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Contents

Rural Community Empowerment through the Utilization of Straw as Compost

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A B S T R A C T

There was a prolonged decline in soil quality due to the excessive use of chemical fertilizers and the lack of empowerment of farmers about these hazards. Meanwhile, the potential to restore soil fertility was very open, one of which is rice straw compost. The aim of this study was to analyze the effect of farmer characteristics and government support on the empowerment of rural community through the utilization of straw as compost in lowland rice plants. The research activity was carried out for three months (April - July 2020). This study was a survey research with a quantitative assessment approach supported by qualitative data and information. A total of 80 respondents selected from 256 were determined by the Slovin formula. Data were collected through direct interview using a closed-ended questionnaire, which had been tested for its validity and reliability. Data were analyzed in two ways, namely: with descriptive statistics to explain the performance of the research variables, and linear regression analysis to determine the factors that influence community empowerment. The result of the study showed that the farmer characteristic and government support had a significant effect on the empowerment. Factors that affect the empowerment of farmers were the characteristics of farmers including; age, education, and farming experience; and government supporting factors consisting of; the role of extension workers, the availability of facilities and infrastructure, as well as the availability of information sources. Therefore, a strategy that can be implemented to optimize community empowerment in the utilization of rice straw for compost is to increase external support to foster farmers either through counseling or other activities.

Keywords Empowerment, Farmers, Compost, Lowland Rice

1. Introduction

As an agricultural nation, agricultural activities have been carried out for a long time, along with technological developments and needs, and intensive agricultural activities are continuously conducted. Along with such agricultural practices, there is a decrease in land or soil quality due to natural processes such as erosion, pollution, and others. Soil contamination will reduce soil quality, such as decreasing soil nutrient content due to erosion. Decreased soil quality can also occur due to excessive and continuous use of manufactured chemical fertilizers for a long time. The impact that will arise from this condition is a decrease in plant productivity on the said land, including in lowland rice cultivation.

In order to prevent the impact caused by decreasing land quality, strategic and systematic efforts to treat the areas experiencing a decrease in land quality are needed. One of the programs offered by the

government, in this case, the Ministry of Agriculture to overcome the decreases of land quality is the use of organic fertilizers with raw materials derived from local plant residues. According to information from local extension workers and documents of Extension Programs from the Cikoneng District Agricultural Extension Center, efforts to promote the use of organic fertilizers have been frequently carried out, and even the practice of producing compost from straw has been carried out. However, farmers, especially lowland rice farmers, still have not used compost as a necessity to compensate for the use of factory chemical fertilizers.

Effendy and Gumelar [1] reported that the use of fertilizers in a balanced manner has not been fully implemented by rice farmers. This means that farmers still rely on the use of factory chemical fertilizers and do not fully understand that the use of factory chemical fertilizers can reduce the quality of paddy fields. This condition is also in line with Effendy and Sudiro [2] that farmers' participation in using fertilizers in a balanced manner in lowland rice is still not satisfactory, meaning that most lowland rice farmers are still reluctant to apply fertilization in a balanced manner to their rice fields. This condition should be stopped or a solution must be sought thus the lowland rice farmers do not rely on factory chemical fertilizers and are encouraged to use organic fertilizer or compost.

Based on the focus of the above problems, the aims of this study were: (a) describe the level of empowerment of lowland rice farmers, (b) analyze the factors that affect the empowerment of farmers in using straw compost, and (C) find strategies to increase the empowerment of rice farmers rice fields through the use of straw compost.

2. Methods

This research applied quantitative research supported by qualitative data and information through direct surveys of the farmers. The research activity was carried out for three months (April - July 2020) in Cimari, Kujang, and Gegempalan Villages, Cikoneng Sub-district, Ciamis Regency, West Java Province, the research location is as shown in Figure 1. Determination of respondents was done by selecting (purposive sampling) due to the limited number of farmers who were willing to take part in the research during the planting season, thus the number of respondent farmers was 76 people representing six farmer groups from three selected villages.
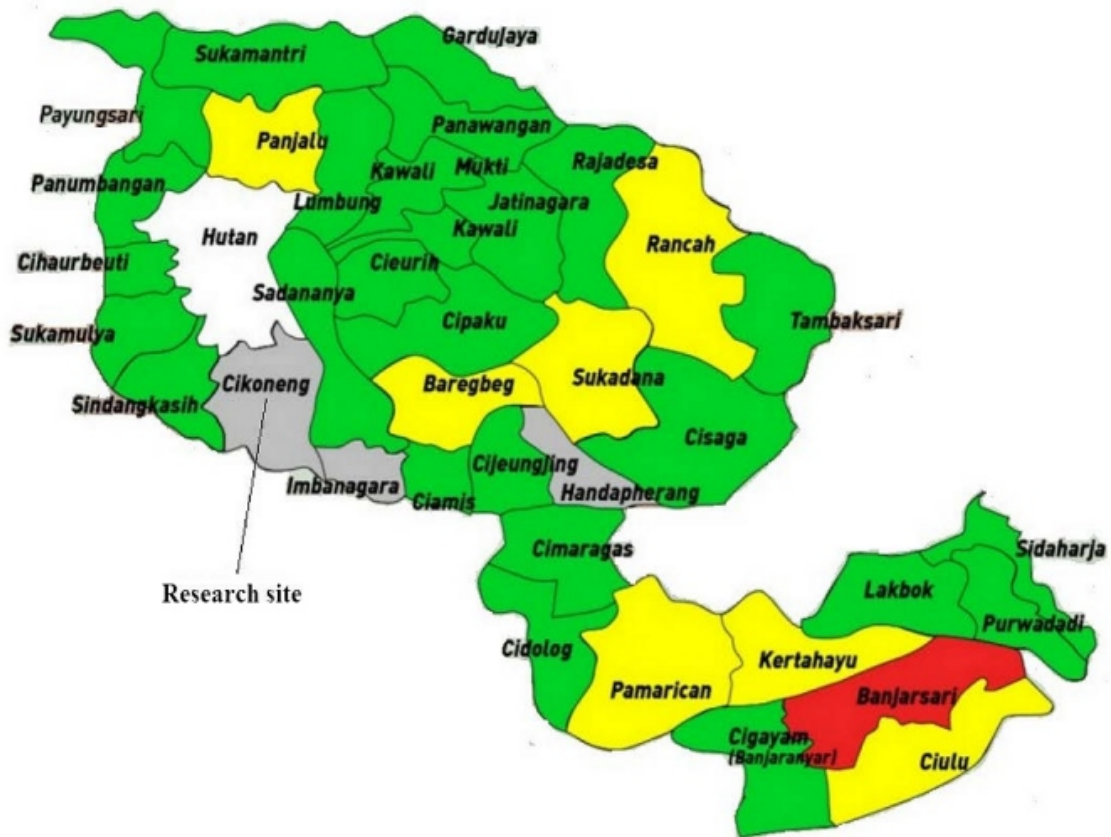
Figure 1. Research location map

The data collected includes primary data obtained directly from respondent farmers through a closed-ended questionnaire that has provided the answer choices. The type of data collected through a questionnaire is in the form of interval data. Before being used to collect questionnaire data, its validity, and reliability were tested. The test results stated that the questionnaire was suitable for use as a means of collecting data with a Cronbach alpha value of 0.823.

Apart from primary data, secondary data was also collected in the form of additional information or data derived from reports or other documents from the local village and sub-district offices.

The data were analyzed in two ways, namely descriptive analysis to explain the performance of each research variable and linear regression analysis to analyze the factors that had an effect on the empowerment of rice farmers. The data analysis was assisted by the SPSS program tool version 26.

Based on the description above, a research framework is built based on the hypothesis that there are a number of variables that can affect the empowerment of a person or community group. Effendy et al. [3] explained the youth involvement to increase the youth empowerment.

Meanwhile, [2] Effendy and Sudiro argued about factors that affect the woman empowerment in land use are external factors and farmer group supports.

Based on these reviews, the research hypothesis consists of two independent variables, namely farmer

characteristics (X1) and government support (X2). Farmers'characteristics include: age, level of education, and farming experience; meanwhile, government support includes: the role of extension workers, the availability of facilities and infrastructure, and the availability of information sources.

3. Results

3.1. Description of Respondent Characteristics

The results of the descriptive analysis of the respondent farmers' characteristics which include age, level of education, and farming experience are presented in Table 1.

Table 1 shows that most of the respondent farmers (75%) are between the ages of 16 and 63, which can be categorized as the productive age group. The majority of respondents' education level (76.25%) is elementary school which is classified as low. Meanwhile, most of the respondent farmers (51.25%) had a long experience of more than 20 years.

3.2. Description of Government Support

The results of descriptive analysis of the variable government support consisting of the role of extension workers, the availability of facilities and infrastructure, and the availability of information sources indicate that generally government support is included in the medium category with an average score of 2.88. A description of government support is presented in Table 2. Table 2 shows that government support obtained a mean score of 2.88, which means that it is classified as sufficient. Although the scores for the support of each indicator are not absolutely different, the highest score is obtained by the role of the instructor (2.91), followed by the availability of information sources (2.90), and the lowest is the infrastructure. These results suggest that the support for infrastructure is considered lower than the role of the extension workers and the availability of information sources.

