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ETHNOBOTANICAL STUDY OF MEDICINAL PLANTS BASED ON LOCAL KNOWLEDGE IN SEDAYU VILLAGE, JUMANTONO, KARANGANYAR, CENTRAL JAVA

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ABSTRACT

Medicinal plants are one of the important aspects of traditional medicine development. The utilization of medicinal plants is a very valuable local wisdom and a culture that needs to be investigated more thoroughly so that knowledge about it does not become extinct. The purpose of this study is to determine the plant species used, the parts of plants used, and how to process them. This research was conducted in May 2023 in Sedayu Village, Jumantono District, Karanganyar Regency, Central Java. The informants of this research were the people of Sedayu Village. The method used was snowball sampling, which focuses on the community of medicinal plant users. The collected data was descriptively, qualitatively, and quantitatively assessed. The results showed that 52 species of medicinal plants were used by the Sedayu Village community, including ginger, kencur, turmeric, red betel, and ciplukan. The most utilized part of medicinal plants was the leaves and the most common processing method was boiling and drinking. This information can be a reference for the village government and the community regarding how important a type of plant as a medicine is to be preserved.

Keywords: medicinal plants, sedayu village, utilization and processing of medicinal plants

INTRODUCTION

Medicinal plants are plants that reduce, eliminate, or cure a person from certain diseases through traditional medicine. Traditional medicine from natural ingredients in the form of simplisia has empirically proven to be used to maintain health and prevent and treat diseases. Experience and skills passed down from generation to generation are the source of information about medicinal plants and their utilization (Lesmana et al., 2018). In general, medicinal plants are plants that contain active substances in their stems, roots, leaves, or other portions, allowing them to be used as ingredients to treat disease (Rusmina et al., 2015). The use of drugs derived from plants or natural ingredients is important because, in general, natural ingredients have fewer adverse effects than synthetic or chemical ingredients. The Indonesian people still demand traditional medicine because it is considered efficacious and relatively cheap (Marwati & Amidi, 2019). Community knowledge about the efficacy of medicinal plants needs to be preserved (Ismiyanti et al., 2021). The World Health Organization (WHO) recommends traditional medicine for public health maintenance, disease prevention, and treatment, particularly for degenerative and chronic diseases. In addition, WHO also supports efforts to improve the safety and efficacy of traditional medicines (Lesmana et al., 2018). Currently, the community's use of plants for treatment is still ongoing. This demonstrates the continuity between humans and nature in the form of human relationships with plants, which is known as Ethnobotany (Dharmono, 2018). Based on this description, the issue arises as to what types of medicinal plants are still utilized by the community of Sedayu Village and how medicinal plants are processed and utilized. It is necessary to research the Ethnobotany Study of Medicinal Plants Based on Local Knowledge in Sedayu Village Community, Jumantono Karanganyar, to gain additional knowledge. This research can serve as information and literature guidelines for future studies in communities that use medicinal plants as constituents in traditional rural medicine, particularly in Sedayu Village.

MATERIALS AND METHODS

This research was conducted in May 2023 in Sedayu Village, Jumantono District, Karanganyar Regency, Central Java. This study used a questionnaire as a data collection instrument. Purposive sampling was used to identify key informants, and snowball sampling was used to select the next respondent based on the direction of the preceding respondent. The number of informants in this study was 26 (male: 9; female: 17), with the majority working as herbal medicine sellers. The method was chosen to find out the species still used by the Sedayu Village community, as well as their ethnobotanical use and sustainability. The types and data sources used in this research were primary and secondary. Then, the data that has been obtained is analyzed using qualitative and quantitative descriptive analysis techniques. Quantitative data analysis uses the following formula (Utomo, 2017):

$$\%\ type\ of\ plant = \frac{\Sigma\ types\ of\ plants\ recommended\ by\ respondents}{\Sigma\ total\ plants}\ x\ 100\%$$

$$\%\ Organs = \frac{\Sigma\ plant\ organs\ used}{\Sigma\ total\ organs}\ x\ 100\%$$

$$\%\ Obtaining = \frac{\Sigma\ source\ of\ obtaining\ plant\ species}{\Sigma\ total\ source\ of\ obtaining}\ x\ 100\%$$

RESULTS AND DISCUSSION

These traditional medicinal plants are important in rural communities in Sedayu Village. The community utilizes medicinal plants based on knowledge of how to use drugs passed down from generation to generation. These medicinal plants were utilized as traditional medicine, an alternative and initial step for treating diseases obtained directly from the forest and yard, and some are planted or cultivated by the community for personal needs. Based on the results of interviews and observations in the field, 52 species of medicinal plants were found and utilized by the Sedayu Village community. The species of medicinal plants are listed in Table 1 below.

Table 1. Types of medicinal plants and plant organs used

No.	Plant Type	Scientific Name	Family	Organs used
1	Alang - Alang	*Imperata cylindrica*	Poaceae	Roots
2	Tamarind	*Tamarindus indica*	Fabaceae	Fruit
3	Red Onion	*Allium ascalonicum*	Liliaceae	Tubers
4	Starfruit	*Averrhoa bilimbi*	Oxalidaceae	Fruit
5	Bidara	*Ziziphus spina-christi*	Rhamnaceae	Leaves
6	Binahong	*Anredera cordifolia*	Basellaceae	Leaves
7	Brotowali	*Tinospora crispa*	Menispermaceae	Stem

8	Java Chili	*Piper retrofractum*	Piperaceae	Fruit
9	Cloves	*Syzygium aromaticum*	Myrtaceae	Flower
10	Ciplukan	*Pysalis angulata*	Solanaceae	Leaf, Fruit
11	Pomegranate	*Punica granatum*	Lytraceae	Leaf, Fruit
12	Insulin	*Smallanthus sonchifolius*	Asteraceae	Leaf
13	Ginger	*Zingiber officinale*	Zingiberaceae	Rhizome
14	Guava	*Psidium guajava*	Myrtaceae	Leaves
15	Distance	*Jatropha curcas*	Euphorbiaceae	Leaf
16	Lime	*Citrus aurantiifolia*	Rutaceae	Fruit
17	Kaffir Lime	*Citrus hystrix*	Rutaceae	Leaves
18	Cumin	*Nigella sativa*	Ranunculaceae	Seeds
19	Katuk	*Sauropus androgynus*	Euphorbiaceae	Leaves
20	Cinnamon	*Cinnamomum burmannii*	Lauraceae	Stem
21	Kedawung	*Parkia timoriana*	Fabaceae	Seeds
22	Coconut	*Cocos nucifera*	Arecaceae	Fruit
23	Moringa	*Moringa oleifera*	Moringaceae	Leaves
24	Basil	*Ocimum sanctum*	Lamiaceae	Leaves
25	Kemuning	*Murraya paniculata*	Rutaceae	Leaves
26	Kencur	*Kaempferia galanga*	Zingiberaceae	Rhizome
27	Cat's Whiskers	*Orthosiphon aristatus*	Lamiaceae	Leaves
28	Turmeric	*Curcuma longa*	Zingiberaceae	Rhizome
29	White Turmeric	*Curcuma zedoaria*	Zingiberaceae	Rhizome
30	Lavender	*Lavandula angustifolia*	Lamiaceae	Flower
31	Lawang	*Illicium verum*	Illiciceae	Flower
32	Lempuyang	*Zingiber zerumbet*	Zingiberaceae	Rhizome
33	Galangal	*Alpinia galanga*	Zingiberaceae	Rhizome
34	Aloe Vera	*Aloe vera*	Asphodelaceae	Leaves
35	Mangosteen	*Garcinia mangostana*	Clusiaceae	Skin
36	Noni	*Morinda citrifolia*	Rubiaceae	Fruit
37	Nutmeg	*Myristica fragrans*	Myristicaceae	Seeds
38	Papaya	*Carica papaya*	Caricaceae	Leaf, Fruit
39	Salam	*Syzygium polyanthum*	Myrtaceae	Leaves
40	Sambiloto	*Andrographis paniculata*	Acanthaceae	Leaf
41	Secang	*Caesalpinia sappan*	Fabaceae	Stem
42	Senggani	*Melastoma candidum*	Melastomataceae	Leaves
43	Lemongrass	*Cymbopogon citratus*	Poaceae	Stem, Leaf
44	Betel Green	*Piper betle*	Piperaceae	Leaves
45	Red Betel	*Piper ornatum*	Piperaceae	Leaves
46	Soursop	*Annona muricata*	Annonaceae	Leaves
47	Srikaya	*Annona squamosa*	Annonaceae	Leaves
48	Suket Teki	*Cyperus rotundus*	Cyperaceae	Tuber
49	Telang	*Clitoria ternatea*	Fabaceae	Flower
50	Temuireng	*Curcuma aeruginosa*	Zingiberaceae	Rhizome
51	Temukunci	*Boesenbergia rotunda*	Zingiberaceae	Rhizome
52	Temulawak	*Curcuma Zanthorrhiza*	Zingiberaceae	Rhizome

Table 1 describes the medicinal plants often used by the Sedayu Village community. Ginger (Zingiber officinale), a rhizome plant belonging to the Zingiberaceae family, had the greatest percentage of medicinal plant utilization at 88.5% (Fig. 1). Ginger rhizome has many well-known health benefits, including anti-inflammatory and antioxidant properties, as well as the ability to relieve nausea. In addition, ginger is also used to treat digestive problems (Linda & Lovadi, 2013). Besides ginger, the plant with the highest medicinal plant use was kencur (Kaempferia galangal) at 84.6%. People in Indonesia frequently use kencur to treat vomiting, vertigo, cough, and other digestive issues. The greatest percentage of medicinal plant use was also found in turmeric (Curcuma longa), at 73.1%. In medicine, turmeric can be used as a pain reliever for women who are menstruating and increase appetite to treat liver disease (Arum et al., 2012)

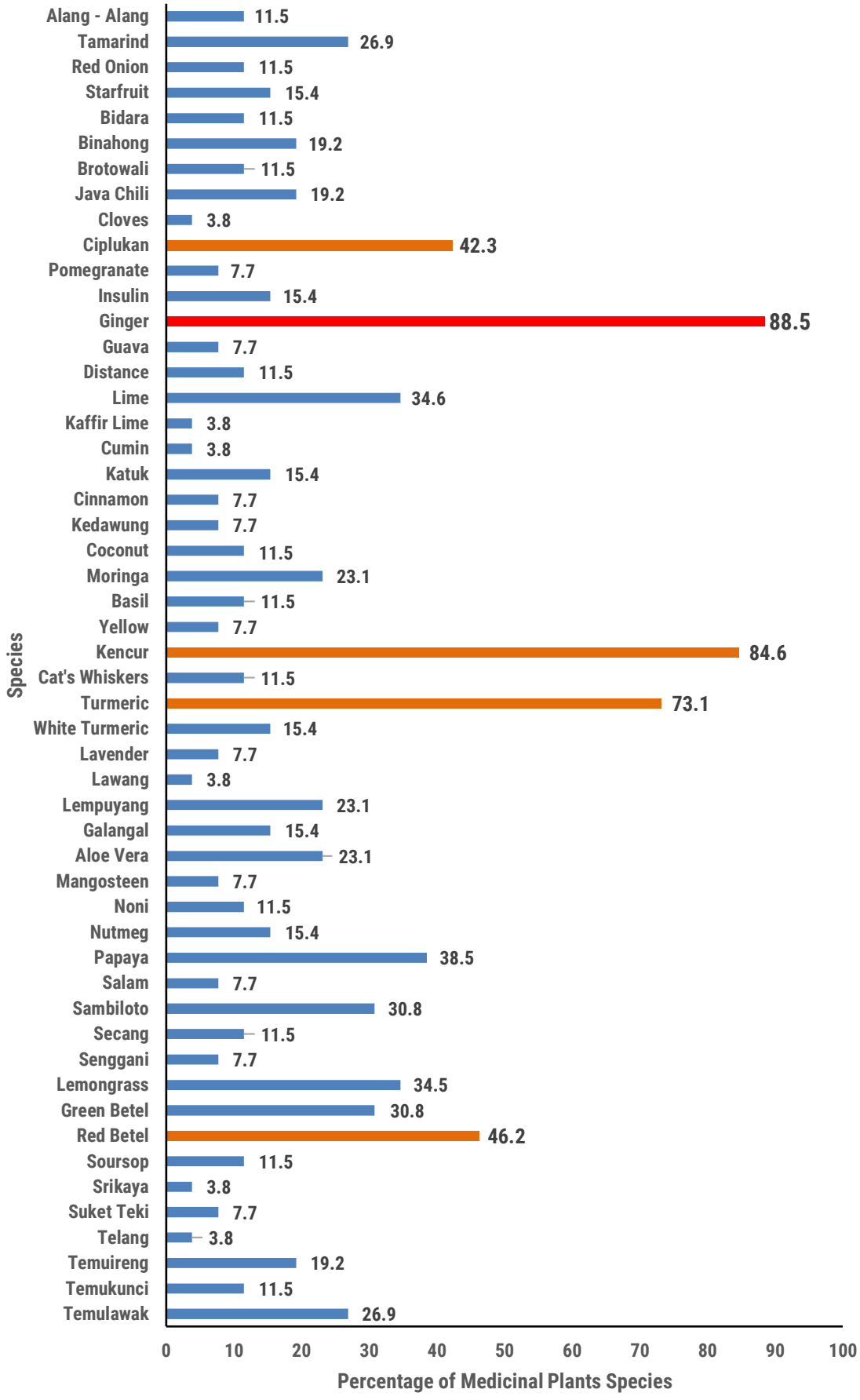

Figure 1. Percentage of medicinal plant types used by the Sedayu village community

Plant parts (organs) used by the Sedayu Village community as medicine were roots, stems, seeds, fruits, flowers, leaves, skin, rhizomes, and tubers. The leaves of 22 varieties of medicinal plants (42.6%) were the most commonly used part (organ) of medicinal plants. Figure 2 depicts the proportion of plant parts that were used as ingredients in medicine.

