and close again. After incubation, the extract was filtered using a funnel lined with filter paper. Then, the filtrate was evaporated at 78°C in the water bath to evaporate the solvent. The extract was stored in a vial.

Microencapsulation of *Dioscorea hispida* tuber extract

Maltodextrin was added as much as 5 grams in each treatment of 20%, 30%, and 40% extract concentrations (Salehi et al. 2019) into a 100 mL glass jar, then add 50 mL of distilled water to each treatment. The mixture was heated using a hot plate for 30 minutes. Extracts that have been mixed with an emulsifier, namely tween 80, as much as 1 mL until homogeneous and forming foam was added to the mixture, and then the liquid material was converted into solids using the *Thin-layer drying* method. Then, it was poured into a Petri dish with a thickness of 3 mm and dried in an oven at 70°C for 13 hours until dry. The resulting encapsulations were then pulverized and sieved.

Microencapsulation observation using SEM (Scanning Electron Microscope)

The encapsulated extract of 20%, 30%, and 40% concentrations were observed for their shape by observing their characteristics. The form of microencapsulation is related to its mode of action. The shape of the microencapsulation can vary depending on the packaging material and the mechanism of the microencapsulation formation. The morphological observation of the microencapsulation was carried out using SEM (*Scanning Electron Microscope*).

Applications of microencapsulated extract in plantations

The extract concentrations were 20%, 30%, and 40%, and 4 species of weeds, namely *T. alata*, *C. pubescens*, *P.amarus*, and *C. mucunoides*. There are 12 combinations of treatments. Every treatment combination had 4 replications. Watering was done in the morning and evening for four weeks following application (Afifah et al. 2024).

Observation parameters in the field

The parameters observed in the field were the degree of (%) of weed damage due to the application of *Dioscorea hispida* extract, which was observed visually using a scoring method of the 2011 Pesticide Commission by Halim (2023) of herbicide efficacy as follows: 0 = no damage 0-5% shape and/or color of leaves and/or abnormal growth, 1 = mild poisoning >5-20% shape or color of leaves and/or abnormal growth; 2 = moderate poisoning >20-50% shape or color of leaves and abnormal growth; 3 = heavy poisoning >50-75% shape or color of leaves and/or abnormal growth; and 4 = very heavy poisoning >75% shape or color of leaves and/or abnormal growth or plant death. Other parameters observed were weed height (cm), measured from the base of the stem to the growing point or tip, observed every week. Other parameters, i.e., root length (cm), weed wet weight (g), and dry weight (g), were observed in the 4th week after application. The bioherbicides are applied around the roots to ease the absorption of the compounds of the microencapsulated extract by plant roots. The wet weight of weeds was observed by pulling out as many weeds down to the roots as the research plots, washing them thoroughly, selecting them according to their type, and weighing them. Weed dry weight is carried out by pulling weeds up to the roots as much as the research plot and washing them thoroughly. Then, the weeds are selected according to their type and dried in an oven for 48 hours at 65°C until they reach a constant weight.

Data analysis

Data obtained from the observations were analyzed using analysis of variance (ANOVA) with the Least Significant Difference (LSD) test at the 5% significance level.

RESULTS AND DISCUSSION

***Dioscorea* species collected in Muna Island, Southeast Sulawesi Province**

Various *Dioscorea* species are growing in several villages on Muna Island, i.e., uwi (*D. alata*), gembili (*D. esculenta*), and gadung (*D. hispida*). There are 3 species of *Dioscorea* found in several villages on Muna Island (Table 1). Two *Dioscorea* species (*D. hispida* and *D. esculenta*) were collected in Tampo village. Three species (*D. hispida*, *D. alata,* and *D. esculenta)* were collected from Wakumoro. Lasehao village has 1 species (*D. hispida*). Oelongko village has 2 species (*D. hispida* and *D. alata*). Napano Kusambi village has only 1 species (D. *hispida)*. The last survey location in Napalakura Village also only has 1 species, *D. alata*.

Morphological characteristics of *Dioscorea* species

*Gadung (*Dioscorea hispida *Dents.)*

Dioscorea hispida has a plant height of 5-10 meters, and a round and spiny stem spread long on the stem and petiole. The stem is green, and the tuber is irregularly round and covered by stiff root hairs. The tuber's skin is brown, and the flesh is white-orange, with the tuber appearing near the surface soil (Figure 2).