3.3. Level of Empowerment

Farmers' empowerment is measured from the aspects of knowledge, attitudes, and skills. The results of the descriptive analysis show that the score for the level of empowerment is 2.50 which is included in the sufficiently empowered category. The details of the scores for each of the empowerment indicators are presented in Table 3.

Table 3 shows that the value obtained by the three indicators of empowerment is not much different, but the highest value is obtained by the knowledge aspect (2.60), followed by attitude (2.54), and the skills aspect of 2.47. These results suggest that skills are rated the lowest by farmers compared with other aspects, thus it needs to be improved hence farmer empowerment also increases

3.4. Factors Influencing Community Empowerment

The results of linear regression analysis showed that the independent variables consisting of farmer characteristics (X1) and government support (X2) had a significant effect at the 95 percent confidence interval (p<0.005). Details of the results of the regression analysis are presented in Table 4.

Table 4 shows that the farmers' characteristics have a significant effect on farmer empowerment with a coefficient of 0.237, likewise government support has a significant effect on empowerment with an influence coefficient of 0.641. This analysis also obtained a determinant factor (R2) of 0.531 and a constant value (a) of 0.101.

These results can be further explained; (1) the determinant factor (R2) is 0.531 or 53.1 percent, meaning that the farmers' characteristics (X1) and government support (X2) contribute 53.1 percent to the level of farmer empowerment, while the remaining 46.9 percent comes from other factors not examined in the study. (2) the constant value is 0.101, it can be explained that if the farmers' characteristics (X1) and government support (X2) are zero, then the level of farmer empowerment is 0.101. (3) the coefficient of influence on farmer characteristics is positive 0.237, meaning that if the coefficient value of government support is zero, then every increase of one unit of farmer characteristics will increase empowerment by 0.237, (4) the coefficient of influence of government support is positive 0.641, meaning that if the coefficient value of farmer characteristics is zero, then every increase of one government support unit will increase empowerment by 0.641. Thus, these results obtain a regression equation model of $Y = 0.101 + 0.237X1 + 0.641X2$.

Table 1. Description of the respondents' characteristics

Group	Group	Total (person)	Percentage (%)
Respondent Age ($X_{1.1}$)			
Not yet productive	0 - 15 years	0	0.00
Productive	16 - 63 years	62	75.00
Not productive/elderly	>64 years	18	25.00
Education Level ($X_{1.2}$)			
Elementary school	0 - 6 years	61	76.25
Junior High School	7 - 12 years	14	17.50
High school	13 – 15 years	5	6.25
Higher education	> 15 years	0	0.00
Experience ($X_{1.3}$)			
Beginner	1 - 10 years	18	22.50
Intermediate	11 - 20 years	21	26.25
Experienced	> 20 years	41	51.25

Table 2. The average score of government support

No	Indicator	Average
1	The Role of the Extension Workers	2.91
2	Facilities and infrastructure	2.81
3	Resources	2.90
	Average	2.88

Table 3. The average score of farmer empowerment

No	Indicator	Average
1	Knowledge	2.60
2	Attitude	2.54
3	Skills	2.47
	Average	2.50

Table 4. Results of the regression analysis

No	Variable	Coefficient	Sig	Description
1	R^2	0.531	-	-
2	Constant	0.101	0.765	-
3	Farmers Characteristic (X_1)	0.237	0.000	Significant
4	Government Support (X_2)	0.641	0.000	Significant

4. Discussion

4.1. Effect of Farmer Characteristics on Community Empowerment

The results of the regression analysis found that the characteristics of respondent farmers (X1) had a significant effect on empowerment, which means that in increasing farmer empowerment, factors related to the farmer's personality should be a concern before taking steps or deciding on a program. In line with this, the results of the descriptive analysis on the personal characteristics of the respondent farmers show that 75.0 percent of the respondent farmers belong to the productive age group (16 - 63 years) and 76.25 percent have long farming experience (> 20 years); although the majority have low levels of education Elementary School).

These results indicate that even though the education level is low, having sufficient experience and being motivated by work spirit to keep doing business will be able to encourage the level of empowerment.

This result is in line with [4], [5], [6], who reported that the personal characteristic component is an important indicator in increasing empowerment. In addition, empowerment can also be determined by factors from outside the characteristics, such as a person's involvement in a program or activity [3], also determined by external factors such as; availability of infrastructure, extension activities, farmer group functions, and access to information [7], [8] [9] [10]. Meanwhile, Permana and Effendy [11] explained

that the empowerment of women farmer groups is significantly influenced by external factors consisting of; support for group members, the availability of facilities and infrastructure, policy support, and outreach activities.

4.2. Influence of Government Support on Community Empowerment

Based on the results of the regression analysis (Table 4), it is known that government support (X2) has a significant effect on the empowerment of a person or community group. This means that government support which consists of; the role of extension workers, facilities andinfrastructure, and availability of information sources determine the level of farmer empowerment. When compared with the results of the descriptive analysis, these results indicate a relationship, government support is in the sufficient category, meaning that it is not fully in accordance with the expectations of the respondent farmers.

Thus, any efforts to increase farmers' empowerment, should consider determined aspects, namely the role of extension workers, the availability of facilities and infrastructure, and the availability of information sources. The more optimal the extension workers carry out their functions and roles, the better the impact on increasing farmer empowerment. Likewise, the availability of infrastructure, as well as the availability of information sources, will have a greater influence on increasing farmer empowerment. These results reinforce the findings of [9], [11] which concluded that the availability of infrastructure, extension activities, support and functions of farmer groups, and access to information greatly determines the empowerment of group members. It is also in line Wijayanti and other research results [12], [13], [14], [15], that government support for a community will have a real effect on the community empowerment.

5. Conclusion

From the descriptions that have been stated above, the conclusions of this study are the characteristics of rice farmers in Cikoneng Subdistrict; age is included in the productive group (16 - 63 years), low level of education (elementary school), and has quite a long farming experience (> 20 years). The factors that influence farmer empowerment are the farmers' characteristics which include; age, education, and farming experience; and government support factors which consist of; the role of the extension workers, the availability of facilities and infrastructure, as well as the availability of information sources. Strategies to increase farmer empowerment in Cikoneng Subdistrict can be implemented by optimizing the support of external factors and paying attention to the characteristics of local farmers.

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The Increase of Organic Shallots (Allium cepa var ascalonicum L.) Production through the Application of Compost on Inceptisol Soils

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A B S T R A C T

This study was conducted at the Experimental Garden of the Faculty of Agriculture, Islamic University of North Sumatra, Jalan Karya Wisata, GedungJohor Village, Medan Johor Sub-district, Medan City, North Sumatra Province at an altitude of ± 25 meters above sea level, with Inceptisol soil type. This study was conducted from March 2021 to May 2021. This study aims to determine the compost type that could increase the production of shallots (Allium cepa var ascalonicum L.) in Inceptisol soil. The study used a non-factorial Randomized Block Design (RAK) with 5 treatments, namely compost type with five replications (25 experimental plots), including P1 = Liquid Organic Pineapple Fertilizer of 4.5 ml/liter of water/polybag, P2 = straw compost of 20 tons/ha (1 kg/polybag), P3 = rice husk charcoal compost of 10 tons/ha (1.5 kg/polybag), P4 = market waste compost of 10 tons/ha (2 kg/polybag), P5 = market waste compost enriched with trichoderma of 10 tons/ha (2 kg/polybag). Parameters observed were the number of tubers per plant, the number of tubers per plot, the weight of the tubers per plant, the weight of the tubers per plot, and the size of the tubers. The results show that the type of compost treatment had a significant effect on the production of shallots. Market waste compost enriched with trichoderma (P5) resulted in the highest production.

Keywords Compost Type, Inceptisol Soil

1. Introduction

The need for Shallots (Allium ascolanicum L.) is very high since they are used in every menu of cuisine. The needs for Shallots in North Sumatra are only 40% met by local production, while the rest is distributed from other regions [16]. In 2019, Shallot production in North Sumatra was 18,072 tons with a harvested area of 2,246 hectares. However, the need for onions in North Sumatra reached 4,057 tons per month [1].

Various efforts have been made by the government of North Sumatra to increase the production of shallots, including planting in the lowlands with Inceptisol soil types. Inceptisol soil is nutrient-poor soil. It can be fertilized using both organic and inorganic fertilizers to increase the productivity of shallots. However, with environmental and health issues, organic fertilizer is an option [18].

Organic fertilizers are sourced from several types and ways of application. The materials to make organic fertilizer can be market waste available in abundance every day, from former rice cultivation in the form of straw, and rice husks available in each growing season.

One of the organic materials for fertilizer which is commonly used is rice straw compost. In this case, compost has the function of improving soil structure, strengthening the binding capacity of aggregates (nutrients) in sandy soil, increasing endurance and water absorption, improving drainage and pores in the soil, and adding and activating nutrients [19].

R. Prabavathi et al [14] stated that the people and government wanted to improve traditional and modern farming methods by converting them to automated services to increase output. In addition, farmers use traditional farming cycles and the latest innovations. Since the global food system is facing severe challenges, there is a need for investment in research to provide new solutions for precision agriculture. Digital technology farming systems have emerged to address contemporary issues in sustainable agriculture and to obtain optimal yields from the right inputs.