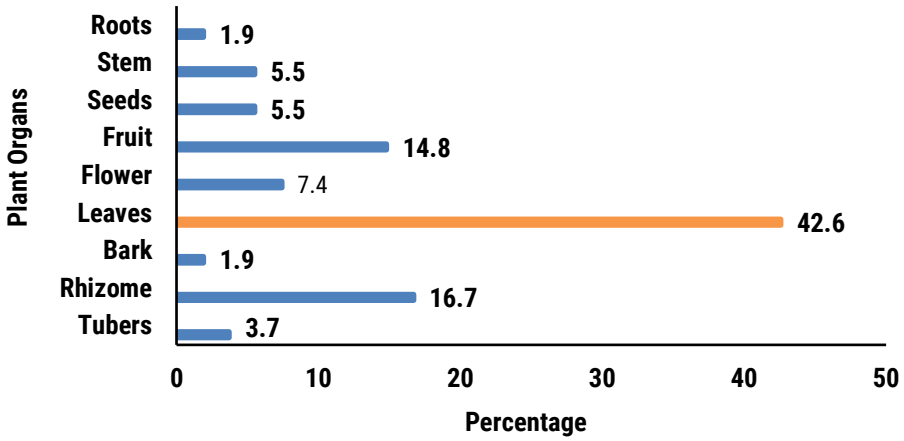

Figure 2. Percentage of Plant Organs Utilized as Medicine

Leaves are more widely used because they are considered easy to obtain and process and have greater efficacy than other plant parts. Furthermore, the most convenient component to extract or gather from the sources is the leaves (Farhatul, 2013). In addition, leaves have a high water content (80%) and contain essential oils, phenols, potassium compounds, and chlorophylls that are capable of curing diseases (Nulfitriani et al., 2013). Similar research results were also found in the Maybrat Tribe community in Sembaro Village, Ayam Maru district, South Sorong Regency, where the leaves are the most widely used part as medicinal plants (Howay et al., 2003). The Dayak Iban community predominantly utilizes leaves from plants for medicinal purposes (Meliki et al., 2013).

The use of other plant parts, such as stems, rhizomes, tubers and roots, are more difficult to collect because they require uprooting and cutting for their utilization, which ecologically affects their amount in nature (Fadilah et al., 2015). This is in accordance with the results of research by Febrianti and Krisnawati (2021), namely, the selection of types and ingredients from medicinal plant parts can be efficacious to reduce or cure certain diseases optimally through processing stages derived from roots, stems, and leaves with the right dosage.

Based on the results of research from 26 respondents with 52 species of medicinal plants, processing can be done in one of five ways: boiled then consumed, brewed then consumed, mashed then applied, squeezed, fermented, or unprocessed. Some varieties of medicinal plants can be processed in multiple ways, such as kencur, which can be consumed directly (without processing), mashed, then applied, or boiled and consumed. If the disease or complaint was distinct, the processing was performed differently. Figure 3 shows the percentage of medicinal plant utilization based on the processing method.

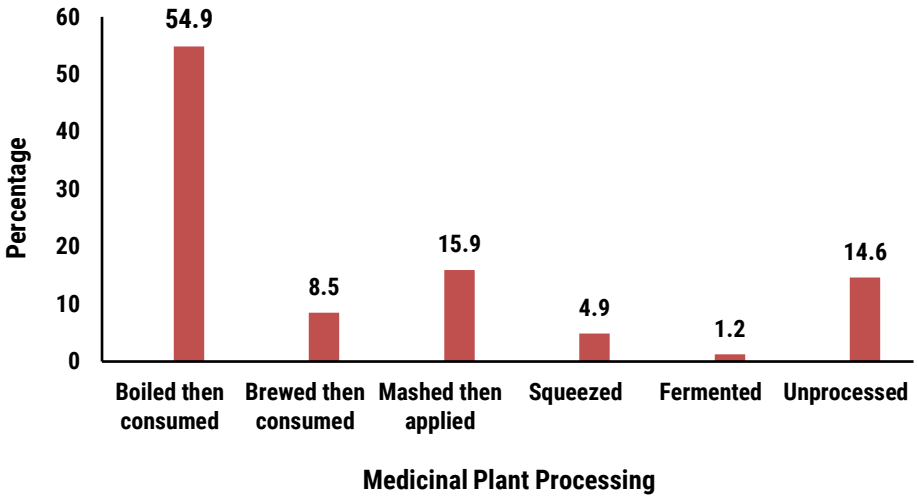

Figure 3. Percentage of medicinal plant processing methods

Figure 3 shows that medicinal plants based on the processing method are mostly boiled and then consumed (54.9%). Similar research results were also found in the Baluran National Park community, where boiling and then consuming plants is the most widely used method (Yaqin, 2020). The Osing Tribe community also shows that boiling and then consuming is the most widely used way of utilizing medicinal plants (Utomo, 2017). The community believes that applying the boiling procedure to medicinal plants eliminates any pathogens present in the plants. Boiling is also believed to be effective because, generally, the diseases treated are types of internal diseases. Another possibility related to the processing method, people who do it more by boiling is because it is easier to take the juice or properties of the plant (Wulandara et al., 2018). The medicinal plant parts used are boiled first and then consumed as an internal disease treatment. Additionally, locals hold the belief that ingesting medicinal plants for internal ailments produces a more rapid effect and reaction than utilizing them topically and even more rapidly than employing other methods to utilize medicinal plants (Efremila et al., 2015).

The advantages of treatment using medicinal plants are that they do not cause side effects compared to using modern drugs or drugs from chemicals. Also, certain herbs are easily available around the yard and easy to make. The process of making traditional medicines is generally very simple, including those that are brewed with water, made into powder and then dissolved in water; some are taken with juice, and the treatment method is generally done orally (drunk) (Azmin et al., 2019). Medicinal plants that are processed in the form of concoctions are generally in the form of jamu, which is formulated with medicinal plants as the basic ingredient and added with other supporting ingredients found in nature (Arisandi & Andriani, 2011).

Due to the benefits of traditional medicine, conservation is one of the means by which medicinal plants must be preserved. Furthermore, knowledge pertaining to the refining and application of medicinal plants must be transmitted orally and through direct practice in order to ensure its eternal sustainability. As awareness of the advantages of medicinal plants grows, it is anticipated that the community will become more engaged in endeavours to preserve these plants, thereby ensuring the continued generation of knowledge as local wisdom.

CONCLUSION

The Sedayu Village community utilizes a total of 52 different species of medicinal plants, with ginger being the most commonly utilized (88.5%). The Sedayu obtain medicinal plants through cultivation, the wild, or the market; leaves are the most utilized plant organs (42.6%). Processing and utilization of medicinal plants by the Sedayu community is mostly done by boiling and then drinking (54.9%). In order to preserve knowledge regarding medicinal plants, the Sedayu community engages in sustainability actions, such as imparting this information to children and local residents through direct or oral practice.

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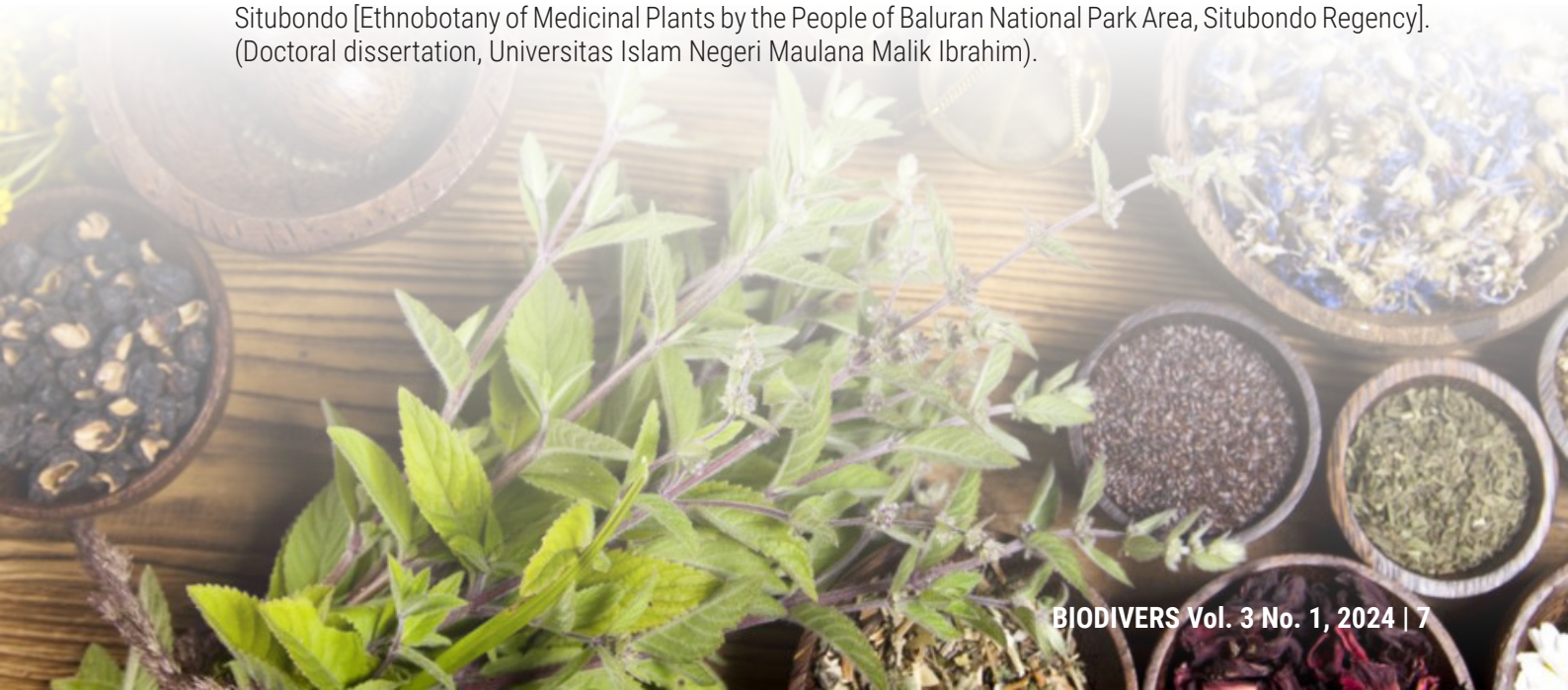