*Uwi (*Dioscorea alata *L.)*

Dioscorea alata has appealing characteristics, such as its green and four-winged stems, the distinct green wing (alatus), and the round stem shape. It has a heart-shaped leaf shape, alternate leaf seating at the bottom and facing at the top, and a right-twisting stem direction. The base and tip of the petiole are purple. It has a smooth leaf surface, a broadly notched leaf base, a flat leaf edge, and a tapered leaf tip. The widest leaf position is in the center (Figure 3).

Its tubers have a round shape with a length reaching 15 cm and diameter of 15 cm, moderate rooting, located mainly around the root neck, and a small part is scattered over the entire surface of the tuber, wrinkled skin surface, brown outer skin color. At the same time, the inside is black, purplish white tuber flesh and gummy. Rooting on the surface of the tuber is slightly located where the roots grow around the root neck.

*Gembili (*Dioscorea esculenta *L.)*

Dioscorea esculenta has a green stem with 2 mm spines. Leaves are green with a single variant and alternate leaf; the heart-shaped leaf blade is widened. The variant leaf shape of the *D. esculenta* is shown in Figure 4. The leaf edges of *D. esculenta* are flat, and the leaf tips are pointed. The distance between the lobes is moderate. *D. esculenta* has spines that are bent downward. The bulbs of the *D. esculenta* are cylindrical. The tubers are branched, with more than five tubers per branch. The surface of the tuber has few roots. The inner skin color of the tuber is white, and the flesh color is white-orange. Wrinkles are visible on the surface of the tuber (Figure 4).

Table 1. The initial collection of *Dioscorea* species on Muna Island, Southeast Sulawesi, Indonesia

Location of collection	Collection name	Tuber collection
Tampo Village	*gadung (D. hispida), gembili (D. esculenta)*	●
Wakumoro Village	*gadung (D. hispida), uwi (D. alata), gembili (Dioscorea esculenta)*	●
Oelongko Village	*gadung (D. hispida), uwi (D. alata)*	●
Lasehao Village	*gadung (D. hispida)*	●
Napano Kusambi Village	*gadung (D. hispida)*	●
Napalakura Village	*uwi (D. alata)*	●

Note: ●: Present, -: Absent

Figure 2. The morphology of *Dioscorea hispida*: A. Habitus; B. Tuber; C. Tuber flesh; D. Stem shape; E. Leaves; F. Roots

Figure 3. The morphology of *Dioscorea alata*: A. Habitus; B. Tuber; C. Tuber flesh; D. Stem shape; E. Leaves; F. Roots

Figure 4. The morphology of *Dioscorea esculenta*: A. Habitus; B. Tuber; C. Tuber flesh; D. Stem shape; E. Leaves; F. Roots

Comparison of morphological characteristics of *Dioscorea* tubers

The properties and characteristics of the tubers of various species of *Dioscorea* are presented in Table 2. The three species of *Dioscorea* that grow on the island of Muna have different tuber characteristics, such as the appearance of tuber shape, size, inner skin color, and tuber flesh color. The differences in the shape of the tubers observed were irregular shapes in *D. hispida*, oval shapes in *D. alata*, and cylindrical shapes in *D. esculenta*.

Morphology profile of microencapsulated extracts by SEM

The microencapsulation morphology of *D. hispida* was presented through an SEM (Scanning electron m*icroscopy*) analysis with 1000x magnification. The spherical shape consists of two layers, namely a transparent-colored layer and a dark-colored layer. The transparent layer is in the center, an image of the internal layer of the tuber extract. The dark layer is on the outside, the encapsulating layer that envelops the transparent layer as a form of the external layer. The dark layer in microencapsulation has a larger area than the transparent layer, which proves that the tuber extract is encapsulated in the e solution. Although there are some irregular shapes of the microencapsulation (*spherical*), the particle distribution has a uniform size in the 30% and 40% treatments (Figure 5).