Adding rice straw compost to the soil is beneficial for improving soil structure and increasing nutrient availability for plants. Straw compost contains C-organic nutrients (20.02), N (0.75%), P (0.12%), K (0.69%), and C/N (23.69) [2]. Furthermore, Prasetiya et al. [12] stated that consistent use of straw compost in the long term will be able to increase the organic matter content of the soil and restore soil fertility. Giving 20 tons/ha showed the highest yields on plant height, tuber diameter, and tuber weight per sample. Based on Hayati [6] stated that straw compost has complete nutrients, but its content is low; so, it needs to be combined with inorganic fertilizers to increase plant growth and production. Rice husk charcoal contains various types of organic acids that are able to release nutrients bound in the mineral structure of the ash. The contents of rice husk charcoal are SiO_2 (52%), C (31%), K (0.3%), N (0.18%), F (0.08%), and calcium (0.14%). It also contains other elements such as Fe_2O_3, K_2O, MgO, CaO, MnO, and Cu in small amounts as well as several types of organic matter.

High silica content can be beneficial for plants because it becomes more resistant to pests and diseases due to tissue hardening [15]. Rice husk charcoal as a substitute for potassium fertilizer is one step in reducing the use of chemical fertilizers. In addition, it can reduce the pollution caused by the waste, such as water pollution and air pollution, and increase the percentage of soil aggregation. Improvements in soil aggregation have not had an impact on improving the percentage of available water pores and slow drainage pores [4].

Market waste is a very potential source of organic matter since it is abundant every day. Its utilization to increase the production of organic shallots needs to be studied, thereby reducing environmental pollution and creating clean, healthy, and comfortable traditional market conditions, as well as overcoming the scarcity of fertilizers [22]. The provision of additional nutrient intake in liquid form will also have an effect on plants. Organic fertilizers can be processed from waste materials, including one sourced from pineapple. Liquid organic fertilizers are mostly applied through the leaves or referred to as foliar liquid fertilizers containing essential macro and micronutrients (N, P, K, S, Ca, Mg, B, Mo, Cu, Fe, Mn, and organic matter) [17].

Therefore, the author has carried out a study entitled "the Increase of organic shallots (Allium cepa var ascalonicum L.) production through the application of compost on Inceptisol soils."

2. Materials and Methods

This study was conducted at the Experimental Garden of the Faculty of Agriculture, Islamic University of North Sumatra, Jalan Karya Wisata, Gedung Johor Village, Medan Johor District, Medan City, North Sumatra Province at an altitude of ± 25 meters above sea level. This study was conducted from March 2021 to May 2021.

The study used a non-factorial Randomized Block Design (RAK) with 5 treatments, namely compost type with five replications (25 experimental plots), including P1= Liquid Organic Pineapple Fertilizer of 4.5 ml/liter of water/polybag, P2 = straw compost of 20 tons/ha (1 kg/polybag), P3 = rice husk charcoal compost of 10 tons/ha (1.5 kg/polybag), P4 = market waste compost of 10 tons/ha (2 kg/polybag), P5 = market waste compost enriched with trichoderma of 10 tons/ha (2 kg/polybag). The parameters observed were the number of tubers per plant, the number of tubers per plot, the weight of the tubers per plant, the weight of the tubers per plot, and the size of the tubers.

3. Results and Discussion

3.1. Number of Tuber per Plant (tubers)

The type of compost (P) had a significant effect on the number of tubers per plant. Data on the number of tubers per onion plant is presented in Table 1.

Table 1. Average Data of Organic Shallot Production using Compost Type Treatment

Treatment of Compost Type	Number of tubers per plant (tubers)	Weight of the tubers per plant S (g)	Weight of the tubers per plot (g)	Number of the tubers per plot (Umbi)	Size of tubers Per Polybag (mm)
P1	12.92 b	8.56 d	49.80 c	73.20 c	7.63 c
P2	12.32 b	10.44 c	61.00 c	69.80 d	8.44 b
P3	12.44 b	10.12 c	58.80 d	73.00 c	8.83 b
P4	14.40 a	13.92 b	76.80 b	81.60 b	10.15 a
P5	15.36 a	16.40 a	95.40 a	87.40 a	10.41 a

Note: Numbers followed by unequal letters in the same column show a significant difference at the 5% level based on Duncan's Distance Test
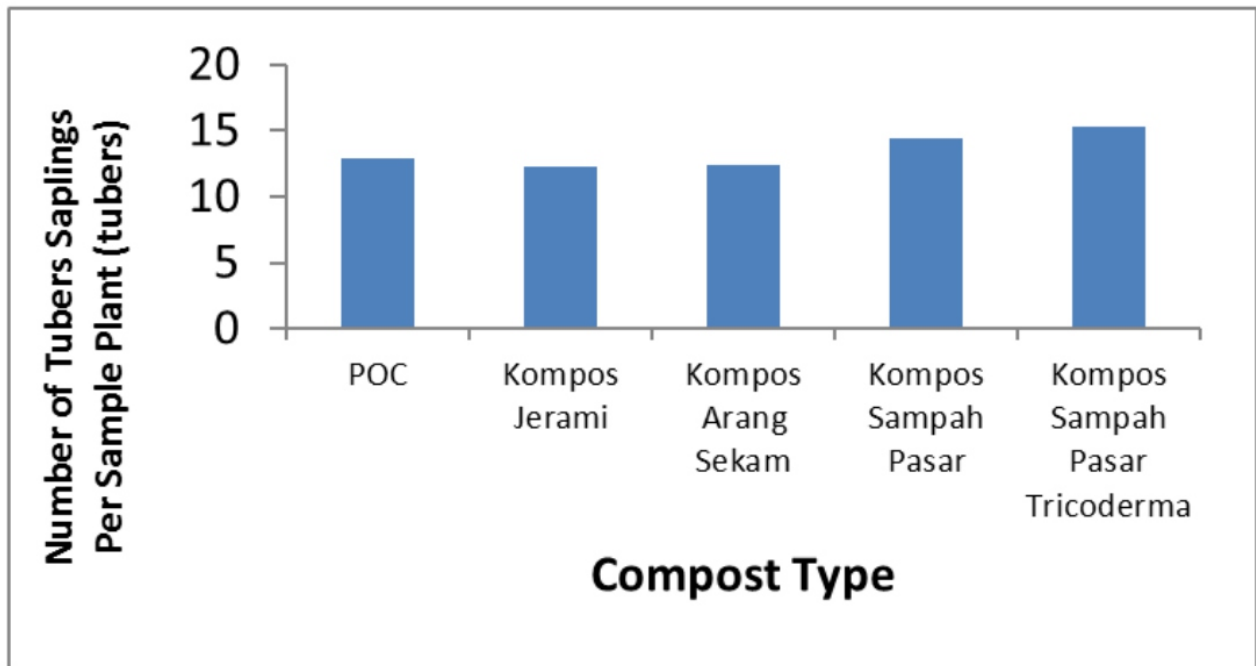
Figure 1. Number of Tubers per Shallot Plant in the Treatment of Compost Type

The treatment of trichoderma market waste compost of 10 tons/ha (P5) resulted in the highest number of tubers per plant (15.36 tubers) compared to giving market waste compost of 10 tons/ha (P4) (14.40 tubers), husk charcoal compost of 10 tons/ha (P3) (12.44 tubers), straw compost of 20 tons/ha (P2) (12.32 tubers), and liquid organic fertilizer of 4.5 ml/liter of water (P1) (12.92 tubers) (Table 1 Figure 1).

3.2. Weight of Tubers per Plant (g)

Compost type (P) had a significant effect on tuber weight per plant. Data on the weight of tubers per plant is presented in Table 1. The compost fertilizer treatment had a significant effect on the weight of tubers per onion plant at harvest, where the treatment of trichoderma market waste compost of 10 tons/ha (P5) resulted in the heaviest tuber weight per plant, i.e., 16.40 g, compared to the application of market waste compost of 10 tons/ha (P4), i.e., 13.92 g, compost charcoal husk of 10 tons/ha (P3), i.e., 10.12 g, straw compost fertilizer of 20 tons/ha (P2), i.e., 10.44 g, and liquid organic fertilizer of 4.5 ml/liter of water (P1), i.e., 8.56 g.

3.3. Weight of Tubers per Plot (g)

The type of compost fertilizer (P) had a significant effect on tuber weight per plot. Data on the weight of tubers per onion plot is presented in Table 1.