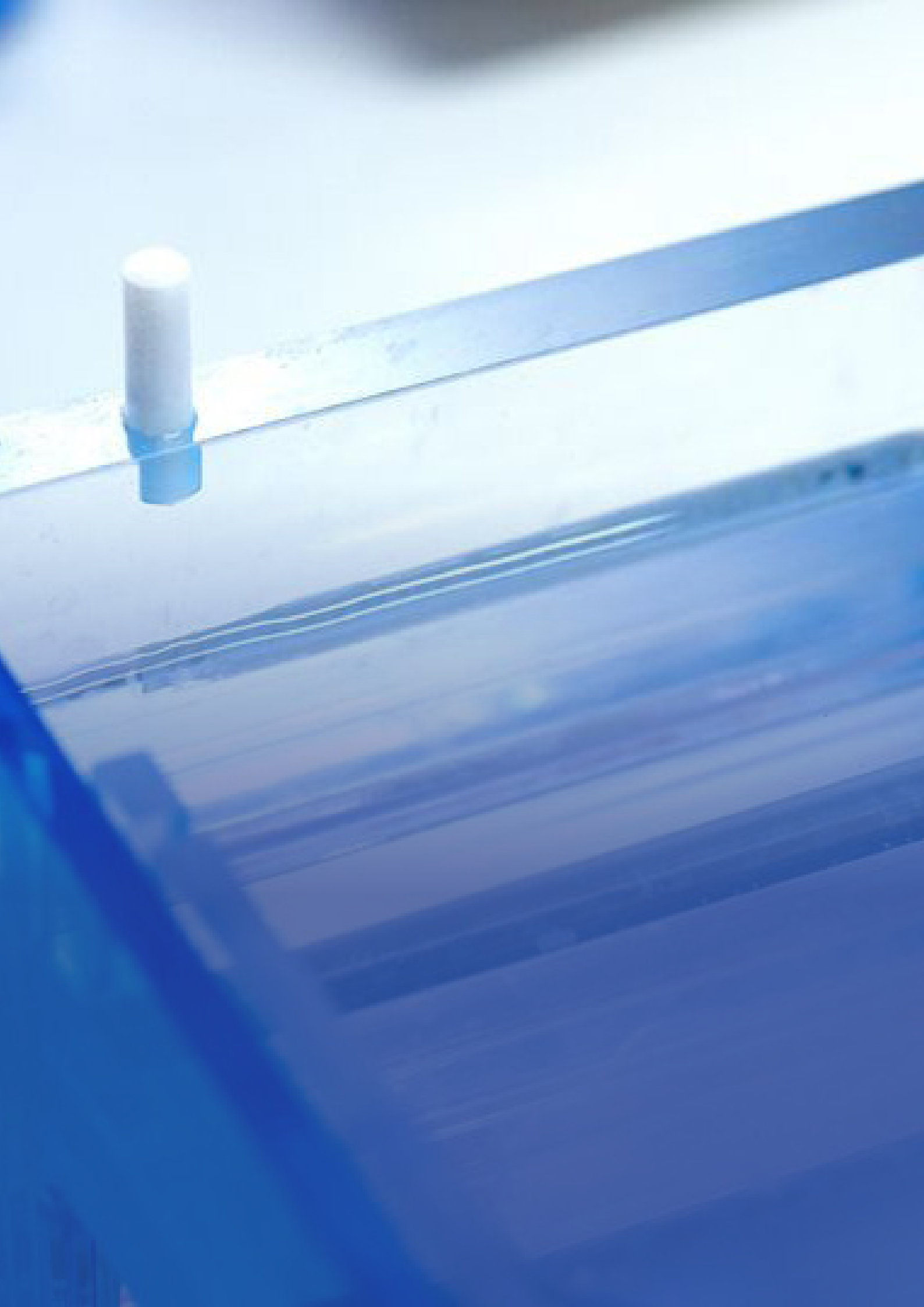

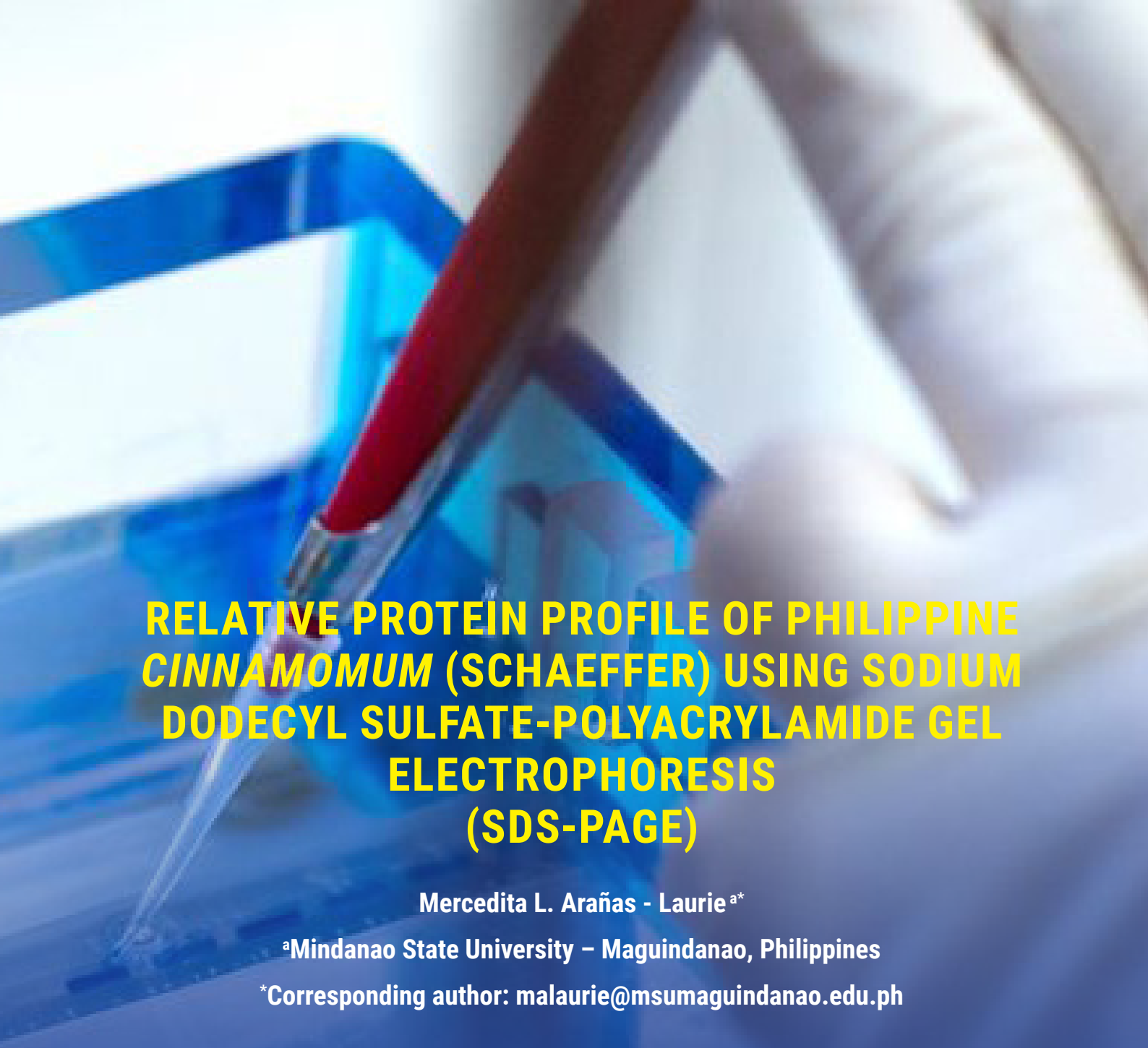

RELATIVE PROTEIN PROFILE OF PHILIPPINE *CINNAMOMUM* (SCHAEFFER) USING SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

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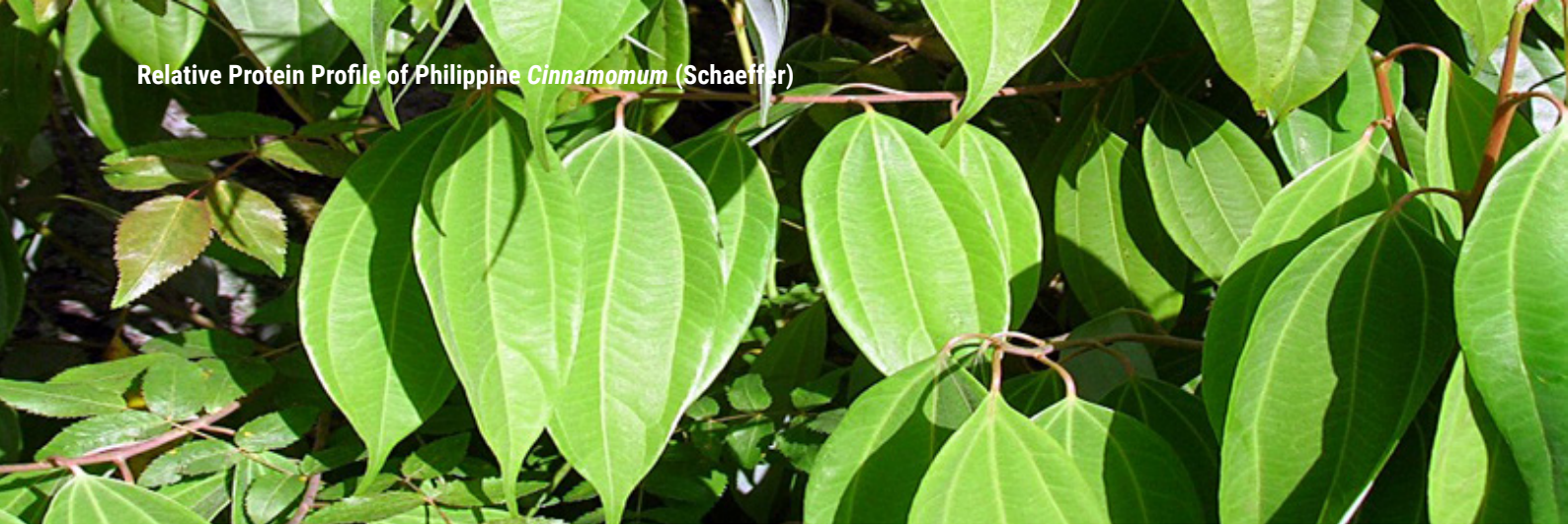
ABSTRACT

Philippine Cinnamomum are trees with aromatic leaves and bark. It has several economic and medicinal uses, but concerns about protein profile are limited. Thus, this study was conducted to determine the relative protein component of the species by Sodium Dodecyl Sulfate- Polyacrylamide Gel Electrophoresis (SDS-PAGE). A total of 27 Cinnamomum species were run in Sodium Dodecyl Sulphate - Polyacrylamide Gel Electrophoresis (SDS-Page) with LSB (Laemli Sample Buffer) and Tris HCI (1:1) as sample buffer. Determining protein profiles is a tool for the systematic classification of the species. Results showed that Philippine Cinnamomum leaf samples have the same component proteins. The SDS-PAGE provided a similar protein banding pattern, although the same bands' intensity varies. Pronounced protein bands were observed corresponding to 50 to 75 kDa and 25 kDa, with additional minor bands indicating a higher protein content. The results in SDS-PAGE showed that the component proteins of Cinnamomum leaves were comparable regardless of geographical source and different stages of growth of the samples. However, the concentration of some component proteins may vary, as indicated by the difference in intensity of protein bands. Sodium dodecyl sulphate Polyacrylamide gel electrophoresis (SDS-PAGE) is a tool in the biochemical method of determining the relative protein profile of Cinnamomum species. It can be used to classify plants up to the species level. Based on the study results, Philippine Cinnamomum has a higher protein content.

Keywords: Philippine Cinnamomum Schaeffer, Relative Protein Profile, SDS-PAGE.



INTRODUCTION

Cinnamomum is a genus of evergreen aromatic trees and shrubs belonging to the laurel family, Lauraceae. The species of Cinnamomum have aromatic oils in their leaves and bark. The genus contains over 300 species distributed in tropical and subtropical regions of North America, Central America, South America, Asia, Oceania, and Australasia. The genus includes many economically important trees (Ravindran et al., 2003).

Cinnamomum has several uses, like spices, flavorings, preservatives, perfume, and soap; some are for liniment and insecticides. Cinnamomum is mostly a folkloric medicinal plant that can cure or relieve several sicknesses and illnesses. It also has anti-fungal (Liu et al., 2001), antibacterial (Kumar et al., 2009), antiviral, and antiseptic properties (Mustapha et al., 2011) and reduces the growth of leukemia and lymphoma cancer cells, colon cancer, and melanoma (Wondrak et al., 2010).

Due to heavy exploitation and deforestation, Cinnamomum plants have been depleted, and some species are placed in the threatened category. Only a few studies have been conducted on protein analysis of Cinnamomum in the Philippines. Relative Protein Profile on Cinnamomum was done through Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for protein analysis to solve these gaps. The results of these investigations can serve as a database for the Relative Protein Profile and be additional data for the correct identification of Philippine Cinnamomum.

The study aims to determine the relative protein profile of Cinnamomum species collected in different mountains in the Philippines. It would provide additional information on the relative protein profile of the Philippine Cinnamomum. Furthermore, the results would be helpful as a basis for policy formulation by the Protected Area Management Board (PAMB) of DENR and the Local Government Unit (LGU) for protecting and conserving the remaining species of Cinnamomum in the Philippines.

METHODOLOGY

This research study includes relative components of the leaf protein of Cinnamomum collected in different places and mountains in the Philippines. The areas of collection of Cinnamomum plants were limited to the following locations: 1) Mindanao: Davao del Sur (Mt. Apo); Socsargen (Mt. Matutum), Davao Oriental (Mt. Hamiguitan); Misamis Occidental (Oroquieta); Misamis Oriental (Naawan); Camiguin Island; Bukidnon (Mt. et al.); Lanao del Sur (MSU-Marawi), 2) Visayas: Negros Oriental (Mt. Guitabon, Mt. Mahayag, and Mt. Kang-Ontol, surroundings of Balinsasayao Twin Lake) and Alcoy, Cebu, 3) Luzon: UPLB Campus and Mt. Makiling.

The laboratory activities were conducted at the Natural Science Research Center (NSRC) Laboratory at Central Mindanao University, Musuan Bukidnon.

Materials used are Silica, dried cinnamon leaf samples, SDS-PAGE Chamber and gel caster, Gel Glass plates and cover, Voltage regulator, Vortex, Centrifuge, Pipettes, Tips and tubes, and Steam bath.

Chemicals used are Deionized water, Methanol, Acetic acid analytical grade, Polyacrylamide, TEMED, APS, Tris HCL, BME, Glycerol, Comassie blue, and Polyacrylamide.

SDS – PAGE BUFFERS:

1.5 M Tris, pH 8.8 (For resolving gel) 250 ml: Cc x N x Mc = grams; Tris, 250 x 1.5 x 0.121 = x; X = grams of Tris needed; X = 45.375 g; Dissolve this to 250 ml with deionize

ed water; Measure pH to 8.8.

1.0 M Tris-HCl, pH 6.8 (For stacking gel); 100 ml: CC x N x Mc = grams Tris; 100 x 1.0 x 0.121 g = x; X = grams of

Tris needed; X = 18.1 g; Dissolve this to 100 ml deionized water. Measure pH to 6.8 with HCl CC1 x N1 = CC2 x N2; 100 x 0.1 = CC2 x 0.121; 10/12 = 0.833 ml ; 833 ul of HCl 99.167 ml deionized water + 833 ul HCl.

1.0 M Tris-HCl, pH 6.8 (For stacking gel); 100 ml: CC x N x Mc = grams Tris; 100 x 1.0 x 0.121 g = x; X = grams of Tris needed; X = 18.1 g; Dissolve this to 100 ml deionized water. Measure pH to 6.8 with HCl CC1 x N1 = CC2 x N2; 100 x 0.1 = CC2 x 0.121; 10/12 = 0.833 ml ; 833 ul of HCl 99.167 ml deionized water + 833 ul HCl.

EXTRACTION BUFFER:

(1:1 LSB with BME and Tris HCl); 7.6 ml (2X Laemli Extraction buffer) + 400 ul BME + 8 ml 50 mM Tris HCl; 2X Laemli Extraction buffer/2 X Sample buffer: (125 mM Tris pH 6.8 + 10% Glycerol + 0.05 % BPB + 0.4 M ME + 2% SDS).

RESOLVING GEL

(12% Polyacrylamide); 30(X) = 12 x 10; X = 4 ml polyacrylamide; 8 ml – good for 2 gels; 2 ml Deionized water; 2.5 ml (1.5 M Tris HCl pH 8.8); 3.3 ml Polyacrylamide (30 %); 100 ul = 0.1 ml 10 % SDS; 4 ul TEMED; (VORTEX – after 20 minutes add); 0.1 ml 10 % APS; (VORTEX and LOAD 3.5 ml per glass plates in a caster); Add 300 ul methanol on top for smooth and even surface; Wait 30 min - 1 hr. for the gel to hardened; Rinse resolving gel with water before adding the stacking gel.

STACKING GEL 4 ml:

2.7 ml Deionized water; 0.5 ml – 1.0 M Tris HCl pH 6.8; 0.67 ml acrylamide solution (Polyacrylamide 30 %); 4 ul TEMED; (Vortex); 40 ul APS (10 %) : add this upon loading; LOAD on top of resolving gel carefully. Install comb, cover with parafilm to avoid dehydration.

0.5 L Staining solution: 200 ml Methanol; 250 ml Deionized water; 50 ml Glacial acetic acid;0.75 g Comassie blue; Mix for at least 4 hrs.; Filter; Store in the dark (cover with aluminum foil 0.5 L Destaining solution: 200 ml Methanol; 250 ml Deionized water; 50 ml Glacial acetic acid.

Flow of Activities:

Field collection, initial identification; preparation of samples/ silica dried; preparation of materials and chemical reagents; laboratory activities; mix reagents for resolving gel, load in glass plates set in gel caster mix reagents for stacking gel, load on top of resolving gel; set glass plates with gel on sds-page chamber; grinding of leaf samples 15-20 mg/tube; add 150 - 200 ul of extraction buffer; vortex; rest for 30 min; waterbath samples in 95Co for two min.; centrifuge at 5 min, 13000 rpm set glass plates with gel on sds-page chamber; Load 15 ul ladder; 15 ul supernatant on wells; RUN in SDS-PAGE Chamber with 1x running buffer at 120 V for 45 min. Remove gel in glass plates carefully, stain, and distain till bands appear clearly, take a picture & laminate the gel. Analyze and interpret the results.

RESULTS AND DISCUSSION

Leaf samples of Cinnamomum from different areas and mountains in the Philippines were collected. A total of 55 samples corresponding to 27 species of collected Cinnamomum were analyzed by Sodium Dodecyl Sulphate – Poly Acrylamide Gel Electrophoresis (SDS-PAGE) with LSB (Laemli Sample Buffer) and Tris HCl (1:1) as sample buffer. Determining protein profile is a tool for the systematic classification of the species.

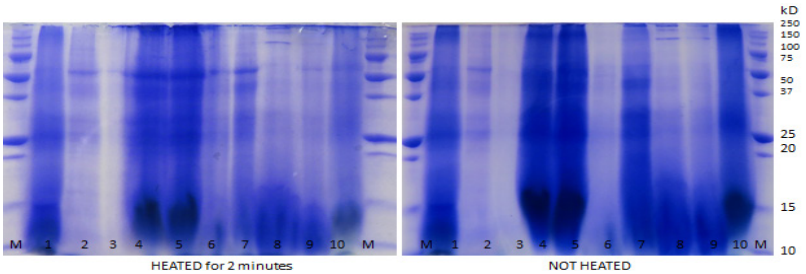


Figure 1. SDS-PAGE (12%) of Cinnamomum cebuense leaf samples at different stages of growth. (M-Molecular weight marker (broad); Lane 1-5 mature age, Lane 6-10 juvenile) Comparisons between heated and non-heated samples.