Weed height (cm)

Table 3 presents the height of broadleaf weeds treated with microencapsulated *D. hispida* tuber extract as bioherbicide four weeks after application. The findings showed that the weed species influence the weeds' height. The height of *T. alata* and *P. amarus* differs significantly, and the height of *C. pubescens* and *C. mucunoides* was not significantly different. However, the weed type and extract concentration interaction does not significantly impact the weeds' height. The results showed that applying various concentrations of microencapsulated *D. hispida* tuber extract for 4 weeks has significant practical implications. The weed species significantly affected weed height, but the interaction between weed species and *D. hispida* tuber extract concentration does not significantly affect weed height.

Table 2. Comparison matrix of tuber properties and characteristics of *Dioscorea* species

Observed properties and characteristics	***Dioscorea* species**		
	D. hispida	***D. esculenta***	***D. alata***
Tubers Shape			
Round	–	–	–
Cylinder	–	–	●
Oval	–	●	–
To finger	–	–	–
Irregular	●	–	–
Tuber length			
5-10 cm	–	–	–
11-15 cm	–	●	–
16-20 cm	–	–	●
21-25	●	–	–
Branching on tubers			
Yes	–	●	–
No	●	–	●
Location of roots on tubers			
Under	–	–	–
Central	–	–	–
Above	–	–	–
Entire surface of the navel	●	●	●
Surface of tuber skin			
Smooth	–	–	–
Finely wrinkled	–	●	–
Roughly wrinkled	●	–	–
Irregular	–	–	–
Porous	–	–	●
Skin color in tubers			
Brown	●	–	–
White	–	●	●
Violet	–	–	–
Dark	–	–	–
Orange	–	–	–
Color of tuber flesh			
White	–	–	–
Orange White	●	–	●
Violet white	–	●	–
Cream	–	–	–
Violet	–	–	–
Tuber latex			
Yes	●	●	●
No			

Note: ●: Present, -: Absent

Root length (cm)

The findings show that the extract concentration significantly affects root length. The root length is also significantly impacted by the weed species. However, the combination of concentration and weed species did not significantly affect root length. The bioherbicide extract application had a tangible effect on inhibiting root length. The 40% bioherbicide concentration effectively inhibited root elongation, as indicated by the average root length. The results showed root elongation was affected by herbicide concentration and weed species. Table 4 shows no interaction effect between the bioherbicide concentration and various species of weeds on root length; however, weed species significantly affect root length. It also shows that the root lengths of *T. alata*, *C. pubescens*, and *C. mucunoides* were not significantly different but different from that of *P. amarus*. Similarly, the extract concentration had a significant effect on root length.

Phytotoxicity level (%)

The treatment of 40% bioherbicide resulted in phytotoxicity on weed after 4 weeks of application (Table 5). The microencapsulated bioherbicide of *Dioscorea* tuber extract significantly affected various broadleaf weeds. The phytotoxicity of broadleaf weeds is presented in Table 6. The results showed that the phytotoxicity of *T. alata* and *C. pubescens* due to the application of *D.hispida* tuber extract were not significantly different, while *C. mucunoides* and *P. amarus* were significantly different. The weed species have a significant effect on the level of toxicity due to the bioherbicide application. The interaction between the weed species and bioherbicide concentration does not significantly affect the level of toxicity. The most sensitive weed against *D. hispida* tuber extract was *P. amarus*, such as changes in the number of leaves and the color and shape of the leaves. These findings emphasize the importance of this research in understanding the physiological effects of controlling weed populations, especially in weed management.

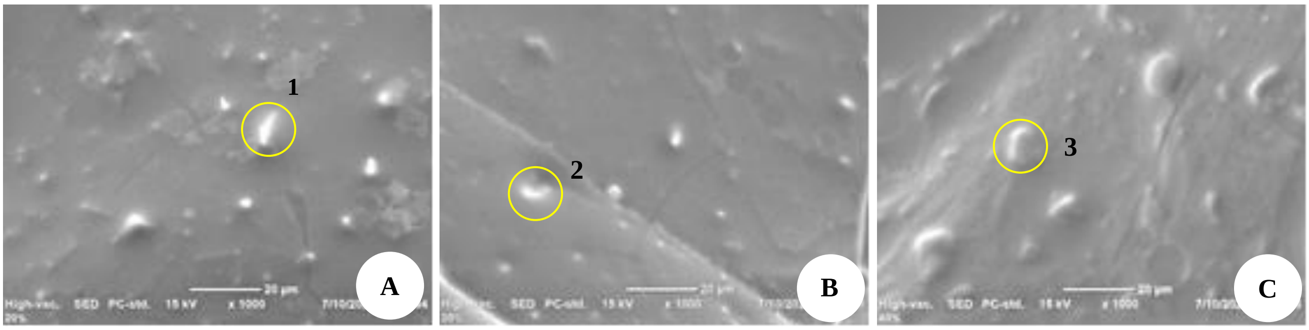

Figure 5. Morphology of microencapsulated extract of *Dioscorea hispida* at 1000x magnification scale. A. 20% treatment showed; 1. Irregular shape; B. and C. 30 and 40% treatment showed; 2. and 3. A round shape