The compost fertilizer treatment had a significant effect on tuber weight per plot. The treatment of trichoderma market waste compost of 10 tons/ha (P5) resulted in the heaviest tuber weight per plot, i.e., 95.40 g, compared to the application of market waste compost fertilizer of 10 tons/ha (P4), i.e., 76.80 g,

husk charcoal compost fertilizer of 10 tons /ha (P3), i.e., 58.80 g, straw compost (P2) i.e., 61.00 g, and liquid organic fertilizer of 4.5 ml/liter water (P1), i.e., 49.80 g (Table 1 and Figure 2)

Figure 2. Weight of Tubers per Plot in The Treatment of Compost Type
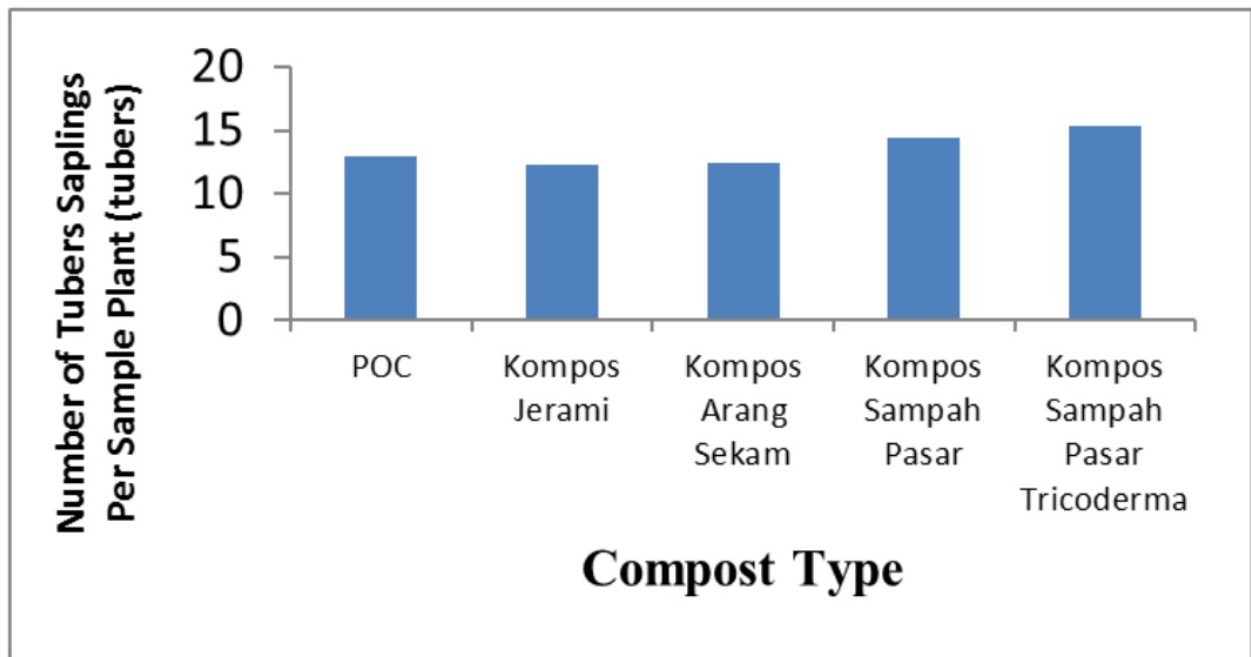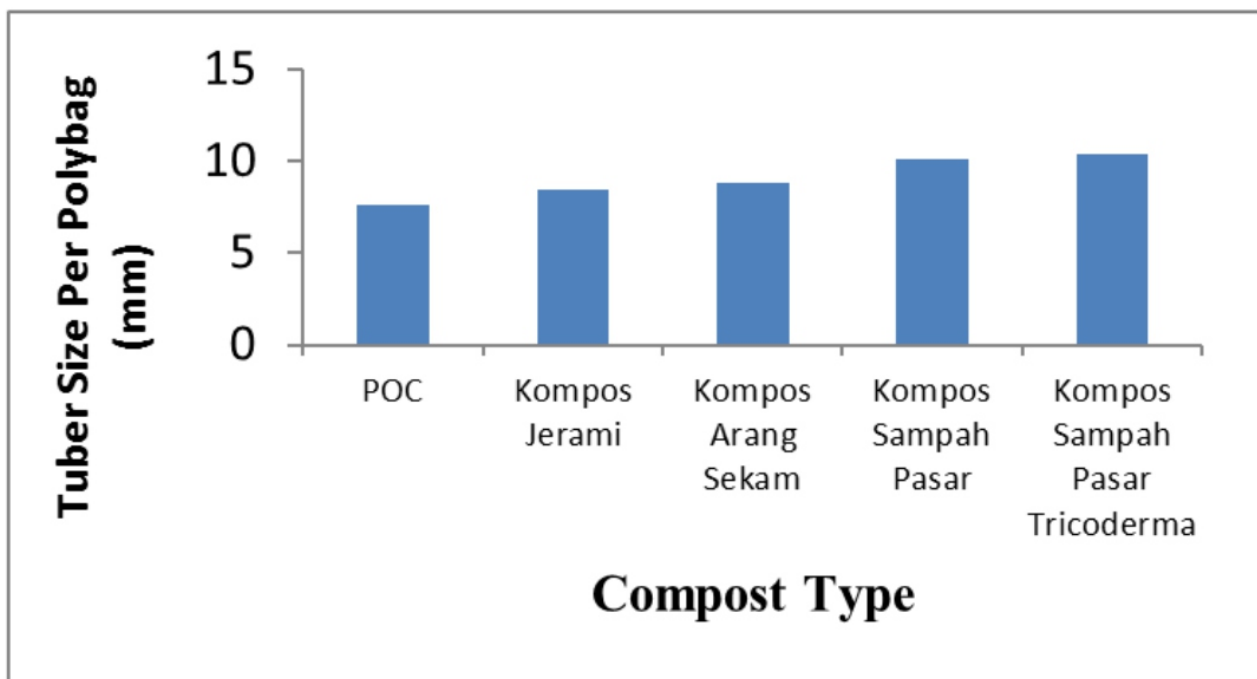

Figure 3. Size of Shallot Tubers in the Treatment of Compost Type

3.4. Number of Tubers per Plot (tubers)

The type of compost significantly affected the number of tubers per plot. Data on the number of tubers per plot is presented in Table 1.

The compost fertilizer treatment had a significant effect on the number of tubers per plot. Market waste

compost fertilizer with trichoderma of 10 tons/ha (P5) produced the highest number of tubers per plot, i.e., 87.40 tubers. It was followed with market waste compost fertilizer application of 10 tons/ha (P4) (81.60 tubers), husk charcoal compost fertilizer of 10 tons /ha (P3) (73.00 tubers), straw compost of 20 tons/ha (P2) (69.80 tubers), and liquid organic of 4.5 ml/liter of water (P1) (73.20 tubers)

3.5. Size of Tubers (mm)

The type of compost fertilizer (P) had a significant effect on tuber size. Data on the size of tubers is presented in Table 1.

The type of compost treatment had a significant effect on the size of tubers per polybag of shallots at harvest, where the treatment of market waste compost of 10 tons/ha (P5) resulted in the largest tuber size per polybag, i.e., 10.41 mm. It is followed with the application of market waste compost fertilizer of 10 tons/ha (P4) (10.15 mm), husk charcoal compost fertilizer of 10 tons /ha (P3) (8.83 mm),straw compost of 20 tons/ha (P2) (8.44mm), and liquid organic fertilizer of 4.5 ml/liter of water (P1) (7.63 mm).

The results show that the size of the tubers matched the description of the onion varieties used, thus the experiment (P5) in this study met the description standard. It is in line with Poerwowidodo [13], stating that plant growth will be optimal if the required nutrients are available in appropriate quantities and forms with the plant's needs.

This is very different from the findings in one of the dairy congresses held in Tuguegarao City, Cagayan in 2018. It was alleged that the same findings were also emphasized, reaffirming the fact that the value or benefit of using dairy cow dung is invaluable. It will recycle and reuse dairy products' manure which can cause environmental pollution and lead to an unprecedented catastrophe caused by global warming [10].

It was highly recommended in the study to adopt vermicomposting using dairy animal waste as an ingredient for the production of vermicompost. It will contribute to the mitigation of environmental pollution, especially gas emissions from dairy animal waste, and as a way of job creation, thereby increasing farmer household incomes and increasing community life.

The type of compost has a significant effect on the production of shallots. The trichoderma market waste compost gives the best results. It is suspected that the trichoderma market waste compost contains organic matter as a nutrient that is sufficient to meet the needs of plants. Elisabeth et al [5] said that the role of organic matter from the plant aspect from the weathering of organic matter can contain organic acids. These acids can increase the availability of nutrients for plants and can be absorbed by plants immediately. The role of Trichoderma sp. is very large in maintaining soil fertility and suppressing the population of pathogenic fungi. Thus, Trichoderma sp. has the potential as an active compost and as a controlling agent for pathogenic organisms [7].

Compost is the result of the decomposition of organic matter by microorganisms. This decomposition is actually a biological process by microorganisms whose energy source comes from organic waste. This biological process runs naturally because microorganisms do need energy to stay alive and reproduce. Sulistyaningsih et al. [17] stated that the bacteria present in the compost extract were quite effective in continuing the process of degradation and decomposition of organic matter. The size and type of compost raw materials affect the decomposition process sooner or later. In addition, the combination of organic waste materials is an important factor in the decomposition process. The more variations of the organic waste mixture, the better the quality of the compost that will be produced [9]. This is in accordance with the results of this study where market waste compost consisting of various kinds of organic materials such as vegetables decomposes faster than straw compost and husk charcoal compost. Elisabeth et al [5] said that the role of organic matter from the plant aspect in the weathering of organic matter can contain organic acids that can increase the availability of nutrients for plants and can be absorbed by plants immediately. Nurhayati et all [11] showed that the application of rice straw compost in the K3 treatment (75 g/polybag) had a significant effect on the moisture content of cocoa seeds by 72.01%, and in the 125 g/polybag treatment it could increase the growth of cocoa seedlings, namely high seedlings, stem diameter, number of leaves, leaf area, root volume, crown and root ratio, and dry weight.