Figure 1 shows that heated samples at boiling temperature for 2 minutes have more distinct and clear bands of each species' protein component than nonheated ones. It showed that heating gave more resolved protein bands. Heating more likely separates the volatile essential oil in Cinnamomum from its component proteins. Heated treatment exhibited a clear and pronounced band, which was carried throughout the study

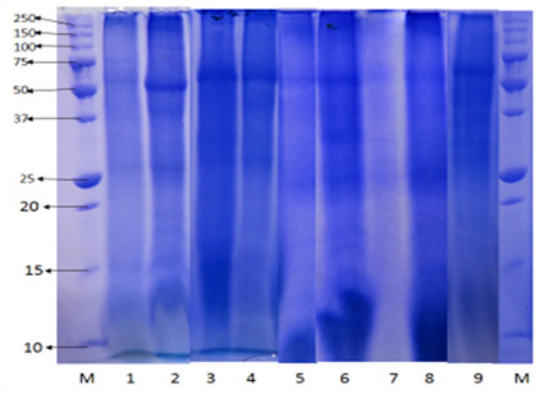


Figure 2. SDS-PAGE (12%) *Cinnamomum* leaf samples from different geographic source. M - Molecular weight marker (broad); 1. *C. sp*.5; 2. *C. mercadoi* (Mis. Occ.); 3. *C. mindanaense* (Bukidnon); 4. *C. oblongum* (Mt. Hamiguitan); 5. *C. loheri*; 6. *C. rupestre*; 7. *C. grifithii*; 8. *C. mercadoi* (Mt. Hamiguitan); 9. *C. sandkuhlii*

Figure 2 shows that the component proteins of all the samples of Cinnamomum are comparable, with distinct bands between 37 kDa and 75 kDa, with minor bands at 12 kDa to 25 kDa. The component proteins are similar, except that some bands are more intense than others.

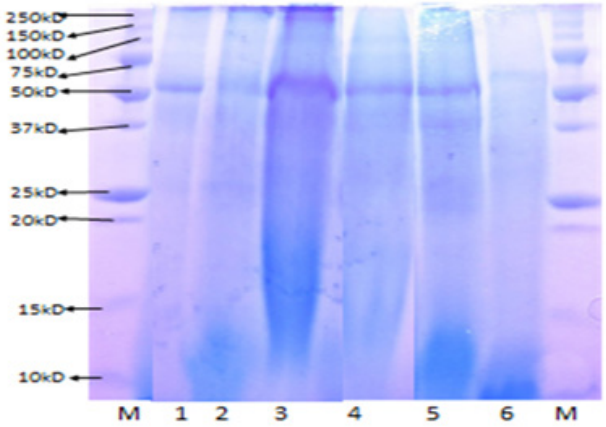


Figure 3. SDS-PAGE (12%) of *Cinnamomum* leaf samples from different geographic source. M - Molecular weight marker (broad); 1. *C. iners* (Bukidnon); 2. *C. mindanaense* (Lanao Sur); 3. *C. burmanni* (S. Cotabato); 4. *C. camphora* (Bukidnon); 5. *C. oblongum* (Bukidnon); 6. *C. sancti-caroli* (Mt. Kiamo, Bukidnon).

Figure 3 shows that the component proteins of all the samples of Cinnamomum are comparable with distinct bands between 50 kDa and 75 kDa, with minor bands at 25 kDa to 37 kDa and 100 kDa to 250 kDa. The component proteins are similar, except that some bands are more intense than others.

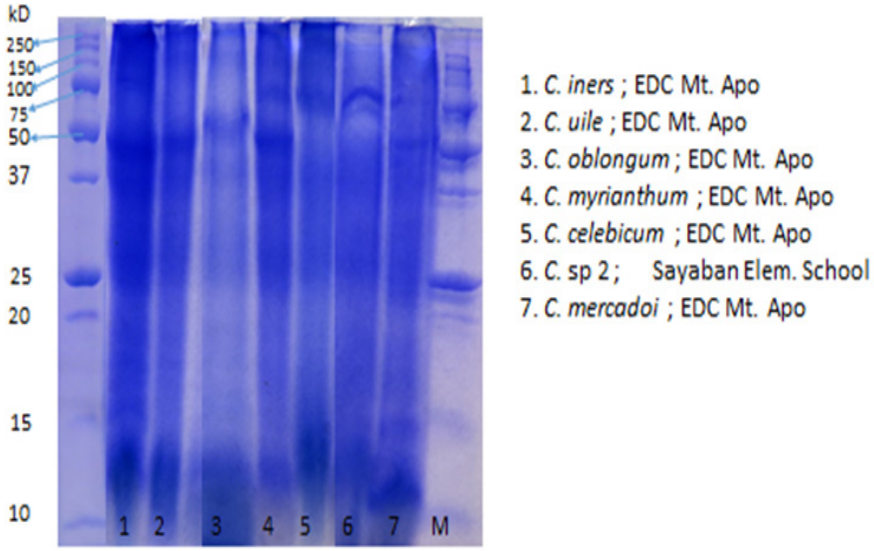


Figure 4. SDS-PAGE (12%) of *Cinnamomum* leaf samples from Mt. Apo. M - molecular weight marker (broad), 1. *C. iners;* 2. *C. utile;* 3. *C. oblongum;* 4. *C. myrianthum;* 5. *C. celebicum; 6. C.* sp.2*;* 7. *C. mercadoi.*

Figure 4 shows that distinct bands of all samples collected at Mt. Apo are at 25 kDa, 50 kDa, and 75 kDa. Minor bands are at 12kDa to 15 kDa and 100kDa to 250 kDa. The component proteins are almost similar, except that some bands are more intense than others.

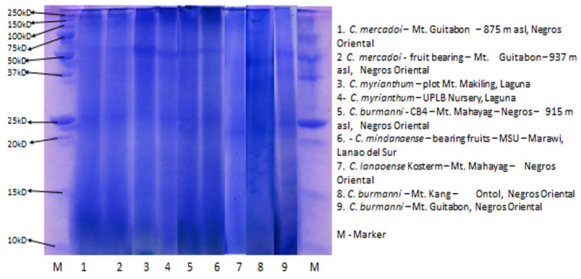


Figure 3. Analysis of Total protein content on SDS-PAGE (12%) showing the variable protein levels of Cinnamomum leaf samples.

Figure 5. SDS-PAGE (12%) of *Cinnamomum* leaf samples from different geographical sources. (M - molecular weight marker (broad), 1. *C.* sp.4 (875 m asl Negros Oriental); 2. *C.* sp.4 (937 m asl Negros Oriental); 3. *C.* sp.7*;* 4. *C.* sp.6*;* 5. *C. ebaloi* (915 m asl Mt. Guitabon Negros); 6. *C. mindanense* (Lanao del Sur); 7. *C. ebaloi* (Mt. Mahayag, Negros); 8. *C. ebaloi* (Mt. Kang-ontol, Negros); *9. C. ebaloi* (Mt. Guitabon, Negros).

Figure 5 shows that the component proteins of all the samples of Cinnamomum are comparable, with distinct bands between 25 kDa and 50 kDa, with minor bands at 12 kDa to 20 kDa and 150 kDa to 250 kDa.

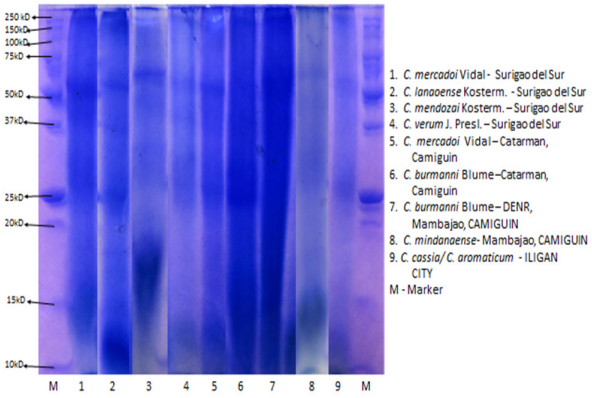


Figure 6**.** SDS-PAGE (12%) of *Cinnamomum* leaf samples from different geographical sources. M - Molecular weight marker (broad); 1. *C. mercadoi* (Surigao); 2. *C. slanaoense;* 3. *C. mendozai;* 4. *C. verum;* 5. *C.* sp.3*; 6. C. burmanni* Catarman Camiguin; 7. *C. burmanni* (Mambajao Camiguin); 8. *C. mindanaense* (Camiguin); 9. *C. cassia*.

Figure 6 shows that the component proteins of all the samples of Cinnamomum are comparable, with distinct bands between 25 kDa and 75 kDa, with minor bands at 12 kDa to 15 kDa and 150 kDa to 250 kDa.

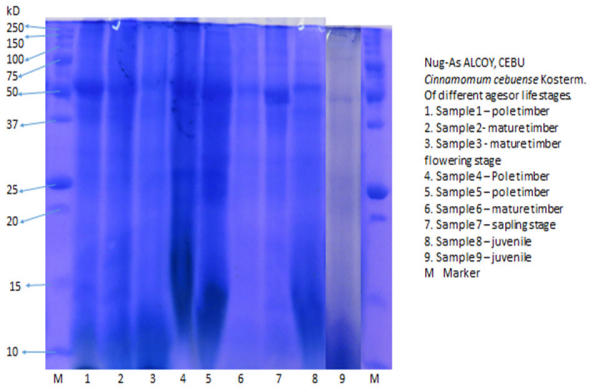


Figure 7**.** SDS-PAGE (12%) of *Cinnamomum cebuense* leaf samples at different stages of growth. (M - molecular weight marker (broad), Lane 1-5 are matured samples, Lane 6-9 are juvenile samples)

Figure 7 shows that the component proteins of all the samples of Cinnamomum are comparable with distinct bands at 37 kDa to 50 kDa, with minor bands at 25 kDa to 37 kDa and 150 kDa to 250 kDa. The component proteins are the same, except that some bands are more intense than others. The results in SDS-PAGE show that the component proteins of Cinnamomum leaves are comparable regardless of geographical source and different stages of growth of the samples. However, the concentration of some component proteins may vary, as indicated by the difference in intensity of protein bands.

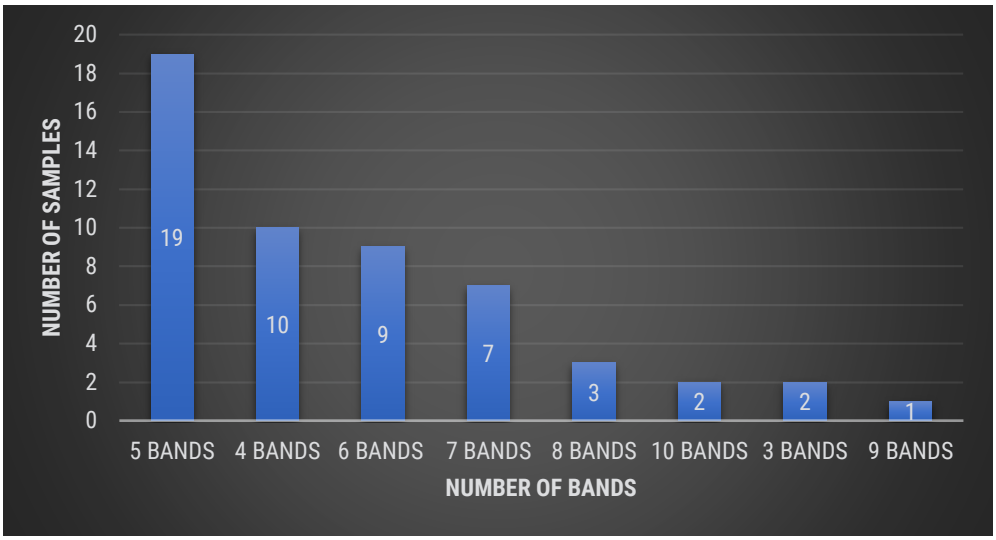


Figure 8. The number of protein bands intensity

Figure 8 shows that 5 bands has the highest number of samples (19), followed by 4 bands with 10 samples, 6 bands with 9 samples, 7 bands with 7 samples, 8 bands with 3 samples, 10 and 3 bands with 2 samples each, and 9 bands has only 1 sample.

Discussion

Sodium dodecyl sulphate polyacrylamide gel electrophores (SDS-PAGE) are the most widely used technique to separate proteins from complicated samples of the mixture. It plays a key role in molecular biology and a wide range of subfields of biological research. In an electric field, when proteins are negatively charged with SDS, proteins migrate toward the negative anode. In SDS-PAGE, the detergent SDS and a heating step determine that the electrophoretic mobility of a single kind of protein is only affected by its molecular weight in the porous acrylamide gel (www.assay-protocol.com).

Determining relative component proteins is a tool for systematically classifying the species. Pragati et al. (2013) also investigated the phylogenetic relationship of nine Ipomoea species, seed proteins were analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was used by Kamel and Loufy (2001) in the taxonomy of the Lauraceae. SDS-PAGE of seed protein criteria in the Lauraceae was confirmed.

This study took several times to extract protein due to gelatinous substances from the leaves, and some heating time was optimized. The best result was from partially dried leaf samples and a steam bath of the extracted samples at 95Co. If the bands are distinct, the protein quantity of 50kD has 750 ng, 20kD, and 100 kD has 150 ng each. These values should be used only to determine a rough approximation of the amount of a protein of interest. Almost all species of Cinnamomum have bands of 20 kD, 100 kD, and 50 kD, which means that Cinnamomum in the Philippines has high protein content.

CONCLUSIONS

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is a biochemical tool for determining the relative protein profile of Cinnamomum species. All species of Cinnamomum have similar protein content but differ only in intensity. Based on the result of the study, Philippine Cinnamomum has a higher protein content.