Table 3. Plant height of broadleaf weeds treated with microencapsulated bioherbicide *Dioscorea hispida* tuber extract at 4 weeks after application

Treatment	**Species of weeds**				**Average (cm)**
	T. alata	***C. pubescens***	***P. amarus***	***C. mucunoides***	
Control	42.75	35.25	11.50	32.75	30.56±15.24
20%	46.25	32.50	13.00	31.25	30.75±14.69
30%	41.25	34.25	12.50	31.00	29.75±11.56
40%	38.00	15.25	12.25	29.25	23.68±18.94
Average (cm)	42.06±19.45[c]	29.31±9.65[b]	12.31±1.19[a]	31.06±4.53[b]	

Note: Mean values followed by the same letter are not significantly different based on the 5% LSD test.

Table 4. Root length (cm) of broadleaf weeds treated with microencapsulated bioherbicide *Dioscorea hispida* tuber extract at 4 weeks after application

Treatment	**Species of weeds**				**Average (cm)**
	T. alata		***P. amarus***	***C.mucunoides***	
Control	7.25	5.69±2.41[ab]	2.00	6.75	5.69[ab]
20%	8.25	6.19±2.83[ab]	1.75	7.25	6.19[ab]
30%	8.25	6.69±3.15[b]	2.00	7.75	6.69[b]
40%	6.50	5.19±2.94[a]	1.75	7.50	5.19[a]
Average (cm)	7.56±2.15[b]	7.00±1.67[b]	1.88±1.02[a]	7.31±1.30[b]	

Note: Mean values followed by the same letter are not significantly different based on the 5% LSD test.

Table 5. Morphological condition of weeds before and after treatment age 4 weeks

Weed Species	Figure		Phytotoxicity scoring result (%)
	Before treatment	After treatment	
T. alata			Toxicity level 15. Damage starts at week 3, showing abnormal leaf shape, color, and yellow spots.
C. pubescens			Toxicity level 15. Damage starts in week 3, showing abnormal leaf shape or color and many yellow spots on the leaves.
P. amarus			Toxicity level 20. Damage starts at week 3 with abnormal leaf shape or color, yellow spots, and shriveled leaves.
C. mucunoides			Toxicity level 15. Damage starts at week 3 with abnormal leaf shape or color and yellowish spots on the leaves.

Table 6. Phytotoxicity measurement of broadleaf weeds treated with microencapsulated bioherbicide *Dioscorea hispida* tuber extract at 4 weeks of age

Treatment	Species of weeds				Average (%)
	T. alata	***C. pubescens***	***P. amarus***	***C. mucunoides***	
Control	2.00	2.00	3.00	1.00	2.00±0.73
20%	2.00	2.00	3.00	1.00	2.00±0.73
30%	2.00	2.00	3.00	1.00	2.00±0.73
40%	2.00	2.00	2.75	1.00	1.94±0.68
Average (%)	2.00±0.00^{b}	2.00±0.00^{b}	2.94±0.25^{c}	1.00±0.00^{a}	

Note: The values were obtained from measurements using the scoring method for phytotoxicity levels from the Pesticide Commission and analyzed using ANOVA. Note: Mean values followed by the same letter are not significantly different based on the 5% LSD test.

Wet weight (g)

Table 7 shows that the wet weight of broadleaf weeds was affected significantly by the species of weed. Still, the interaction between the type of weed and bioherbicide concentration does not significantly affect the wet weight. Among the various broadleaf weeds in this study, the highest wet weight was found in *T. alata* at 7.97 g and the lowest in *P. amarus* at 1.74 g. Based on Table 7, the weed species affected the wet weight, but the interaction between the weed species and extract concentration did not significantly affect the wet weight. The results also showed that the wet weight *C. pubescens* and *T. alata* were not significantly different, whereas *C. mucunoides* and *P. amarus* were significantly different.