The shallot plant is a pseudo-trunked plant with very thin stems called discs. On the disc, there are buds that can become new plants called lateral shoots or tillers which will form new discs to form new tubers [21].

In this case, the formation of the disc until the formation of tubers really needs nutrients, where the nutrients that are needed in the preparation of tissues are Phosphorus and Potassium which play a role in activating growth enzymes. Based on Poerwowidodo [13] stated that plant growth will be optimal if the required nutrients are available in quantities and forms according to plant needs.

The availability of nutrients (N, P, K) contained in the compost gives a positive response to the growth of tubers, which will be absorbed and carried to the leaves to be assimilated in the process of photosynthesis. One of the products of this photosynthesis is fructans, in which Liliaceae plants store fructans in tubers [21]. An indirect mechanism is also developed by Trichoderma sp to increase plant growth and yield through the production of phytohormones and exopolysaccharides. Trichoderma sp is known as PGPR which produces phytohormones cytokinins and gibberellins [8].

It is suspected that the addition of nutrients from the combined material for each type of compost will be better than one type of compost material. Cahaya and Nugroho [3]showed that the mixture of vegetable waste and goat feces turned into compost faster than the other variables.

4. Conclusion

1. Different types of compost produce different components of shallot production.

2. The trichoderma market waste compost P(5) produced the best results for the production of shallots.

3. The type of compost has a significant effect on the number of tubers per plant, the weight of tubers per plant, the weight of tubers per plot, the number of tubers per plot, and the onion tuber size.

4. The treatment of trichoderma market waste compost showed a higher number of tubers than other fertilizer treatments.

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Optimum Formulation Substrate for Oyster Mushroom Cultivation Using Linear Programming Model

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A B S T R A C T

The oyster mushroom (P. ostreatus), also known as the grey oyster mushroom, is currently one of the popular edible wood mushrooms consumed by people in Malaysia. Under Malaysia's National Agro-Food Policy (2011-2020), mushrooms have been identified as high-value commodities. Mushroom cultivation has become an immense potential agriculture activity in Malaysia, where oyster mushrooms are looked at as highly valued crops with low-cost technology that can bring high returns within a short time. Commonly, mushroom cultivation uses sawdust as oyster mushroom media. However, due to the increasing price of the commercial substrate of sawdust, growers are looking for a low-cost alternative substrate. Currently, researchers are looking at the potential of agriculture waste as an alternative medium for mushroom cultivation. For example, agriculture waste of empty oil palm fruit bunch and rice straw as a substrate for oyster mushrooms. Using Microsoft Excel Solver, the linear programming method was used to represent the substrate formulation. The results from this model produced an optimum 1 kg substrate formulation at a minimum cost of RM0.56 per kg. This proposed formulation satisfied the minimum and maximum nutrient requirements for oyster mushroom growth.

Keywords Linear Programming, Substrate Formulation, Oyster Mushroom

1. Introduction

There are more than 1000 species of oyster mushrooms (Pleurotus sp.) worldwide. The oyster mushroom (P. ostreatus), also known as the grey oyster mushroom, is currently one of the popular edible wood mushrooms consumed by people in Malaysia. Under Malaysia's National Agro-Food Policy (2011-2020), mushrooms have been identified as high-value commodities. This is reflected by the local markets, where it is reported that the daily demand for fresh mushrooms is around 50,000kg while the supply is only 24,000kg [1]. The oyster mushroom commodity will be grown intensively with government support.

Like any other plants, oyster mushrooms also need nutrients to grow, such as carbohydrates

(cellulose,hemicellulose and lignin), protein, fat, minerals, and vitamins. Commonly, in Malaysia, the commercial cultivation of oyster mushrooms utilises rubber tree sawdust as the medium. According to NST Business [2], there is a shortage of rubber tree sawdust due to the limited availability of rubber trees. For example, in Austria there have also been spiking trends in the price of sawdust for the past twenty years as reported in Kranzl et al. [3] and it is still increasing. This price increasing trend is similar in Malaysia, one of the global exporters of rubber trees and other timbers. The price of sawdust increased due to many factors: the limited source and the logistic cost to deliver the materials.

Oyster mushroom growers, especially small-medium growers, are having a hard time looking for another sawdust alternative where it should be low in price and sustainable. A study of substitution materials that can replace sawdust asthe main ingredient of the mushroom oyster plant medianeeds to be done. The selected substitute material should have characteristics like sawdust and sufficient nutrient content to support the growth of oyster mushrooms. In presentstudies, researchers actively look at agriculture waste as a medium to grow oyster mushrooms .

2. Substrate Ingredients for Oyster Mushroom Cultivation

Mushrooms can be grown on a wide range of substrates, and the selection of substrates is based on their availability and cost. A substrate is an essential part of mushroom production. According Onyeka et al. [4], the period of mycelium running, pinhead development, the quantity of fruiting, the cropping time, the primordial diameter, and the biological efficiency of oyster mushrooms are all influenced by the substrate medium. Hence, the ingredient selection in substrate formulation for mushroom cultivation is vital to ensure the mixed planting media can provide different kinds of nutrients and minerals required for mushroom growth.

2.1. Main Substrate

Sawdust is a by-product of lumber and wood industries and has been commonly used as a medium substrate for oyster mushroom cultivation. In a study by Shah et al. [5], sawdust reached maximum yield, biological efficiency, and quantity of fruiting bodies, making it the ideal substrate for growing oyster mushrooms. Oyster mushroom is being grown commercially in Malaysia using sawdust from rubber trees as the foundation media. However, the shortage of rubber trees has created a significant challenge for mushroom growers [6]. A new alternative substrate should be investigated to alleviate the lack of sawdust from rubber trees. Therefore, much research has been conducted to find the effectiveness of other agricultural by-products on mushroom growing. As described by Muswati et al. [7], mixing substrate can maximise mushroom yield by optimising compositional features such as water holding capacity and enhanced medium structure to achieve an optimal carbon-to-nitrogen (C/N) ratio that increases substrate efficiency. Hence,farmers can achieve their goal yields by combining

substrates that are in scarce supply.

Other lignocellulosic wastes from oil palm wastes can be utilised as an alternate substrate to cultivate the oyster mushroom. Some studies have done work on the potential of growing oyster mushrooms on palm oil mesocarp fibre, and the result proved it as an excellent substrate for Pleurotus species cultivation [6,8,10]. Empty fruit bunches (EFB), palm press fibre (PPF), an oil palm frond (OPF), sugarcane bagasse, and corn cob have a significant amount of hemicellulose, cellulose, and lignin. They can be used as a low-cost alternative to growing oyster mushrooms [8]. EFB is made up of 26.49% lignin, 32.57% cellulose, and 27.7% hemicellulose [9]. Besides containing cellulose, hemicellulose, and lignin, EFB also lacks sap, a wood component that may inhibit mushroom development [10]. The combination of rubber tree sawdust with both shredded PPF and EFB showed tremendous potential as substrates for developing oyster mushrooms [6]. However, EFB and PPF are ineffective as individual substrates for oyster mushroom cultivation. In a study conducted by Tabi et al. [11], Substrate A (100% EFB) has an insufficient amount of nitrogen (0.2%) for mycelium development, while Substrate C (100% PPF) has the lowest mushroom yield due to the low carbon content (47.2%). Therefore, fewer nutrients are available for the fruiting bodies to grow.

Rice straw is a common Pleurotus substrate in Asia due to its composition of slow-digesting carbohydrates [12]. The nutrients in rice straws are similar to those in sawdust. Rice straw is made up of 27% hemicelluloses, 39% cellulose, 13% lignin, and 9% dust [13]. Somashekhar [14] have presented research on several agricultural wastes, and according to the findings, ragi straw yielded 1.41 kg of mushroom, followed by 1.23 kg of rice straw. Finger-millet husk and rice straw performed better in terms of the number of days required for an entire spawn run in an experiment conducted by [12]. According to Utami [13] the concentration of rice straw addition that can be used to replace sawdust in oyster mushroom planted media was 15%:60%.

2.2. Supplementation

Lignocellulosic wastes are often deficient in protein, making them unsuitable for mushroom cultivation thatrequires the addition of nitrogen, phosphate, and potassium [15]. Adding supplements to the mushroom substrate isessential to improve mushroom development and yield, especially for substrates with low protein content [16].Supplementation positively affects mycelia growth andmushroom production [17].

Nitrogen supplementation is critical for mushroom growth and yield because the C/N ratio affects spawnrunning and fruiting body growth [15]. Most nitrogen levelin substrates is between 0.5-0.8 %, as in Chanakya et al.[18]; therefore, adding organic nitrogen helps to increase mushroom yields. Organic materials such as rice bran, wheat bran, and molasses are part of additives. Additives are protein and nitrogen-rich ingredients added to substrates to help mushrooms grow and produce more [19].

The effects of seven different additives, wheat bran, rice bran, soybean flour, de-oiled soybean meal, mustard cake, cotton seed cake, and cotton seed meal on mushroom yield, were investigated by [20]. The result concluded that adding 1% de-oiled soybean meal and 2.5% cotton seed cake to wheatstraw is the best way to increase Pleurotus ostreatus var. Florida yields. Salama et al. [21] evaluated the effects of wheat bran, rice bran, urea and zinc sulphate on oyster mushroom cultivation. The result shows that oyster mushroom yield and quality were improved by adding either wheat or rice bran to rice straw substrates.