ACKNOWLEDGMENT

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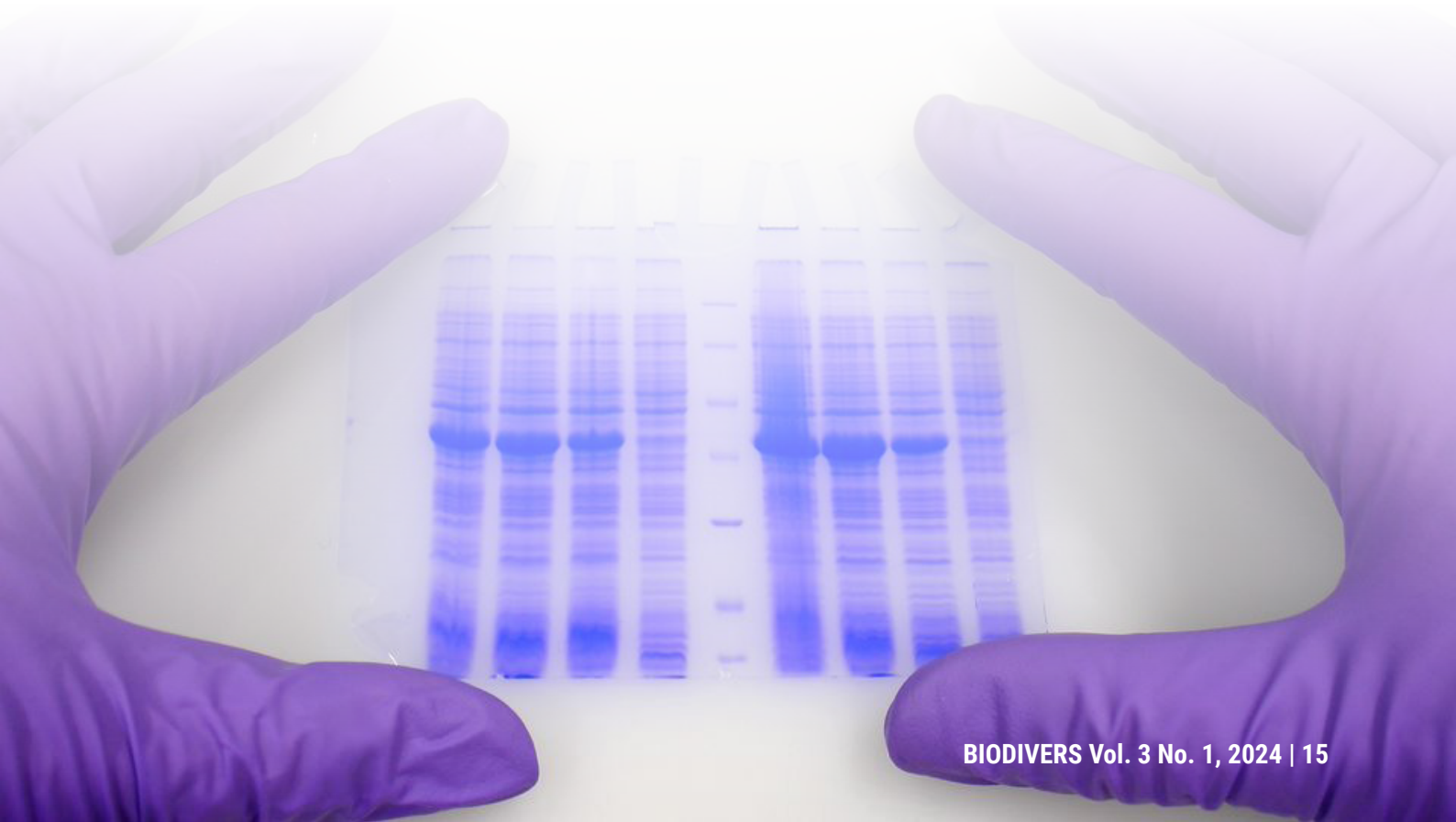

THE EFFECT OF POLYSTYRENE PAPER STYROFOAM FEED ON SURVIVORSHIP AND GROWTH OF *Tenebrio molitor* L.

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ABSTRACT

Polystyrene Paper Styrofoam (PSP) styrofoam waste is one type of plastic waste that is difficult to degrade. However, recent research reported that *Tenebrio molitor* L. can degrade PSP Styrofoam. The degradation depends on the composition of PSP styrofoam as a part of feeding. This research aimed to get the composition of good feed to increase the degradation rate of the PSP styrofoam waste, but it still had a good effect on the survivorship and growth of *T. molitor* L. larvae. This research was an experimental laboratory and was designed using a control and seven treatments with 3 replications. The parameters observed were survivorship, growth in body length, biomass, and waste degradation rate. The data obtained was analyzed using ANOVA and Duncan test. The results showed that differences in PSP styrofoam waste feed had a significant effect on survivorship ($p=0.00$), on the growth of body length ($p=0.002$) and biomass ($p=0.00$), and waste degradation rate ($p=0.02$). The research revealed that the lower the composition of PSP styrofoam and the higher the composition of the concentrate combination, the higher the survivorship, growth, and waste degradation rate. The result shows that 1 kg *T molitor* L. larvae can degrade 0.25 kg of PSP styrofoam waste for 60 days. The findings can be used to solve the problem of styrofoam pollution.

Keywords : degradation, larvae, styrofoam, *Tenebrio molitor* L.

INTRODUCTION

Styrofoam is a product made from raw polystyrene and is one of the plastic types. The number of styrofoam waste products increases as the human population increases. Polystyrene is divided into three types: Expanded Polystyrene (EPS), Extruded Polystyrene (XPS), and Polystyrene Paper (PSP). Generally, PSP styrofoam is used as a food tray because of the advantages of its flexible material, can maintain the freshness and temperature of the ingredients in it, and is practical, and cheap (Swamilaksita et al., 2018). PSP styrofoam also has a weakness, its nature is difficult to degrade and takes about 500 years to fully decompose. A once-designed PSP styrofoam design increases the waste that can pollute the soil, water, and air (Bilal et al., 2021). These things cause PSP styrofoam waste which must be solved. However, based on recent research, there are some biological agents that have the potential to degrade PSP Styrofoam, such as bacterial (Ho et al., 2018) and fungus (Ho et al., 2018; Onodera et al., 2001).

Tenebrio molitor L. larvae is an insect (Coleoptera) that reported has high potential to degradate the PSP styrofoam waste (Peng et al., 2020; Tsochatzis et al., 2021) and others type of plastic (Peng et al., 2020; Zhang et al., 2022) . In society, the potential for these larvae is still not fully understood and properly harnessed. T molitor larvae are known as mealworm which has characteristic golden-yellow and holometaboles (Hong et al., 2020). T molitor larvae reported has capability to degraded PSP styrofoam due to the larvae's intestine containing bacterial symbions which produce extraculicular enzyme to degrade the PSP styrofoam fragment to small molecule (Fabreag & Familara, 2019). The aspect of nutritions is main factor to optimize microbial activity of the larvae (Palmer et al., 2022). However, there is no data regarding feeding composition using PSP styrofoam waste on survivorship and growth of the larvae, and capability the larvae to degraded the waste. Degradation of PSP styrofoam will be faster due to availability of proper nutrition in feed which stimulate to optimize microbial activity in the produce degradation enzymes (Fabreag & Familara, 2019). Feeding composition using concentrate design as a source for metabolic energy (Ferrari et al., 2019) and fresh chayote as a source of minerals and water (Sakung et al., 2020; Subchan et al., 2022).

This research is to investigate the effect of different composition of polystyrene paper (PSP) styrofoam waste feed on survivorship, growth, and waste degradation rate of the T molitor larvae. By investigating the impact of styrofoam feed on the survivorship and growth of *Tenebrio molitor* larvae, the study sheds light on the feasibility of using these larvae as a means of degrading styrofoam waste. This research is significant as it offers insights into the efficacy of utilizing *Tenebrio molitor* larvae as a bio-degradation for styrofoam waste, potentially leading to the development of eco-friendly waste management strategies. Furthermore, by addressing the environmental concerns associated with styrofoam pollution, the study contributes to ongoing efforts to reduce plastic waste and promote sustainability in waste management practices.

MATERIALS AND METHODS

This research was conducted from February to April, 2021 at the Center Development Advance Sciences and technology (CDAST) University of Jember. This research uses Completely Randomized Design (CRD) consisting of control and seven treatments with 3 replications. The feed composition used included Control (K): (90% concentrate + 10% chayote), P1 (100% PSP styrofoam), P2 (90% PSP styrofoam + 10% chayote), P3 (80% PSP styrofoam + 10% concentrate + 10% chayote), P4 (70% PSP styrofoam + 20% concentrate + 10% chayote), P5 (60% PSP styrofoam + 30% concentrate + 10% chayote), P6 (50% PSP styrofoam + 40% concentrate + 10% chayote), P7 (45% PSP styrofoam + 45% concentrate + 10% chayote). Feeding quantity (grams) adjusted by 0.08 x latest total wet biomass x 7 days x feed percentage (concentrate/ PSP styrofoam/ chayote).

Preparation phase

Tools and materials that were used including container (23×9.5×6 cm^3) for T molitor larvae used during the research. The analytic balance are required to count biomass and PSP styrofoam waste mass. An electronic digital caliper to measure the larvae body length. The dry styrofoam PSP waste taken from the final processing place must be soaked in a mixture of water and charcoal chaff (1:1) for the previous three days (served as adsorben) and then cut into pieces.

Tenebrio molitor L. larvae were purchased from a farmer in Jember. *Tenebrio molitor* L. larvae were reared in a plastic tray (37×30×11 cm^3) fed with concentrate so that at the time of sorting it has reached the desired length and wet biomass of 2-2.6 cm and 0.07-0.14 grams. The final test research pan used were 24 pieces, with 50 larvae in each pan. Aliquot pans as many as 8 pieces, with 125 larvae in each pan. The total larvae used amounted to 2400 individuals.

THE RESEARCH STAGE

Parameters observed were survivorship (%), length growth (cm/week) as well as biomass (gram/week), and degradation rate (gram/week). Measurement once a week for 6 weeks. The survivorship is shown from the quantity of the larvae and pupas in the final weeks of research. The growth is indicated by the increase in the body length or biomass between the last week and first. The degree of degradation is measured based on the mass of the beginning and the end of the styrofoam PSP styrofoam in each pan per week.

Preparation of the aliquot methods

The process of obtaining dried biomass by baking 25 larvae/week from 8 aliquot pans in oven at 65 (±0.5)˚C. Once it is an oven, it is measured totally dry biomass and divided by 25. The wet biomass in 8 pans is compared to aliquot, compared with an estimated biomass of biomass that leads to a regression equation. Each treatment has a different allometric regression equation. This regression equation, used as a formula for finding dry biomass in a final test (24 pans). Allometric equations for measuring dry biomass are (Daba & Soromessa, 2019):

$$y = a + bX$$

Note:

y : dry biomass

a : constant

b : coefficient

X : wet biomass

Data analysis

The data obtained from the research was then analyzed by Analysis of Variance (Anova) and Duncan test using the SPSS 25 program.

RESULTS AND DISCUSSION

After carrying out 1 control and 7 treatments, the results of the comparison of differences in mass, survivorship and level of degradation are obtained as shown in Figure 1.

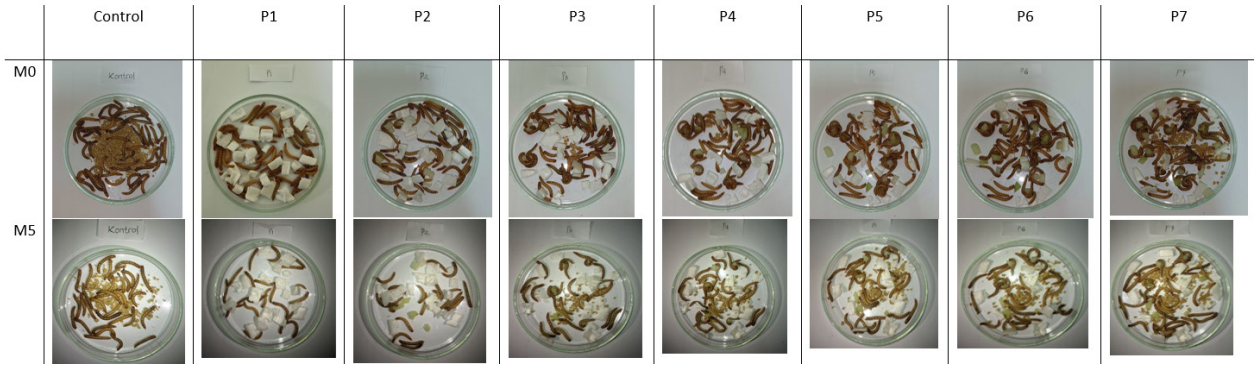

Figure 1 differences in mass, survivorship and level of degradation.

The results of the comparison of differences in length of *Tenebrio molitor* as shown in Figure 2.

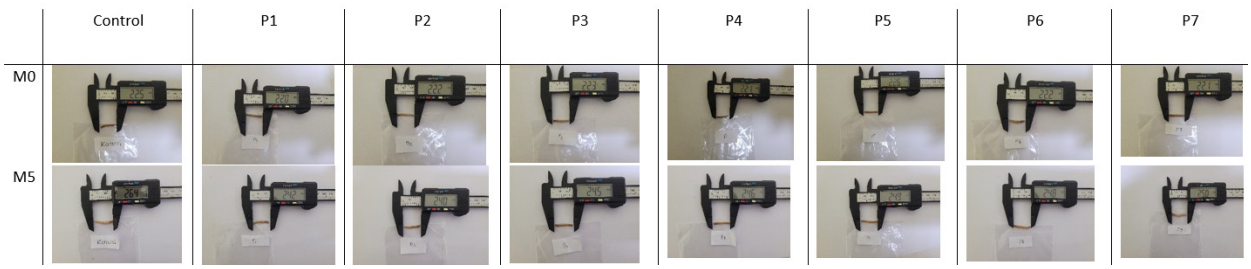

Figure 2 differences in length of *Tenebrio molitor* at week 0 (M0) and week 5th (M5) for all treatments.

T molitor larvae's survivorship

Based on data analyse using Anova, it shows that the difference composition of PSP styrofoam waste feed was significant effect (F = 1787; df = 7; p = 0.000) on the survivorship (%) of the T molitor larvae. Table 1 shows that Control and P7 had the greatest survivorship (61.33% and 57.33% respectively). P7 shows no difference significantly with P5 (54.67%) and P6 (55.33%). P1 (42%) shows the smallest survivorship and no difference significantly with P2 (44.67%). P2 shows no difference significantly with P3 (48%) and P4 (48.67%).

Table 1. Differences the *T. molitor* survivorship (%) among treatments

Treatments	Mean ± SD (*)
K	61.33 ± 3.06 ^{d}
P1	42.00 ± 2.00 ^{a}
P2	44.67 ± 2.31 ^{ab}
P3	48.00 ± 2.00 ^{b}
P4	48.67 ± 2.31 ^{b}
P5	54.67 ± 4.16 ^{c}
P6	55.33 ± 3.06 ^{c}
P7	57.33 ± 6.78 ^{cd}

*) Note: Note: mean and standard deviation (SD) followed by the same alphabet indicate a different result based on Duncan test 5%.

The research revealed that higher the composition of PSP styrofoam waste feed, the lower composition of concentrate which effected on decreasing the survivorship. The low percentage of survivorship due to the composition of the feed given imprecise and lacking in several essential nutrient substances. The concentrate contains carbohydrate that will be converted into glucose molecules. The glucose being a source of energy will be absorbed by intestine larvae and used for metabolism process and mobility. If the amount of glucose exceeds the body's needs, it will be stored in the form of glycogen or fat as energy reserves. The research findings indicate that an increase in the proportion of PSP styrofoam waste feed leads to a decrease in the concentration of concentrate. To optimize degradation while maintaining the well-being of *Tenebrio molitor*, it may be beneficial to carefully balance the feed composition, ensuring that essential nutrients are provided in sufficient quantities. By adjusting the feed composition to provide adequate nutrition, we can enhance degradation rates without compromising the health and survival of the larvae. The best feed composition identified was P7 (45% PSP styrofoam + 45% concentrate + 10% chayote).