Dry weight (g)

The treatment of microencapsulated bioherbicide on various broadleaf weeds significantly affects the dry weight of broadleaf weeds. Table 8 presents the dry weight of broadleaf weeds treated with bioherbicide. The highest dry weight of broadleaf weeds treated with bioherbicides was obtained in *T. alata* (3.54 g), and the lowest was in *P. amarus* (0.29 g). It showed that the dry weight of *C. pubescens*, *T. alata*, *C. mucunoides*, and *P. amarus* differed significantly. However, the interaction between bioherbicide treatment and different weed species did not significantly affect dry weight.

Table 7. Wet weight of broadleaf weeds treated with microencapsulated bioherbicide *D. hispida* tuber extract at the age of 4 weeks.

Treatment	Species of weeds				Average (g)
	T. alata	*C. pubescens*	*P. amarus*	*C. mucunoides*	
Control	7.25	7.50	1.58	6.48	5.70±2.63
20%	9.00	7.63	1.20	6.43	6.06±3.23
30%	8.25	7.63	1.43	6.55	5.96±2.87
40%	7.38	6.15	2.75	5.05	5.33±2.64
Average (g)	7.97±1.53[c]	7.23±1.00[c]	1.74±1.44[a]	6.13±1.62[b]	

Table 8. Dry weight (gr) of broadleaf weeds treated with microencapsulated bioherbicide of *D. hispida* tuber extract at the age of 4 weeks after application.

Treatment	Types of weeds				Average (g)
	T. alata	*C. pubescens*	*P. amarus*	*C. mucunoides*	
Control	3.83	3.10	0.44	2.78	2.53±1.38
20%	3.52	3.18	0.27	2.47	2.36±1.34
30%	3.40	3.18	0.22	2.55	2.34±1.38
40%	3.43	2.45	0.21	2.35	2.11±1.42
Average (g)	3.54±0.60[d]	2.98±0.58[c]	0.29±0.12[a]	2.54±0.75[b]	

Discussion

Comparison of the morphological characteristics of Dioscorea *tubers*

The tubers of *Dioscorea* were used as a source of local food from generation to generation in Muna regency. Previous research conducted by Ariani and Geo (2019) showed that the Muna tribe utilizes *D. alata* and *D. esculenta* with the local names 'ghofa' and 'mafu' as the sources of carbohydrates by consuming their tubers. *D. hispida* is known as 'kolope' by local people and grows in the forest. People rarely use it as a food source because it has more sap and causes itching when it comes into contact with the skin.

Table 1 shows that one or more Dioscorea species could be found in each village. It indicated that *Dioscorea* tubers are abundant and commonly found in the forest. Still, the community has not utilized them much because they do not know the proper processing methods (Padhan and Panda 2020). *D. hispida* tubers are abundant in the area, so training is needed for the community to utilize *D. hispida* tubers as various processed products, such as chips, to reduce dioscorein content. The people in Muna usually boil and fry *Dioscorea* tubers and add salt to remove the sap from the tubers.

D. alata, *D. esculenta*, and *D. hispida* have different morphological characteristics. The morphology of *D. hispida* differs from the other 2 species; it has thick and stiff root hairs and brownish tuber skin. *D. alata* tubers are purplish and have sap or mucus (Lathifah et al. 2024). *Dioscorea* esculenta tubers have skin and flesh color variations from white to orange (Susila et al. 2020), and the color of the inner skin ranges from light brownish white to light yellowish (Pasireron et al. 2021). The tuber surface is rough, smooth, and slippery. The roots on the surface of the tuber have thorny characters on the surface of the tuber and cracks on the surface.