Rice bran has been used widely as a nitrogen source for oyster mushroom substrate [22,10,23,9]. However, the growth of mycelium at the first stage of mushroom growth may be hampered by a high amount of rice bran [10]. Guerrero et al. [24] assessed the effect of different levels of rice bran supplementation on oyster mushroom growth and production. The results show that supplementing fermented sawdust with 15% of rice bran together with 1% brown sugar and 1% lime is the best for growing oyster mushrooms like Pleurotus Florida and Pleurotus Sajor-caju. Khan et al. [26] suggested utilising wheat bran and rice bran at a rate of 10%, while for cottonseed meal, soybean cake and groundnut cake at a rate of 3-6% on a dry weight basis of the substrate.

Lime ($CaCO_3$) is a crucial component in mushroom cultivation and has been utilised frequently in various studies to improve the pH of the substrate. For a successful oyster mushroom harvest, pH is critical. Most mushrooms thrive at pH levels close to neutral or light basic [26]. Khan et al. [26] concluded that oyster mushrooms produce a sufficient yield on cotton waste containing 2% of lime.

This study aims to evaluate the suitability of several types of lignocellulosic wastes that are ample in Malaysia for replacing rubber tree sawdust in oyster mushroom cultivation. Because of the differences in the ability of such substrates to improve nutritional and environmental requirements, as well as variations in cellulose, hemicellulose, and lignin content, productivity and biological efficiency were increased in some mixtures when compared to wheat straw alone [23]. Following that, this study aimed to find the optimal formulation substrate mixture to assess oyster mushroom growth, yield, and economic feasibility of small-scale production by utilizing only locally accessible agro-industrial by-products.

2.3. Nutritional Requirement for Oyster Mushroom Growth

Pleurotus spp. get their nutrients from a host substrate or agricultural wastes high in lignin, cellulose and hemicellulose [20]. Carbon, nitrogen as well as other minerals in the substrate such as S, Mg, Ca, K, P, and some lower-level minerals such as Mn, Fe, Zn, Mo, and Cu with an ash percentage of 2.5 to 15.7, is essential as a source of nourishment for Pleurotus spp [27]. Mushrooms need carbon and nitrogen for structural and energy requirements [28]. Cellulose and hemicellulose are carbon sources, while protein and amino acids are nitrogen sources [29].

Therefore, any agricultural waste containing cellulose, hemicellulose, or lignin can be a suitable substrate for growing oyster mushrooms [30].

Total nitrogen (N), total carbon (C), and the carbon/nitrogen ratio (C/N) are essential elements that influence mycelium colonisation and fruiting body development in oyster mushrooms [12]. Depending on the mushroom species, the required amount of each nutritional component varies.

2.3.1. Nitrogen Content

Nitrogen is an essential nutrient for mycelium growth [11]. Nitrogen is required for cellular development and various metabolic processes, including the synthesis of proteins and enzymes [20]. Supplementing nitrogen with organic sources helps to improve mushroom biological efficiency [31]. However, an excessive amount of nitrogen in the substrate can encourage the growth of mould Mishra et al. [25] and thus prevent mushroom growth [7].

Supplement addition can raise the temperature of the substrate by 2-3 degrees Celsius or even more [25]. Therefore, it is crucial to find the optimal nitrogen level in the media culture for mushrooms depending on their species.

2.3.2. Carbon Content

Carbon sources are essential components of the nutritional media to promote the best growth of fungi [32]. The carbon compounds that fungi use for sustenance offer the energy the fungus needs to carry out its life functions [33]. In straw nitrogen, carbon can easily be obtained fromcellulose, hemicellulose, and lignin; however, it is mainly bound and cannot be accessed until it is released by enzymes [20]. Palm pressed fibre, sugarcane bagasse, and corncob have high levels of lignin, hemicellulose, and cellulose, which contribute to the carbohydrate or carbon source needed for the growth of oyster mushrooms [8].

2.3.3. C/N Ratio

C/N ratio is crucial in determining the best substrate composition for oyster mushrooms. C/N ratio is essential as it influences the fermentation process Abella et al. [34], mycelium growth, fruiting body formation, and development [30]. Though oyster mushroom demands more carbon and less nitrogen, most of the primary substrate materials, such as cereal straw, cotton waste, and sawdust, require the addition of nitrogen sources such as wheat and rice bran to achieve the appropriate C/N ratio for oyster mushroom [34]. Each mushroom species needs an optimal C/N ratio in the culture substrate for growers to attain the best output in the shortest time possible [35].According to Miles and Chang [33], a C/N ratio of 32- 150 is best to produce Pleurotus spp. To attain an optimal C/N ratio, the kinds, and formulations of substrates for mushroom culture should have a balance of carbon and nitrogen [36]. The ideal nutrient ratio of culture media components stated in a study by Lee and Cho [37] is a medium

with total nitrogen and carbon levels of 0.65-1.11% and 47.0-49.1%, respectively

3. Method to Formulate Substrate

Multiple studies have utilised mixture design to find the mushroom substrate's optimal proportion. Mixture design is a response surface experiment used to see whether there is a combination of ingredients that create an optimal response to either maximise or minimise a property [38]. Simplex-centroid designs are one of the common methods in mixture design for identifying a unique collection of components at various centroids that maximise or minimise the response variable based on the goal function. Kasina et al. [39] applied the simplex-centroid designs method to find the optimal local substrate mixture that maximises oyster mushroom yield. Simplex-centroid designs were proven highly efficient and successfully identified and established the ideal substrate combination for oyster mushroom production.

The simplex-lattice design is the most often utilised approach for substrate screening among all mixture-design methods as it provides a thorough analysis of the relationships in different aspects and the goal values, as well as the quantitative relationships between the various substrates and evaluation indicators, which are produced by using regression analysis [40]. D-optimum approach of the simplex-lattice design was used to optimise Grifola frondosa cultivation on crop straw (corn cob, corn straw, rice straw, and soybean straw) as a substrate, and the optimised model determined the use of crop straw as a substitute for sawdust in the substrate composition [41]. Wu et al. [42] applied a simplex-lattice design to their study to find the optimal proportion of agro-residues consisting of wheat straw, corn straw and soybean straw as the primary substrate to replace sawdust and cottonseed hulls in the production of Pleurotus pulmonarius.

A mathematical model is a popular approach to finding the ideal proportional ingredients mixture. The standard optimisation methods used widely in finding an optimal composition for fertiliser are the goal programming model [43-45] and the linear programming model [45,46]. However, the use of this method in formulating mixture substrate has been less applied in mushroom cultivation. Only one study can be found utilising goal programming in finding the optimal medium composition for oyster mushroom growth which was reported in [48]. Therefore, in this study, the optimum formulation substrate for oyster mushrooms by using a linear programming model is discovered.

3.1. Methodology

The model for this study is taken from a study done by Aldeseit [46] where the author utilised the LP technique in formulating the optimal composition of the three synthetic fertilisers. The developed model's primary goal is to reduce the cost of formulated oyster mushroom substrate. The mathematical model in this study is built using the sets and parameters listed below. Let,

n = Total number of ingredients used in the model

u = Index for the type of ingredients in the model, where u = 1, 2, 3, ..., n

v = Index for the type of nutrient in the model, where v = 1, 2

Yu = The cost (per kg) of ingredients u The cost (per kg) of ingredients u

xu = The amount of ingredient u in the formulated substrate

auv = The amount of nutrient v contain in ingredient u

Cv = Maximum requirements of nutrient v in the formulated substrate

bc = Minimum requirements of nutrient v in the formulated substrate

du = The maximum inclusion rate of ingredient u in the formulated substrate

eu = Minimum requirements of nutrient u in the formulated substrate

The model is provided in the following format: Minimize

$$Z = y_u x_u \qquad (1)$$

Following are the limitations that apply to the objective function:

$$\sum_{u=1}^{n} x_u = 1 \ kg \qquad (2)$$

Constraint (2) represents the total amount of formulated substrate for oyster mushroom must satisfy 1 kg.

$$c_v \geq \sum_{u=1}^{n} a_{uv} x \geq b_v \qquad (3)$$

Constraint (3) represents the nutrient requirement limit of nutrient v contain in the formulated substrate for oyster mushroom.

$$d_u \sum_{u=1}^{n} x_u \geq x_u \geq e_u \sum_{u=1}^{n} x_u \qquad (4)$$

Constraint (4) represents the maximum and minimum inclusion rate of ingredients in the substrate.

$$x_u \geq 0 \qquad (5)$$

Constraint (5) is a non-negativity constraint that ensures that the optimal value of ingredients in the substrate is not negative.