Growth of body length

Based on the results of the Anova test, it shows that the difference in the composition of PSP styrofoam waste feed had a significant effect (F = 5.5; df =7; p = 0.002) on the growth of body length (cm/week) T molitor larvae.

Table 2. The differences growth of body length (cm/week) of T molitor larvae among treatments

Treatments	Mean ± SD (*)
K	0.381 ± 0.108[b]
P1	0.026 ± 0.042[a]
P2	0.097 ± 0.133[a]
P3	0.003 ± 0.002[a]
P4	0.050 ± 0.167[a]
P5	0.034 ± 0.072[a]
P6	0.004 ± 0.088[a]
P7	0.180 ± 0.015[a]

*) Note: Note: mean and standard deviation (SD) followed by the same alphabet indicate a different result based on Duncan test 5%.

Based on Table 2, shows that the various composition treatments of the PSP styrofoam waste feed have different results. Control had the greatest growth of body length (0.381 cm/week), while the P3 (0.003 cm/week) showed the smallest growth of body length and no different significantly with P1 (0.026 cm/week), P2 (0.097 cm/week), P4 (0.05 cm/week), P5 (0.034 cm/week), P6 (0.004 cm/week), P6 (0.004 cm/week), P6 (0.004 cm/week), P6 (0.004 cm/week), P6 (0.004 cm/week), and P7 (0.18 cm/week).

Based on the research, it was found that the different treatments of PSP styrofoam waste feed had a significant effect on growth of body length (cm/week) and biomass (grams/week) of T molitor larvae. A good feed composition was contained a high percentage of concentrate. This is because the concentrate contains essential amino acids such as lysine which plays a major role in protein synthesis and methionine which plays a role in tissue growth (Q. Yang et al., 2020) and nitrogen balance (Ferrari et al., 2019). Thus, a deficiency in the percentage of concentrate will cause a deficiency of essential amino acids which can lead to a decline in the protein synthesis process and small growth.

Growth of biomass

Based on the results of the Anova test, it shows that the difference in the composition of PSP styrofoam waste feed had a significant effect (F =27.895; df =7; p = 0.00) on the growth of biomass T molitor larvae.

Table 3. The differences growth of biomass (grams/week) of T molitor larvae among treatments.

Treatments	Mean ± SD (*)
K	0.0155 ± 0.0026[c]
P1	0.0013 ± 0.0004[a]
P2	0.0026 ± 0.0007[a]
P3	0.0043 ± 0.0006[a]
P4	0.0016 ± 0.0008[a]
P5	0.0013 ± 0.0004[a]
P6	0.0042 ± 0.0014[a]
P7	0.0088 ± 0.0032[b]

*) Note: mean and standard deviation (SD) followed by the same alphabet indicate a different result based on Duncan test 5%.

Based on Table 3, shows that the various composition treatments of the PSP styrofoam waste feed have different results. Control had the greatest growth of biomass (0.0155 grams/week), while P1 (0.0013 grams/week) and P5 (0.0013 grams/week) showed the smallest growth of biomass and no different significantly with P2 (0.0026 grams/week), P3 (0.0043 grams/week), P4 (0.0016 grams/week), and P6 (0.0042 grams/week), but different significantly with P7 (0.0088 grams/week).

Giving a high PSP styrofoam feed composition and a low concentrate feed composition gives a consequence a fewer available and digestible amino acids. In addition, the results of the depolymerization of PSP styrofoam carbon will be converted into 47.7% CO_2, 49.2% feces, and only 0.5% assimilated as biomass (Matyja et al., 2020). Matter this shows that PSP styrofoam as feed only provides a little energy but can still make T molitor larvae grow

in length (cm/week) and biomass (grams/week). T molitor larvae fed with high composition PSP styrofoam did not form many folds in its body structure (S. S. Yang et al., 2018). These are the reasons why Control (90% concentrate + 10% chayote) and P7 (45% PSP styrofoam + 45% concentrate + 10% chayote) continued to show good growth. P1 to P6 show different growth with ideal conditions. This can occur due to internal factors such as the digestibility of each individual.

Degradation rate of the PSP styrofoam waste

Based on the results of Anova test, it shows that the difference in the composition of PSP styrofoam waste feed had a significant effect (F = 2.33; df =7; p = 0.02) on degradation rate (grams/week).

Table 4. The differences degradation rate of PSP styrofoam waste by the T molitor larvae among treatments

Treatments	Mean ± SD (*)
K	0.00 ± 0.00 [a]
P1	0.09 ± 0.12 [ab]
P2	0.18 ± 0.23 [ab]
P3	0.25 ± 0.36 [b]
P4	0.25 ± 0.36 [b]
P5	0.26 ± 0.37 [b]
P6	0.29 ± 0.38 [b]
P7	0.33 ± 0.38 [ab]

*) Note: mean and standard deviation (SD) followed by the same alphabet indicate a different result based on Duncan test 5%.

Based on Table 4, shows that the various composition treatments of the PSP styrofoam waste feed had different results in the degradation rate (grams/week) of PSP styrofoam. P7 (0.33 grams/week) shows no different significantly with P1 (0.09 grams/week), P2 (0.18 grams/week), P3 (0.25 grams/week), P4 (0.25 grams/week), P5 (0.26 grams/week), P6 (0.29 grams/week), and at the Control treatment there is no degradation. Based on the research, it was found that the different feed treatments had a significant effect on the degradation rate (grams/week) of PSP styrofoam waste. The T molitor larvae can digest polystyrene which is the raw material for PSP styrofoam because their intestines contain Exiguobacterrium sp. YT2 strain that secretes extracurricular enzymes. This enzyme can catalyze the depolymerization process of styrofoam fragments and also reducing the size of the molecules (Matyja et al., 2020). The degradation process of PSP styrofoam that occurs in the intestines of T molitor larvae causes changes in the chemical structure of polystyrene from hydrophobic to hydrophilic so that it can be digested. Enzymatic activity is also carried out by gut microbes. Urbanek, et al. (2020) found that there were seven classes, Gammaproteobacteria, Bacilli, Clostridia, Acidobacteria, Actinobacteria, Alphaproteobacteria and Flavobacteria, which were the most abundant microbiome groups in the intestine of *T. molitor*, while the dominant genera were Enterobacter, Lactococcus and Enterococcus. While Brandon et al. (2021) discovered eight unique gut microorganisms associated with PS biodegradation including Citrobacter freundii, Serratia marcescens, and Klebsiella aerogenes.

The PS degradation process occurs because *T. molitor* as the host and the microbiome in its gut collaborate to create an environment conducive to the plastic biodegradation process. This was proven by Brandon, et al. (2021) that *T. molitor* secretes emulsifying factors (30–100 kDa) that mediate the bioavailability of plastics. The gut microbiome of *T. molitor* secretes factors (<30 kDa) that enhance respiration on polystyrene (PS).

Based on the results that have been obtained, it shows that the lower the composition of PSP styrofoam feeding, the higher the concentrate causes the waste degradation rate (grams/week) to be higher. In addition, the higher nutrient can optimize microbial activity in producing extracurricular enzymes (Sari et al., 2019) so that the degradation rate of PSP styrofoam waste is faster. Based on the difference in feed composition of the eight treatments, P1 (100% PSP styrofoam) showed the lowest degradation rate is 0.09 grams/week, while K (0% PSP styrofoam) no degradation process occurs. P7 (45% PSP styrofoam + 45% concentrate + 10% chayote) showed the highest degradation rate of styrofoam PSP waste is 0.33 grams/week or 0.047 grams/day. P7 used 50 larvae so each individual can degraded 0.00094 grams/day/individual. It means, 4.432 individuals or roughly 1 kg T molitor larvae can degrade 0.25 kg of PSP styrofoam waste for 60 days. This composition study is important because if *T. molitor* larvae only uses styrofoam (100% PSP), it shows changes in larval development caused by a decrease in insufficient food supply (Matyja, et al., 2020).

Tenebrio molitor still has significant potential to be applied in outdoor waste management, albeit requiring further research and careful approaches. Although typically found in its natural habitats, some studies support the adaptability of these beetles to new environments, including outdoor settings. Recent studies also suggest that *Tenebrio molitor* may have the potential to degrade non-organic waste in outdoor environments, such as styrofoam. Based on research conducted by Ribeiro et al., (2018), although *Tenebrio molitor* depends on abiotic conditions throughout its life, it turns out that *Tenebrio molitor* has a high adaptability. *Tenebrio molitor* is able to live in extreme dry conditions and can survive by eating substances with low water content. With the appropriate technological developments and interdisciplinary cooperation, the implementation of *Tenebrio molitor* in outdoor waste management can be an effective and sustainable alternative. Additionally, it necessitates environmental regulation and the development of monitoring and control techniques.

Optimizing the feed composition to enhance the degradation rate of *Tenebrio molitor* involves several key steps. Firstly, initial research on larval nutritional requirements is conducted to understand the food requirements necessary for optimal growth and activity. Subsequently, various feed ingredients that potentially meet these needs are systematically evaluated, including various types of protein sources, carbohydrates, fats, and additional nutrients. Following this, a series of feeding trials is conducted to test different combinations and proportions of feed ingredients in effectively promoting larval growth and activity. Throughout the trials, parameters such as feed consumption rate, larval growth, and waste degradation efficiency are continuously monitored and evaluated. By employing this approach, the feed composition can be adjusted and optimized to achieve the best outcomes in terms of waste degradation rate by *T. molitor*. This method enables the development of more effective and efficient feed formulations, thereby enhancing the potential application of larvae in organic waste management overall.

The different treatment of PSP styrofoam waste feed had a significant effect on the survivorship (%) of T molitor larvae. The addition of high feed supplements could improve the larval body condition. The nutrient needs of the larvae will also be met so as to increase the probability of survivorship (%) of T molitor larvae. On the other hand, feeding the PSP styrofoam waste with a high composition to the larvae, will not be able to finished their life cycle (Yang et al., 2018). This condition can happen because the lack of nutrients in the feed will cause T molitor larvae to not grow to the standard threshold and cause death (Wu et al., 2018). This research lasted for 42 days or 6 weeks, while T molitor larvae could only survive well for one month trial with styrofoam feed (Yang et al., 2015). These are some of the situations that caused T molitor's survivorship to become low.

CONCLUSION

The composition of PSP styrofoam, consentrate, and chayote affected highly significant on survivorship ($p<0.001$) and growth ($p<0.01$) of T molitor and PSP styrofoam degradation rate ($p=0.02$). The treatment which contains lower the composition of the PSP styrofoam, the higher the composition concentrate which contains high nutrients, resulting higher in survivorship, the body length growth, biomass growth, and degradation rate. The good composition of feed after control (P0) is P7 (45% PSP styrofoam + 45% concentrate + 10% chayote). T molitor in the treatment of P7 showed a survival rate of 57.33 (6.78) % and dry biomass growth of 0.0088 (0.0032) grams/week. The result shows that 1 kg T molitor L. larvae can degrade 0.25 kg of PSP styrofoam waste for 60 days. Therefore, these findings indicate substantial potential for promoting sustainable waste management practices. reducing the negative impacts of styrofoam pollution on the environment. The feed composition P7 is considered the optimal blend for mitigating the negative environmental impact of styrofoam pollution, as it exhibits a high degradation rate while also maintaining a balanced nutritional profile for the larvae and preserving their viability. The efficient degradation process by *T. molitor* not only reduces the amount of styrofoam waste polluting the environment but also produces residues that are more easily biodegradable. This can alleviate pressure on landfills and mitigate the risk of environmental contamination. Furthermore, the use of *T. molitor* as a waste degradation agent also holds promise for larger-scale applications. Implementing *T. molitor*-based waste management systems in places such as waste treatment facilities or areas with high levels of styrofoam pollution could yield significant impacts in reducing the amount of waste ending up in the environment.

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Bamboo Utilization on Peleng Island, Indonesia: Unveiling Local Knowledge and Diverse Applications

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ABSTRACT

Bamboo is a useful plant with significant cultural and economic importance, particularly in small Indonesian islands, such as Peleng Island in Central Sulawesi. This paper explores bamboo utilization on Peleng Island, aiming to broaden our understanding of bamboo use on small Indonesian islands and shed light on local wisdom in bamboo utilization. The research was conducted on Peleng Island in 2019. Information on bamboo utilization was gathered through in-depth interviews with residents. Bamboo materials were collected and sent to the Herbarium Bogoriense for identification. The data were analyzed descriptively. There are six bamboo species utilized on Peleng Island, namely Bambusa vulgaris, Dendrocalamus asper, Gigantochloa atter, Neololeba atra, Schizostachyum brachycladum, and Schizostachyum lima. Bamboo on Peleng Island is used for building materials (roofs, pillars, and wall weaving), cooking traditional foods like nasi jaha and bambu suman, and for various other uses (ornamental plants, water containers, stairs, poles for picking fruits/cloves, pest traps, and fish trap).

Keywords: Banggai Kepulauan, bamboo, local wisdom, Peleng Island

INTRODUCTION

Bamboo worldwide is estimated to have 123 genera (Ahmad et al., 2021), with between 1439 (Widjaja et al., 2014) and 1662 species (Canava et al., 2017). In Indonesia, 24 genera and 175 bamboo species have been reported (Damayanto & Ferirenta, 2021), with 105 species reportedly endemic to the area (Widjaja, 2019). New species recently published have increased the number of bamboo species in Indonesia (Ervianti et al., 2019; Muzakki, 2020; Widjaja, 2020, 2023).

(Bamboo is a versatile plant that) As a versatile plant, humans have utilized bamboo since ancient times (Hsiung, 1987; Dlamini et al., 2022; Mohan et al., 2022). It is not only used as a building material but also for crafts, art, food, and musical instruments. In Indonesia, bamboo holds significant cultural and economic importance, particularly in regions situated on small islands. Due to their compact size, these areas often contend with limited natural resources. Peleng Island, located in the Banggai Kepulauan Regency, Central Sulawesi, is one of Indonesia's small islands with great bamboo potential.. There are eight bamboo species reported on Banggai Kepulauan, namely Bambusa tuldoides, B. vulgaris, Dendrocalamus asper, Gigantochloa atter, Neololeba atra, Schizostachyum brachycladum, S. lima, and Thyrsostachys siamensis (Damayanto & Rahmawati, 2020). Most of these species are significantly utilized in Banggai Kepulauan, particularly in Peleng Island. In this paper, we explore the utilization of bamboo on Peleng Island, aiming to expand our understanding of how bamboo is used on small islands in Indonesia. Our findings are expected to offer new insights into the local wisdom associated with bamboo utilization in these areas.