The tuber of *D. alata* and *D. hispida* was bigger than that of *D. esculenta* because the photosynthesis involved in tuber growth led to an increase in tuber size. Photosynthesis produces glucose, an energy source and a raw material for growth. Higher photosynthates in the tuber resulted in more extensive tuber growth. A study by Hamaoka et al. (2022) showed differences in the shape and size of tubers tend to be elongated (cylindrical), rounded, or oval, which is determined by the direction of photosynthesis translocation. Tubers with a larger diameter than the length indicate that the sink capacity is accumulated in the diametral direction and vice versa. Differences were also found in the flesh and skin colors of the tuber. The flesh of the tuber of *D. alata* and *D. hispida* is white-orange. However, the color of the inner skin of *D. hispida* is brown, and *D. alata* is white. Genetic factors and the influence of environmental factors such as temperature, humidity, and different soil conditions cause this variation. Plants grown in various ecological conditions tend to adapt to the surrounding environment. Plants' morphology varies because environmental factors are more dominant in influencing plants than genetic factors (Cao et al. 2021).

Morphology of microencapsulated tuber extract with various concentrations

Figure 5 shows the morphology of microencapsulated *D. hispida* tuber extracts at 20%, 30%, and 40% concentrations have different shapes. It relates to the encapsulation mechanism that attracts allelochemical compounds in the tuber extract. The microencapsulated extract has a ball shape but is irregularly round. The round shape of non-aggregate microencapsulation indicates that microencapsulation is physically stable (Misni et al. 2021). Microencapsulation consists of amphiphilic polymers containing hydrophobic compounds in the core and hydrophilic compounds surrounding the particles to prevent. Small spheres that are evenly distributed with a uniform particle distribution do not unite to form flocculation, indicating a polydispersion index. The smaller the polydispersion index value, the more uniform the distribution of particles in a component is in layer thickness and size. Meanwhile, the irregular shape of microencapsulation can be caused by the coating material (viscosity and solubility), the ratio of the core to the coating, and the inlet air temperature (Kori et al. 2022).

The morphology of microencapsulated extract indicates that a 40% concentration has an effective coating. The microencapsulated bioherbicide derived from *D. hispida* tubers demonstrated the most significant effect on broadleaf weed growth four weeks after application, with *T. alata* reaching 42.06 cm in height and *P. amarus* the smallest (12.31 cm). The dose of bioherbicide also affected the height. The negative control group had the highest height (30.56 cm), while the treatment group of 40% bioherbicide had the lowest plant height (23.68 cm). The findings of this study showed that the weed species and the bioherbicide concentration have different responses to the growth of broadleaf weeds. A study by Bashar et al. (2022) showed that the concentration of bioherbicides has various effects on the weed because the phytotoxic compounds contained in bioherbicides can reach target organisms through evaporation or decomposition with specific mechanisms depending on the organs and their chemical properties.

The effect of microencapsulation of tuber extract on various parameters

Applying different concentrations of microencapsulated *D.hispida* extract had a significant effect on root length. The weed species with the highest morphological damage was *P. amarus*. The research by Pranata et al. (2023) showed that the higher the dose of bioherbicide, the more bioactive herbicide is exposed, which affects the weed growth and further causes the weed to die. Bioherbicides mainly inhibit the function of essential enzymes (Table 3).

The interaction between the treatment of bioherbicide and weed species had the most effect on *P. amarus*, which has the shortest root. Previous studies showed that the average root length of *P. amarus* is 1-6 cm, *T. alata* is 6-9 cm, *C. pubescens* is 7-12 cm, and *C. mucunoides* is 7-12 cm (Stefanowska and Miroslawa 2022: Prasetyorini 2019; Rahman and Nur 2019; Feitoza et al. 2018), One factor that affects root elongation is the disruption of hormone activity that inhibits cell division. The phenolic compound in *Dioscorea* tubers disrupts cytokinin hormone activity, which results in inhibited cell growth and enlargement so that the formation of potential shoots (*plumules*) and radicles is inhibited (Amarasekara and Wickramarachchi, 2021). The findings of this study showed that the administration of bioherbicides affects root elongation by reducing the low rate of root cell growth (Zanfano et al. 2022) (Table 4).