The model's expansion is written as follows:

$x1$ = The amount of rice straw in the formulated substrate

$x2$ = The amount of EFB in the formulated substrate

$x3$ = The amount of PPF in the formulated substrate

$x4$ = The amount of sawdust in the formulated substrate

$x5$ = The amount of rice bran in the formulated substrate

$x6$ = The amount of limestone in the formulated substrate

By referring to Table 1, the extension of the objective function can be stated as follows,

Minimize

$$Z = 0.5x_1 + 0.5x_2 + 0.8x_3 + 1.5x_4 + 0.8x_5 + 1.2x_6$$

Subject to:

1. Constraint (2) can be written as follow

$$x1 + x2 + x3 + x4 + x5 + x6 = 1$$

2. The expansion for Constraint (3) can be expressed as follows, using Tables 1 and Table 3 as references. Nitrogen minimum limits in the total substrate,

$$0.0065x_1 + 0.002x_2 + 0.014x_3 + 0.0032x_4 +$$
$$0.0073x_5 + 0.000x_6 \geq 0.0065$$

3. Nitrogen maximum limits in the total substrate,

$$0.0065x_1 + 0.002x_2 + 0.014x_3 + 0.0032x_4 +$$
$$0.0073x_5 + 0.000x_6 \leq 0.00111$$

4. Carbon minimum limits in the total substrate,

$$0.5176x_1 + 0.488x_2 + 0.472x_3 + 0.4604x_4 +$$
$$0.1487x_5 + 0.000x_6 \leq 0.47$$

5. Carbon maximum limits in the total substrate,

$$0.5176x_1 + 0.488x_2 + 0.472x_3 + 0.4604x_4 +$$
$$0.1487x_5 + 0.000x_6 \leq 0.491$$

6. The expansion for Constraint (4) can be expressed as follows, using Table 2 as reference. Rice straw minimum inclusion rate

$$x_1 \geq 0.15 \sum_{n=1}^{6} x_u$$

7. Rice straw maximum inclusion rate

$$x_1 \leq 0.6 \sum_{n=1}^{6} x_u$$

8. EFB maximum inclusion rate

$$x_2 \leq 0.5 \sum_{n=1}^{6} x_u$$

9. PPF maximum inclusion rate

$$x_3 \leq 0.5 \sum_{n=1}^{6} x_u$$

10. Rice bran maximum inclusion rate

$$x_5 \leq 0.15 \sum_{n=1}^{6} x_u$$

11. Limestone inclusion rate

$$x_6 = 0.02 \sum_{n=1}^{6} x_u$$

12. The expansion for Constraint (5) can be expressed as follows,

$$x_u \geq 0, \text{ where } u = 1, 2, 3, ..., 6$$

This model is then solved using Excel Solver Application.

Table 1. Type, Nutrient Contents and Approximate Costs (per kg)

Ingredients	Type	N (%)	C (%)	Cost (RM/kg)
Rice Straw	Main	0.65	51.76	0.50
EFB	Main	0.2	48.8	0.50
PDF	Main	1.4	47.2	0.80
Sawdust	Main	0.32	46.04	1.50
Rice bran	Supplement	0.73	14.87	0.80
Limestone	Supplement	-	-	1.20

Source: [49,6,48,50] and market survey

Note: N-Nitrogen, C-Carbon, EFB-Empty Fruit Bunches, PPF-Palm press fibre

Table 2. Minimum and Maximum Rate of Each Ingredient in the Substrate

Ingredients	Minimum Inclusion (%)	Maximum Inclusion (%)
Rice Straw	15	60
EFB	0	50
PDF	0	50
Sawdust	0	100
Rice bran	0	15
Limestone	2	2

Note: EFB-Empty Fruit Bunches, PPF-Palm press fibre

Source:[13,6,10,26]

Table 3. Minimum and Maximum Rate of Each Ingredient in the Substrate

Nutrient	Minimum Requirement (%)	Maximum Requirement (%)
Nitrogen	0.65	1.11
Carbon	47.0	49.1

Source: [37]

4. Proposed Formulation Substrate for Oyster Mushrooms

Six types of ingredients in the substrate were considered in formulating substrate for oyster mushrooms. The six ingredients were rice straw, EFB, PPF, sawdust, rice bran and limestone, denoted as x_1, x_2, x_3, x_4, x_5 and x_6, respectively. They were chosen due to their accessibility and availability in Malaysia. One of the benefits of using this substrate is that it is readily available and cost-effective. The results displayed in this section were produced by using Microsoft Excel Solver.

Table 4 displays the optimal amount of ingredients and its total cost per kg. The total cost of the formulated substrate was obtained by 'sumproduct' the optimal amount of each ingredient in the substrate with the cost of ingredient per kilogram.

Based on the result, to achieve an optimal substrate for oyster mushrooms, the mixture must be composed of 0.6 kg of rice straw, 0.222 kg of EFB, 0.15 kg of PPF, 0.008 kg of rice bran and 0.02 kg of limestone. Rice straw has the most significant ratio in the substrate, probably due to its high nitrogen level and lower cost than other main substrates. Sawdust was not chosen probably due to its high cost, and the other lower-cost ingredients were able to provide sufficient nutrients for the substrate.

Table 4. Ingredient Amount in Formulated Substrate for Oyster Mushrooms

Ingredients	Cost (RM/kg)	Amount (kg)
Rice straw	0.5	0.600
EFB	0.5	0.222
PDF	0.8	0.150
Sawdust	1.5	0
Rice bran	0.8	0.008
Limestone	1.2	0.020
Total	0.5614	1

Table 5 shows the formulated substrate's nutrient content and the required nutrient limit that should be contained in the oyster mushroom substrate.

Table 5. Nutrient Content of the Formulated Substrate and the Required Nutrient Limit in Substrate

Nutrients	Amount (kg)	Minimum Required	Maximum Required
Nitrogen	0.0065	0.0065	0.0111
Carbon	0.491	0.47	0.491
C/N ratio	75.538		

The formulated substrate has satisfied the minimum and maximum nutrient requirement for an oyster mushroom that was suggested in [37]. When formulating mushroom substrate, it is crucial to consider the ratio of carbon-to-nitrogen (C/N) in the substrate. Miles and Chang [33] state that the optimal C/N ratio for oyster mushroom production is 32-150. The C/N ratio for the formulated substrate is 75.538, and it is within the considered range. Therefore, this formulated substrate is safe to be utilised for oyster mushroom cultivation as all the conditions needed are fulfilled.

5. Conclusions

The formulated substrate that consists of 0.6 kg of rice straw, 0.222 kg of EFB, 0.15 kg of PPF, 0.008 kg of rice bran and 0.02 kg of limestone was shown to have a nutrient content that is needed by mushroom optimally in order to grow. Compared to current practice for mushroom substrate, sawdust used is 50% and 50% supplemented ingredients. The optimal mixtures do not contain sawdust which is intelligent profit to the growers. The proposed optimal mixtures, too, have shown an affordable cost for mushroom growers, which estimated cost is RM0.56 per kg.

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Adulteration Test of Chicken DNA (Gallus gallus) by the Multiplex PCR Method Using a Specific Primer for Mitochondrial DNA Co1

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A B S T R A C T

Food adulteration cases continue to increase in line with the increasing public need for food. Multiplex PCR is one type of PCR method that is often used to detect adulteration in food. This study aims to confirm the performance of primers that have been designed based on the CO1 gene that specifically amplifies chicken DNA by a primer specificity and sensitivity test using single and multiplex PCR method. The results showed that the primer had specific properties because it is capable of amplifying the target DNA according to its size. The sensitivity test showed that the CO1 primers for chicken have a sensitivity of up to 10-3 ng/µl similar to the pig's D-loop primers, while the CO1 primers for horses have a sensitivity of up to 10-2 ng/l similar to the Cyt b for dogs. Sampling test using five types of meatballs by the multiplex PCR method showed that the samples detected animal DNA that matched the respective raw materials for making it, while sampling using commercial meatballs showed that only three samples contained bovine DNA and it could be concluded that the other two samples had been adulterated with chicken meat.

Keywords Adulteration, DNA, Multiplex PCR, Primer, Chicken

1. Introduction

Adulteration or food counterfeiting can be described as a deliberate act to add, replace, alter, and misrepresent a food product and, food packaging and to provide incorrect information on labels for the purpose of deceiving consumers in order to obtain economic benefits [1]. Animal food products are one of the food products that often counterfeited. This is because the selling price of animal food products is relatively more expensive when compared to vegetable food products [2]. Chicken meat and its processed products are a good source of animal protein because it contains complete essential amino acids and has a relatively cheaper price compared to bovine or goat [3]. This not only causes an increase in public consumption of chicken meat, but also has the potential to increase counterfeiting of processed chicken products [3]. Cases of counterfeiting not only occur with the addition of non-halal animal meat to processed chicken or halal meat products, but also can be use as an adulterating material for a product, such as addition of chicken in meatball which isclaimed as made from bovine [4].

Detection of counterfeiting in food products can be done with DNA-based detection [5]. DNA generally has stable properties at high temperatures and pressures. Besides that, DNA can also be found in almost all parts of the cell of organisms [6]. PCR is a molecular method for duplicating pieces of

DNA up to millions of times in a relatively short time [7]. This multiplication is inseparable from the use of enzymes and a pair of primers that are specific to the target DNA [8]. Multiplex PCR is a PCR technique used to amplify various targets in one reaction [7] by using several pairs of primers so the test process can run faster and can save energy and costs [9]. Primer design is one of the processes that must be considered in the multiplex PCR method, especially the annealing temperature of each primer so that cross-hybridization does not occur [10]. The use of mitochondrial DNA molecules as the basis for primer design is recommended compared to nuclear DNA, because the cell consist more mitochondrial DNA than nuclear DNA and it has high specificity for each species because it is only derived from the DNA of the female parent [11]. The genes most often used as markers of animal or meat species include cytochrome b (cyt b), 12S and 16S ribosomal RNA subunits and the displacement loop (D-loop) region [12].