METHODS

The research was conducted from June to July 2019 on Peleng Island, Banggai Kepulauan Regency, Central Sulawesi Province, Indonesia (Figure 1). Information on bamboo utilization on Peleng Island was obtained through in-depth interviews (Table 1) with local residents. Bamboo materials were also collected to create herbarium specimens for identification purposes. These bamboo specimens were sent to the Herbarium Bogoriense (BO) for processing and further identification. Bamboo identification was conducted using references from the bamboo collection at BO and literature such as Widjaja (1987, 1997, 2001a, 2001b), Widjaja et al. (2005), and Dransfield & Widjaja (1995). The data were then analyzed descriptively.

Table 1. List of questions about bamboo and its use on Peleng Island

No.	Questions
1	How many bamboo species are recognized in this village?
2	How frequently do you use bamboo for personal purposes?
3	Where do you typically source your bamboo?
4	Which parts of the bamboo (roots, young shoots, culms, leaves, or all parts of them) have you utilized for personal use?
5	What are the uses of those bamboo parts?
6	Can you show the bamboo utilization products in this village?
7	Can you describe or demonstrate how to make those products?
8	Do you regularly consume young shoots of bamboo? If so, which bamboo species are consumed?
9	Have you ever used bamboo for medicinal purposes? If yes, which bamboo species and parts are used?
10	What are the traditional or local beliefs regarding bamboo in this village?

RESULTS AND DISCUSSION

There are six bamboo species used on Peleng Island in daily life, namely Bambusa vulgaris, Dendrocalamus asper, Gigantochloa atter, Neololeba atra, Schizostachyum brachycladum, and Schizostachyum lima (Figure 2–3). This constitutes approximately 75% of the eight bamboo species found on Peleng Island (Damayanto & Rahmawati, 2020). The utilization of bamboo on Peleng Island can be categorized into three types, namely (1) as a building material (Figure 4), (2) for food preparation, and (3) for other purposes. As a building material, bamboo in Peleng Island is usually used as house roofs, pillars, and wall weaving. The traditional roofs of houses on Peleng Island are made from materials such as bamboo, rattan, and sago palm leaves. These roofs are known as ato in Peleng Island (Figure 5). The ato is made from bamboo G. atter, locally known as bambu peling. This bamboo is used as the main support pole (Figure 6) for sago palm leaves (Metroxylon sp.). Bambu peling is abundantly available on Peleng Island, making it a popular choice for ato support poles. According to Dransfield & Widjaja (1995), G. atter has long been known as a building material in Southeast Asia.

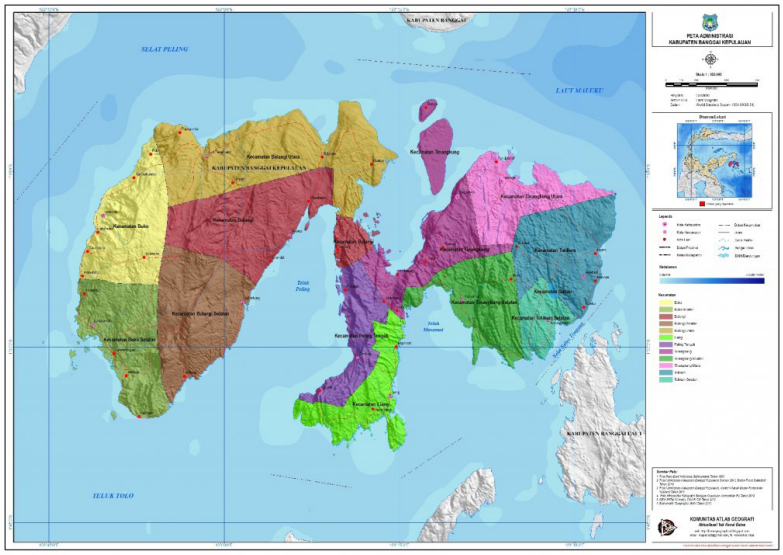

Figure 1. Research Location on Peleng Island, Banggai Kepulauan Regency, Central Sulawesi Province (Source: BPKPPST, 2015)

Figure 2. *Bambusa vulgaris, Dendrocalamus asper*, and *Gigantochloa* atter (from left to right)

Figure 3. *Neololeba atra, Schizostachyum brachycladum*, and *Schizostachyum lima* (from left to right)

Figure 4. Bamboo for building materials in Peleng Island

The ato is crafted by folding mature sago palm leaves and placing them precisely on the bamboo support poles, then securing them with rattan (*Calamus* sp.) (Figure 6). In regions like Kokolomboy, Leme-leme Darat Village in Bulagi Subdistrict, and Alul Village in Buko Subdistrict, where natural forest cover is abundant, residents still use sliced rattan to tie the ato. Despite its increasing scarcity, rattan remains relatively accessible in these areas. However, in Alul Village and Kokolomboy, rattan is no longer found in the surroundings, and villagers must go deep into the forest to gather rattan. Fortunately, residents of Alul Village have begun cultivating rattan around their gardens. In areas without natural forests, such as Tinangkung and North Tinangkung Subdistricts, materials for sewing ato, such as bamboo *N. atra*, locally known as bambu lonas, are used. Bambu lonas is preferred for its flexibility and durability, making it suitable for sewing *ato*.

Figure 5. Traditional roofs, called *ato*, in Peleng Island

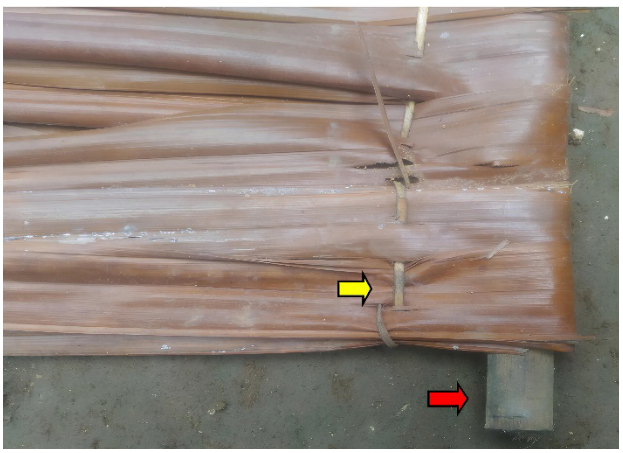

Figure 6. *Ato* with the main support pole from bamboo culm (red arrow) and rope from sliced rattan (yellow arrow)

Sago palm leaves are chosen as ato material due to their abundance on Peleng Island, their wide leaf blades, which allow for the creation of many ato with relatively few leaves. Most local residents can now purchase ato at traditional markets. These ato are sold at an average price of Rp. 3,000 per sheet, with a length of approximately 2 meters. On average, ato can last for 3-5 years, depending on the rainfall in the area. If damaged, only the sago palm leaf part of the ato must be replaced, as the bamboo support poles are usually still strong and can be reused. This is one of the local wisdom of the people of Peleng Island that directly contributes to the sustainable utilization of bamboo.

Most houses on Peleng Island still use bamboo and wood boards as the main building materials. Specifically for pillars, the preferred bamboo species is *D. asper*, locally known as pontung (betung in Indonesia). Bambu pontung has long been known for its strong structure compared to other bamboo species, making it ideal for building pillars. However, due to the difficulty in finding *bambu pontung* in Peleng Island, *bambu peling* (*G. atter*) is still commonly used. Widjaja (2001a) states that *D. asper* has been known as a building material in the Java region, especially for pillars, and some are used for furniture, chopsticks, toothpicks, paper, and musical instruments. The culm diameters of this bamboo are reported to reach 20 cm (Widjaja et al., 2005), making it very suitable for building pillars. Dransfield & Widjaja (1995) state that the culms of *D. asper* have very thick walls and are very strong and flexible, making them also frequently used in bridge construction.

As mentioned earlier, most houses on Peleng Island still use bamboo and wood boards as the main building materials, including for the walls of their homes. For those who are more affluent in Peleng Island, building a house with wooden boards or even concrete is relatively easy. Therefore, the walls of their homes can be made of wooden boards or bricks. However, for less financially capable people, weaving from bamboo is usually used for house walls (Figure 7). The bamboo species used for this purpose is *S. lima*, locally known as *bambu toi* in Tinangkung and North Tinangkung Subdistricts or lambangan in Bulagi and Buko Subdistricts. In addition to *S. lima*, bamboo species such as *S. brachycladum*, known locally as *bambu lemayu* or *lamayu*, are also often used for wall weaving. Bamboo *S. lima* is chosen for wall weaving because it is abundant on Peleng Island. This bamboo also has thin walls (up to 4 mm) with very long internodes that are suitable for weaving.

Figure 7. Bamboo weaving for house walls in Peleng Island

In addition to being used as a building material, bamboo on Peleng Island is also utilized as a container for cooking food. *Nasi jaha* (Figure 8) and *bambu suman* are examples of traditional foods from Peleng Island that are typically cooked and served in bamboo containers. The process of making *nasi jaha* and *bambu suman* is very similar to the process of making *lemang*, which is famous in the Sumatra and Malaysia regions. If *lemang* is made from rice (Dransfield & Widjaja, 1995), then *nasi jaha* is made from glutinous rice, and *bambu suman* is made from *taro*. Making *nasi jaha* begins with preparing straight bamboo culms with long internodes and thin walls. The bamboo species used is *S. brachycladum*. This bamboo species is often used in making lemang (Dransfield & Widjaja, 1995; Widjaja, 2001a) because the characteristics of its culms are very suitable for speeding up and maintaining the cooking process.

Figure 8. *Nasi jaha* in the bamboo culms (left) and the slice of *nasi jaha* (right) in Peleng Island

The mature culms of *S. brachycladum* are cut in the middle internode to obtain long internodes with not-too-thin walls. If the walls are too thin, they will easily break when cooked later. The node in the bottom part of the bamboo culm is left, while the top part is removed to serve as the opening for inserting food ingredients. After the bamboo's inside is washed, it is lined with young banana leaves. The process of inserting banana leaves must be done carefully to avoid tearing the leaves. Mature banana leaves are not recommended for making nasi jaha because they easily tear when inserted into the narrow bamboo hole. Glutinous rice that has been washed is then inserted into the bamboo hole but not to the point of filling the space inside the bamboo culm. Glutinous rice is usually purchased in the Luwuk region (mainland Sulawesi); if it is not available in large quantities, it is mixed with regular rice. After that, coconut milk is added until the glutinous rice is submerged. The top hole of the bamboo is stuffed with banana leaves; then, it is burned in hot embers (not directly in the fire).

Cooked nasi jaha is indicated by the loss of water content (coconut milk) inside. After cooking, nasi jaha is cooled, then split and cut into pieces (Figure 8). The price per piece in traditional markets on Peleng Island is an average of Rp. 1,000. Making bambu suman is similar to nasi jaha, but the ingredients used are taro. Taro is peeled, washed, and grated, then mixed with grated coconut, and placed in bamboo before being burned. Before burning, salt and a little water can be added according to taste and a little water. In areas with limited water, such as Alul Village, Bulagi Subdistrict, the making of *nasi jaha* and *bambu suman* usually does not use coconut milk or water. To overcome this, the bamboo species used as containers is B. vulgaris, locally known as aok tuu. Bamboo *B. vulgaris* has thicker culms and shorter internodes compared to *S. brachycladum* (Widjaja, 2001a; 2001b; Widjaja et al., 2005), so the burning process uses direct fire. The thick culms of *B. vulgaris* contain enough water, so when burned, they release enough water to aid in cooking.

In addition to *nasi jaha* and *bambu suman*, the young shoots of *D. asper, B. vulgaris*, and *G. atter* are commonly used as vegetables on Peleng Island (Figure 9). These young bamboo shoots are prepared by carefully removing the outer layers until a smooth, whitish surface is revealed, then thinly sliced and washed. Known for their delicious and sweet taste, the young shoots of D. asper have been enjoyed for generations (Widjaja, 1987; Dransfield & Widjaja, 1995; Widjaja, 2001a; Damayanto, 2018). Similarly, *B. vulgaris* is prized for its tasty young shoots (Widjaja et al., 2014), although locals note a slightly bitter flavour than *D. asper*. The young shoots of *G. atter* are also popular and widely used as vegetables (Widiarti, 2003), and they are appreciated for their delicious flavour (Widjaja et al., 2014).

Figure 9. The vegetables from young shoot of bamboo in Peleng Island

Various uses of bamboo on Peleng Island, besides being used as a building material and food, have also been revealed in this study. Bamboo species such as S. brachycladum have been used as ornamental plants, water containers, and stairs. Bamboo S. lima is used as a pole for picking fruits and cloves, and a pole filled with sticky sap to trap pests on plantation crops. Bamboo B. vulgaris is also known to be used as a fish trap or locally known as kalason.

CONCLUSION

There are six bamboo species used on Peleng Island in daily life, namely Bambusa vulgaris (aok tuu), Dendrocalamus asper (pontung or betung), Gigantochloa atter (bambu peling), Neololeba atra (bambu lonas), Schizostachyum brachycladum (bambu lemayu or lamayu), and Schizostachyum lima (bambu toi or lambangan). Bamboo on Peleng Island is extensively used for three main purposes: as a building material for roofs, pillars, and wall weaving; for cooking traditional foods like nasi jaha and bambu suman; and for various other uses such as ornamental plants, water containers, stairs, pole for picking fruits/cloves, pest traps, and fish trap.

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NPK NUTRIENT STATUS IN SOIL AND LEAVES OF *PIPER NIGRUM* L UNDER DIFFERENT GROWTH CRITERIA

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ABSTRACT

The White pepper (*Piper nigrum* L.) is Indonesia's leading spice commodity. As a production center, Bangka Belitungstill needs help in cultivation, including nutrient availability. Nutrients N, P, and K are macro elements that determine the growth of plants, including pepper. This research aims to identify the status of N, P, and K nutrient, in P. nigrum plants by observing leaf visuals, nutrient analysis, and plant tissue analysis. Significance of study: Knowledge regarding the absorption of nutrients by plants and their distribution in various parts of the plant (for example, leaves, branches, fruit, and roots) will be useful for agronomists in designing efficient fertilizer application strategies. The experimental methods used in this research were observation and laboratory analysis methods. The results of the observation data show that NPK nutrients in land with excellentgrowth criteria have an N-total of 0.22%, P_2O_5 available is 383.2 mg/kg and available K_2O of 6.4 mg/kg. Plant growth lacking nutrients criteria has a total N content of 0.20%, P_2O_5 of 115.1 mg/kg, and K_20 of 7.1 mg/kg. while plant tissue has an N-Total content of 2.41%, P-Total of 0.26%, and K-total of 2.47% for plants with excellent growth criteria and N-total, P-total, and K-total respectively 2.79, 0.32, and 3.95 mg/kg for plant tissue with poor growth criteria.