Applying *D. hispida* tuber extract as a bioherbicide caused phytotoxicity in weed plants, which is indicated by plant abnormalities compared to negative control treatment., such as alteration in color and form, wilting, yellowish-brown lesions leaves, leading to the dropping of leaves (Kim et al. 2023). Table 6 showed that the phytotoxicity score for *T. alata*, *C. pubescens*, and *C. mucunoides* weeds was 15%, while *P. amarus* was 20%. This variation arises from different defense systems or resistance levels among these weed species, leading to varied symptomatic responses. A previous study by Strilbyska et al. (2022) indicated that allelochemicals decrease the permeability of cell membranes. Consequently, this can allow toxic substances to enter the plant, interfering with its defense mechanisms and metabolic functions. Dioscorin in *D.hispida* tuber extract causes plant death through physiological disorders. A study by Castillo et al. (2021) showed that disocorin in *D. hispida* tubers could reach 2000 mg/kg, and it can cause genetic changes. Applying bioherbicide also caused chlorosis symptoms, spots on the leaves, and bud death. A study by Bednarz et al. (2023) showed that allelochemicals can cause disease, necrosis, and chlorosis, which inhibit growth and suppress photosynthetic activity.

The impaired water absorption affects the growth of roots and inhibits photosynthesis, resulting in reduced plant absorption capacity, thus affecting wet weight. Islam et al. (2024) stated that toxic chemical compounds in plants can hinder growth and development and cause morphological damage. This is because the bioactive elements in the plants effectively disrupt water absorption and inhibit weed metabolism (Table 7). Several factors influence the outcomes of treatments on different weed species, one of which is the defense system unique to each weed type. According to Wang et al. (2024), it relates to physiological and morphological adaptations that help prevent or reduce the entry of harmful compounds into the plant when exposed to toxic substances. Each weed species has defense mechanisms, such as forming protective layers on the surface of leaves or roots, which can limit the absorption of harmful substances. Physiological changes resulting from treatment may lead to abnormal growth (dwarfism) and color changes in the leaves, stems, or roots. These changes signal disruptions in the plant's metabolic processes, impacting the weed's ability to absorb water and nutrients. Ultimately, these factors influence the wet and dry weight of the weeds and the root length. These findings are consistent with the research of Martinez et al. (2021), which suggests that growth decreases for each parameter observed, with the growth rate slowing down depending on the weed type and the concentration used.

The effect of microencapsulation of Dioscorea *tubers on weed growth*

The administration of various concentrations of microencapsulation of *D. hispida* tuber extract resulted in different growth of 4 species of weeds, namely *T. alata, C. pubescens, P. amarus*, and *C. mucunoides*. The differences in root length were found in the treatment of 40% concentration of bioherbicide. It could be caused by alkaloid compounds in the microencapsulation of *D. hispida* tuber extract. *D. hispida* tubers contain toxic dioscorin alkaloids (Hazrati et al. (2020). Dioscorin and diosgenin were derived from alkaloids and effectively inhibit weed growth, namely height, length, and biomass. The alkaloid content in *D. hispida* tubers is 31.4%, and *Dioscorea alata* L. is 12.52% (Indrawati et al. 2020). The growth rate that decreased the most was *P. amarus*. This is due to the different defense factors of each weed species (Motmainna et al. 2023). They state that the differences in inhibitory effects on several species of weeds occur due to the presence of morphological and physiological defense system factors against environmental stress. Morphological adaptation is based on the inhibition or prevention of the entry of harmful compounds into the plant, such as the presence of lignin. Lignin in the plant cell wall prevents the entry of allelochemical compounds into the membrane (Hasan et al. 2018).

In conclusion, there are 3 species of *Dioscorea* on Muna Island, i.e., *D. alata*, *D. esculenta*, and *D. hispida*. These three species have specific characteristics, namely the color of the tuber flesh. *D. alata* and *D. hispida* have orange-white tuber flesh, while *D. esculenta* has purple-white tuber flesh. The microencapsulated tuber extracts have different shapes at different concentrations of bioherbicide. The microencapsulated tuber extract at the concentration of 20% has irregularly shaped capsules, while the 30% and 40% concentrations show round shapes. The dark layer in microencapsulation has a larger area than the transparent layer, proving that the tuber extract is encapsulated in the encapsulation solution. The application of a 40% microencapsulated tuber inhibits weed growth with a toxicity level of 15%-20% out of 75% (ranging from abnormal to dead).), especially on *P. amarus*. It also inhibits root elongation. The potential of *D. hispida* as a bioherbicide needs further exploration. One of the species collected from Muna Island belongs to Dioscorea. The tubers can be consumed and used as medicinal ingredients. To promote the sustainability of this species, the author intends to investigate the use of *D. hispida* tubers in microencapsulation technology applications to manage weed growth and address related issues using natural herbicides.

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