Keywords: Deficiency, Deficiency Symptoms, Potassium, Nitrogen (N), Phosphorus (P), White pepper

INTRODUCTION

Piper nigrum L has strategic value as the most popular spice and makes a very popular spice (Srinivasan et al., 2007). Piper nigrum L., a flowering vine from the family Piperaceae, is valued for its dried berries called peppercorns, which are known to have health benefits and are used as a spice and seasoning.

White pepper production tends to decline. Meanwhile, from the export side, fluctuating developments are visible. Based on the Revealed Comparative Advantage (RCA) value, Indonesian Piper nigrum appears to be competitive for export purposes to Germany, the Netherlands, France, Italy, and Belgium. Meanwhile, the Export Product Dynamics (EPD) value shows that the competitive position of Indonesian Piper nigrum in the Netherlands, Germany, and Belgium is in the Retreat position. On the other hand, Indonesian White pepper has the competitive position of Rising Star in Italy, Falling Star in France, and Lost Opportunity in America. Finally, based on the Trade Specialization Index (TSI) value, it can be shown that Indonesia is very competitiveness as an exporter of whole White pepper. Competitiveness analysis using Revealed Comparative Advantage (RCA), Export Product Dynamics (EPD), and Trade Specialization Index (ISP) (Balqis & Yanuar, 2021).

Yudiyanto et al., 2014; Ropalia et al., 2022, stated that one of the causes of the low productivity of Muntok white pepper recently, apart from increasing farming costs, is also due to the decline in soil fertility where White pepper farming is done. White pepper cultivation requires sufficient nutrients for growth and unfavorable environmental conditions, especially related to decreased soil fertility and unsuitable weather. Paduit et al. (2018) stated that the high response to N, P, and K, recommendations for effective fertilization, the need for plant nutrient absorption and disposal must be clearly understood to gain efficiency in white pepper cultivation. Large amounts of nutrients are required to produce and maintain economic yields of white pepper. To achieve high yields, farmers must apply nutrients in sufficient quantities to meet the total nutrient requirements of the plant.

Knowledge regarding the absorption of nutrients by plants and their distribution in various parts of the plant (for example, leaves, branches, fruit, roots, etc.) will be helpful for agronomists in designing efficient fertilizer application strategies. Another problem is related to the low price of P. nigrum, which encourages lower production costs and a cleaner environment (Sulok et al, 2020). P. nigrum is cultivated in various soil types with varying pH and fertility. Ideal conditions require loose soil rich in humus and important plant nutrients, with good drainage, sufficient water.

Among the nutrients consumed by black pepper, N uptake is the highest, followed by K and Ca, and the number of nutrients lost from the soil will follow the order: N>K>Ca>Mg>P>S>Fe>Mn>Zn (Srinivasan et al., 2007). Plants require the most significant amount of N among the three main/primary nutrients (the others are P and K). N, P, and K have many functions, including promoting rapid growth, increasing leaf size and quality, and enhancing fruit and seed development; they form a single component of many important components in plants, including amino acids, which are the building blocks of proteins and enzymes, which are involved in most biochemical process catalysts. So it is necessary to study the condition of Piper nigrum plants based on the growth status of to leaf visuals, soil N, P, and K nutrients, and leaf tissue nutrients, to determine the N, P, and K nutrient status on the two P. nigrum growth criteria.

MATERIALS AND METHODS

Two park conditions were determined using different criteria to be observed in depth. Two (2) adjacent garden locations were selected with good and low growth criteria, marked by the visual appearance of pepper growth morphology, production criteria of 2 to 4 tons per hectare to determine growth criteria (≤2 tons low production, 2-3 tons sufficient production and ≥ 4 tons produced well. Twenty trees were used, with each tree observing 20 symptomatic leaves on each tree. The leaf deficiency or toxicity criteria are observed in detail by comparing the visible symptoms with the table of toxic and nutrient deficiency symptoms (McCauley et al., 2011). Nutrient status assessment is carried out by analyzing the macronutrients N, P, and K and creating criteria for high, sufficient and low nutrient status (SRI, 2005). The detected nutrients are compared with those absorbed by the plant (analysis of old leaf plant tissue).

Table 1. Observation stages

Stages	Outer	Achievement indicators	Method
1. Determine the criteria for studying location gardens	2 criteria for good and bad growth of White	Plants with good and bad growth criteria can be distinguished	Visually based on morphological observation of plants
2. Observation of leaf symptoms. Using determinant keys to determine plant leaf symptoms (McCauley AM, Jones C, Jacobsen J. 2011. Plant Nutrient Functions and Deficiency and Toxicity Symptoms. Module. United States: Montana State University)	Observe the symptoms of young leaves and old leaves by comparing them based on the observation table for deficiencies and toxic macro and micro nutrients	Deficit and toxic nutrients are determined based on visible symptoms	Visual observation of leaves by comparing visual symptoms with a table of nutrient deficiency and toxic symptoms
3. Soil sample collection	macro micro nutrient status detected	analyzed for macronutrients N, P and K	Table Method Types of macro and micro nutrients
4. Plant tissue analysis Sampling old leaves	To observe mobile nutrient uptake and for immobile nutrients samples were used from old leaves.	Nutrient uptake calculations are carried out by comparing media nutrients with the nutrients contained in leaf plants	Nutrient uptake efficiency is calculated using the formula = amount of plant nutrients (top and roots) / amount of nutrients in the media

The criteria used to recognize the chemical fertility status of soil analysis results are based on standardized soil status criteria by the Indonesian Soil Research Institute (SRI, 2005). Table 2 below contains a table of soil criteria in the very low, low, fair, high, and very high range.

Table 2. Soil media status criteria standardized by the Indonesian Soil Research Institute (2005)

Soil Characteristic	Very Low	Low	Currently	High	Very High
C- Organik (%)	<1	1-2	2.01-3	3.01-5	>5
N-Total (%)	<0.1	0.1-0.2	0.21-0.5	0.51-0.75	>0.75
P2O5 Bray 1 (ppm)	<10	10-20	21-40	41-60	>60
K2O HCL 25% (me/100g)	<10	10-20	21-40	41-60	>60
pH H20 < 4.5	4.5-5.5	5.6-6.5	6.6-7.5	7.8-8.5	>8.5
very (acid)	(acid)	(slightly acid)	(neutral)	(slightly alkaline)	(alkaline)

Source : Soil Research Institute (2005)

RESULTS AND DISCUSSION

Detect deficiency and toxic symptoms in old leaves

Table 3. Results of visual observations of Piper nigrum leaves

Deficiency Symptoms	CGrowth No Good	Toxic Symptoms	Old Leaves	
			Good Growth Criteria	Growth No Good
Nitrogen*		Nitrogen**		
The color of the leaves becomes pale,	√	The plant is dark green and lush, but it usually has a small root system (shallow and limited). Burning symptoms occur on the edges of the leaves, and is followed by tissue death on the strands between the veins of the leaves.	X	x
Leaf tissue becomes dry and dies,	√			
Plant growth is stunted and stunted,				
Phosphor		Phosphor		
Leaf veins The color of the leaves is dark green and the surface looks shiny reddish.	√	Necrosis and death of growing points. Chlorosis on the leaf blades between the veins of young leaves and symptoms of scorching on the edges of old leaves.	X	√
Leaves are short – short.	√			
The edges of the leaves, branches, and stems become smaller and purplish red and gradually turn yellow.				
Potassium		Potassium		
Old leaves will shrivel or curl, turn brownish red, and dry out like they have been burned	√	Excess potassium (K) disrupts the absorption of Ca and Mg, stunting plant growth. Thus, the plant experiences a deficiency. At first, the leaves appear puckered, with edge leaf yellowing, visible spotting of dirty-colored chocolate, and leaf death.	X	x
Transparent yellow spots appear on the leaves.	√			
Susceptible to disease	√			
Magnesium ***		Magnesium		
Orange-yellow interveinal chlorosis on older leaves	x	Necrosis (tissue death) in plant leaves.	X	x

* (Wahyuni, Darma, & Wayahdi, 2017), ** (Wiraatmaja, 2017), *** (McCauley et al., 2011)

Symptoms of N, P, and K deficiency were detected in poor growth conditions, but toxic symptoms of P were also detected in good growth criteria (Table 3). Meanwhile, toxic symptoms are only found in phosphorus symptoms. Figure 1 below shows visuals of old plants and leaves.

(1a)

(1b)

(1c)

(1d)

Figure 1. Piper nigrum growth conditions (1a) Good growth criteria and No Good Growth (1b), and leaf conditions with visible symptoms (1c and 1d)

Soil nutrient analysis, leaf tissue analysis and absorption efficiency

Table 4. Results of N, P, and K nutrient analysis based on Piper nigrum growth status

Soil Characteristic	Settlement Status	Method	Unit	Measurement	Criteria
pH H 2 O	Good	Extract 1 : 5 ICBB/ MU/11.004.2 (Potentiometry)	-	6.5	neutral
	No Good			7.0	neutral
C Organic	Good	ICBB/MU/11.004.14 (Walkley & Black / Gravimetric)		4.05	high
	No Good			3.72	high
C/N	Good	calculation		18	
	No Good			19	
N-Total	Good	ICBB/MU/11.004.12 (Kjeldahl)	%	0.22	currently
	No Good			0.20	low
P_2O_5 Available	Good	ICBB/MU/11.004.4 (Olsen) ICBB/MU/11.004.5 (Bray I)	mg/Kg	383.2	high
	No Good			115.1	low
K_2O Available #	Good	DTPA – AAS	mg/Kg	6.4	very low
	No Good			7.0	very anxious

A low level of nitrogen is a criterion for poor growth, but nitrogen is relatively sufficient for good growth (Table 3). Another condition that can be seen is the phosphorus status, which is quite good for good growth criteria, but if the growth is not good, phosphorus is detected to be low. Meanwhile, the potassium content is relatively very low based on the criteria (Table. 2).

Table 2 is used to determine the adequacy of nitrogen, phosphorus, and potassium nutrients obtained based on soil analysis at the research location. The criteria used are based on the Soil Research Institute (SRI, 2005). Table 5 shows the results of N, P, and K nutrient analysis in old leaves. Nutrient content figures in White pepper leaves were compared with standard data for nutrient adequacy in corn.

Table 5. Results N, P and K tissue nutrient analysis leaf

Type Nutrient	Settlement Status	Method	Unit	Mark Results Observation	Standard Enough Nutrienton Corn	Unit
Leaf Old						
C- Organic	Good	Gravimetry		49.11	45	%
	No Good			47.12		
N	Good	ICBB/MU/11.003.2 (Kjeldahl, Titrimetry)	%	2.41	2.7-4.0	%
	No Good		%	2.79		
P	Good	ICBB/MU/11.003.3 (Spectrophotometer UV vis)	%	0.26	0.25-0.50	%
	No Good		%	0.32		
K	Good	ICBB/MU/11.003.4 (AAS)	%	2.47	1.70-3.0	%
	No Good		%	3.95		

Organic C in P. nigrum leaves with good and bad growth criteria is 49.11 and 47.12% (Table 5). This value is within the criteria for normal carbon presence. According to Salisbury & Ross (1992), the range of carbon in the dry weight of plant tissue can reach 45%. Nitrogen makes up approximately 1-5% of the plant body. Observation results for corn with sufficient N criteria ranged from 2.70-4.00%, P= 0.25-0.05% and K 1.70-3.00%. Meanwhile, soybeans can reach enough in the range of N 4.00 to 5.50, Phosphorus 0.26-0.5%, and Potassium around 1.70-2.5%. (Plant Nutrition Manual) (Salak & Steinhilber, 2010). According to Salisbury & Ross (1992) the concentration of N in dry tissue can reach 1.5%, P 0.2%, and K 1.0%, while carbon can reach 45% of the dry weight of plant tissue.

Table 4 shows that P. nigrum with good growth has lower N, P, and K than leaf tissue with poor growth criteria. This is thought to be caused by the mobility nature of these nutrients. Nutrient analysis was only carried out on old leaves and was not observed on young leaves. Observations on old leaves based on the mobility characteristics of dead nutrients are classified as mobile nutrients so that the initial symptoms will appear on old leaves (McCauley, 2011). So, it is estimated that nutrient transport determines the nutrient content in old leaf tissue to young leaves,

which are relatively faster to leaf and have good growth criteria. K+ plays a role in several physiological functions, including controlling cell growth and wood formation, xylem-phloem water content and movement, nutrient and metabolite transport, and stress responses (Sardans & Peñuelas, 2021). Soil nutrient conditions with good and bad growth criteria were detected to be very low.

Many symptoms are similar; for example, nitrogen (N) and sulfur (S) deficiencies can vary depending on the location, growth stage, and severity. Multiple symptoms of deficiency and poisoning (toxicity) can occur at the same time. More than one deficiency or toxicity may produce symptoms, or one nutrient deficiency may occur due to excess of another nutrient. For example, excess P can cause Zn deficiency. Types of plants, and even several varieties of the same type, differ in their ability to display symptoms of deficiency and poisoning.

The role of nutrients N, P, and K play in almost all metabolic processes. As determined by its function, N influences plant growth rate and quality (Njira and JNabwami, 2015; Njirah, 2015). Phosphate (Pi) is an important macronutrient for plant life. Several regulatory components involved in Pi homeostasis have been identified, showing great complexity at the cellular and subcellular levels. Determining the Pi content in plants is crucial to understanding this regulation (Kanno et al., 2016). A balanced supply of essential nutrients is one of the most important factors in increasing crop yields. A review of the nutritional management of black pepper has been comprehensively reviewed by (Srinivasan et al., 2007).

Potential factors that can cause pseudo-symptoms include disease, drought, excess water, abnormal genetics, herbicide and pesticide residues, pest attacks, and the effect of soil compaction. Hidden symptoms. Plants sometimes experience nutrient deficiencies without showing visual symptoms. Field symptoms that show different from ideal (actual) symptoms. When tested in the field or controlled for the role of certain elements, many plants did not produce the expected symptoms.

CONCLUSION

NPK nutrients in very good growth criteria have an N-total of 0.22% (medium), P_2O_5 available is 383.2 mg/kg (high), and available K_2O of 6.4 mg/kg (very low). Meanwhile, plant growth with poor growth criteria has an N-total content of 0.20% (Low), P_2O_5 of 115.1 mg/kg (Low), and K_20 of 7.1 mg/kg (very low). Meanwhile, plant tissue with good growth criteria has an N-total content of 2.41%, P-total of 0.26 percent and K-total of 2.47 percent. Meanwhile, for P. nigrum with poor growth, the leaf nutrient content of N-total, P-total, and K-total was 2.79%, 0.32%, and 3.95% respectively

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