This study aims to examine the adulteration and contamination of chicken DNA in processed meat products and to determine the specificity and sensitivity of the specific primer cytochrome c oxidase subunit 1 (CO1) as a result of the research design in identifying chicken DNA using the multiplex PCR method.

2. Materials and Methods

2.1. DNA Sample
In this study, the DNA samples used were fresh meat and processed meat products, namely meatballs. Fresh meat consists of chicken (Gallus gallus), dog (Canis lupus familiaris), pork (Sus scrofa domesticus) and horse (Equus caballus). The meatball samples consist of chicken meatballs, dog meatballs, pork meatballs, horse meatballs and a mixture of chicken, dog, pork and horse meatballs made by the researcher and commercial bovine meatball samples obtained from supermarkets and meatball shops in Malang, East Java, Indonesia.

2.2. Primer
The primers used in the study consist of the chicken CO1 gene primer designed by the researcher, the pig D-loop gene [13], the horse CO1 gene [14], the dog Cyt b gene [15] and the bovine 12S rRNA gene [16]. The primer specifications were showed in Table 1. The primer for chicken identification was designed during this study based on the cytochrome c oxidase subunit 1 (CO1) of mitochondrial DNA of chicken, whereas the design of other primers were obtained from some other papers.

2.3. Chicken Forward and Reverse Primer Design
The design of a specific primer for chickens (Gallus gallus) was conducted based on the nucleotide

sequence of the CO1 gene in chickens contained in GenBank (NCBI with access number GenBank ARJ60440.1). The DNA sequence was downloaded in FASTA form and then aligned with other animal species using software Clustal X. Alignment was carried out using BioEdit software to obtain a conserved area.

Table 1. Primer specifications

Primer	Sequence Order (5` to 3`)	Number of Nitrogen Bases	Amplicon Size (bp)	Source
CO1 (Chicken)	Forward: 5'-C TTTACCTAATTTTCGGCAC -3'	20	306	Study
	Reverse: 5'-TTCTACGGTAGATGAGGCTA-3'	20		
D-loop (Pig)	Forward: 5'-TACTTCAGGACCATCTCACC-3'	20	835	Haunshi *et al.,* 2009
	Reverse: 5'-TATTCAGATTGTGGGCGTAT-3'	20		
CO1 (Horse)	Forward: 5'- CACCAGCCCTATCCCAATAT -3'	20	113	Hakiki, 2020
	Reverse: 5'- GAGAAGCATGGTAATGCCTG -3'	20		
Cyt b (Dog)	Forward: 5'-CCTTACTAGGAGTATGCTTG-3 '	20	101	Rahman *et al.,* 2014
	Reverse: 5'-TGGGTGACTGATGAAAAAAGA-3'	20		
12S rRNA (Bovine)	Forward: 5'-ACCGCGGTCATACGATTAAC-3 '	20	155	Cahyadi *et al.,* 2018
	Reverse: 5'-AGTGCGTCGGCTATTGTAGG-3 '	20		

2.4. DNA Isolation Using the Method of Chloroform:isoamyl alcohol

DNA isolation was initiated by weighing 20 mg of fresh meat and meatball samples and added with STE buffer (0.1M NaCl, 0.001M EDTA and 0.01M Tris-Cl). The samples were crushed using micropestle until completely destroyed, added 40 µl SDS 10% and 20 µl pro-K and vortexed for 20 seconds. The sample solution was incubated in a thermomixer at 55 °C overnight at 800 rpm.

Incubated samples were centrifuged for 10 minutes at a speed of 12000 rpm at a temperature of 29°C, separated between the supernatant and pellet solution. The supernatant containing crude DNA solution was then taken and placed in a new tube with a size of 1.5 ml and added 1x volume of Chloroform:Isoamyl alcohol (24:1) as much as 400 µl and 40 µl of 5M NaCl, then homogenized and centrifuged, on the supernatant formed by adding 1x volume of Chloroform:Isoamyl alcohol (24:1) as much as 300 µl. Then the mixture was inverted and centrifuged, separated as much as 200 µl of the supernatant formed into a new tube and added 600 µl of cold ethanol (temperature 4°C) and 40 µl of 5M NaCl, then the mixture incubated at -20°C for 2.5 hours. After incubation, the samples were centrifuged at 12000 rpm at 4°C for 5 minutes. The pellets obtained were tapped to release and added 70% ethanol, and the mixture was centrifuged at 12000 rpm at 4°C for 5 minutes. The supernatant was discarded and the pellet was tapped so that it was released and dried using a thermomixer at 55°C, after drying add50 µl of TE buffer pH 7,6. The samples were then stored at -4°C for 15 minutes to measure the concentration and purity of the DNA obtained using nanodrops [17].

2.5. DNA Amplification Using Single PCR and Multiplex PCR Methods

The DNA amplification of the samples was carried out to test the performance of the specific primers that had been designed through specificity and sensitivity tests using the single PCR and multiplex PCR methods and then confirmed using electrophoresis. The primer specificity test was conducted to see the specific nature of a primer in amplifying the target DNA. The primer sensitivity test was carried out to determine how small the target DNA concentration could be detected by the primer. The concentrations of chicken DNA used were 10 ng/µl, 10-1 ng/µl, 10 -2 ng/µl, 10-3 ng/µl, 10-4 ng/µl, and 10-5 ng/µl.

PCR amplification was carried out using single PCR and multiplex PCR methods. In the single PCR, the PCR mix formulation used had a total volume of 10 µl, consist of My Taq Red Mix 5 µl, primer forward 0,5 µl, primer reverse 0,5 µl, nuclease free water 3 µl and DNA sample 1 µl. As for the multiplex PCR method, the PCR mix formulation used had a total volume of 21 µl, consist of 10 µl My Taq Red Mix, 0,5 µl forward primer, 0,5 µl reverse primer, 3 µl nuclease free water and DNA sample 1 µl. The PCR program used include hot start 95°C 5 minutes, continued with 30 cycles of denaturation 94°C 1 minute, annealing 52°C 1 minute, extension 72°C 1 minute, final extension 72°C 7 minutes and cooling 4°C.

3. Results and Discussion

3.1. Chicken Forward and Reverse Primer Design

Primer design was manually done using Clustal X2 and BioEdit. The first step in the primer design process was to perform multiple alignments of the chicken CO1 gene sequence with other animal CO1 gene sequences. The chicken CO1 gene sequence (Gallus gallus access code GenBank KX987152.1) along 1550 bp was aligned with the dog CO1 gene (Canis lupus familiaris access code GenBank KJ522809.1) along 1544 bp, pig (Sus scrofa domesticus GenBank access code KJ789952.1) along 1544 bp and mice (Rattus norvegicus access code GenBank NC_001665.20) along 1544 bp. The primers designed at the time of the study consisted of forward primers and reverse primers. The forward primer attachment sites started from the sequence of the 55th base to the 75th base, while for the reverse primer attachment site starting from the 341st base sequence to the 361st base with the number of amplicons being 306 bp. Optimal primer length ranges from 18-30 bases [18], and the optimal GC percentage for the PCR process generally ranges from 40-60%. The low % GC content of the primers can cause the primers not to adhere effectively to the DNA template [19]. The optimal melting temperature ranges from 50-65°C, and the optimal annealing temperature ranges from 37-60°C [18]. Based on Table 2, it can be seen that the primers that have been designed have met the criteria of a good primer, where the base length of the designed forward and reverse primers is 20 bp, the GC percentage is 40-45%, the annealing temperature is 51-53°C, the melting temperature is 56-58°C and the length of targeted amplicons is 306 bp.

Table 2. Criteria for the specific primer design of the chicken CO1 gene (Gallus gallus)

Primer Type	Primer Sequence	Base Length (bp)	%GC	Ta (°C)	Tm (°C)	Number of Amplicons
Primer Forward	5'-CTTTACCTAATTTTCGGCAC-3'	20	40	51	56	
Reverse Primer	5'-TTCTACGGTAGATGAGGCTA-3'	20	45	53	58	306

3.2. PCR Optimization

The primer used during the research was optimized by the gradient PCR method to determine the most optimum annealing temperature, the annealing temperature used consisted of 5 different temperatures, the temperature was selected based on the calculated melting temperature (Tm) value, and the selected temperature range was 48°C, 50°C, 52°C, 54°C, and 56°C. The annealing temperature optimization with this gradient method has the aim of getting the optimal PCR product [20].


Figure 1. Visualization of PCR Annealing Temperature Optimization. M: 100 bp marker, 48-56: Chicken DNA band formed at annealing temperature of 48-56 °C

Based on the results of visualization using 2% agarose (Figure 1), the chicken DNA bands were formed in all optimization temperature ranges with thick single bands. The temperature chosen as the temperature with optimal amplification for the PCR process was 52 °C because of the thick single band shape and the size that matched the target amount, which was 306 bp. The optimum temperature chosen based on the optimization results with the gradient method is a single DNA band that is clearly formed, not blurry or smeared and thick [21].

3.3. Primer Specificity Test for Single PCR Method

This specificity test was carried out to avoid primer identification errors and ensure that the primers that have been designed have properties that are specific to the target DNA. In this study, chicken DNA and other animal DNA including dogs, pigs and horses were used to test the specificity of the designed

chicken CO1 primer.
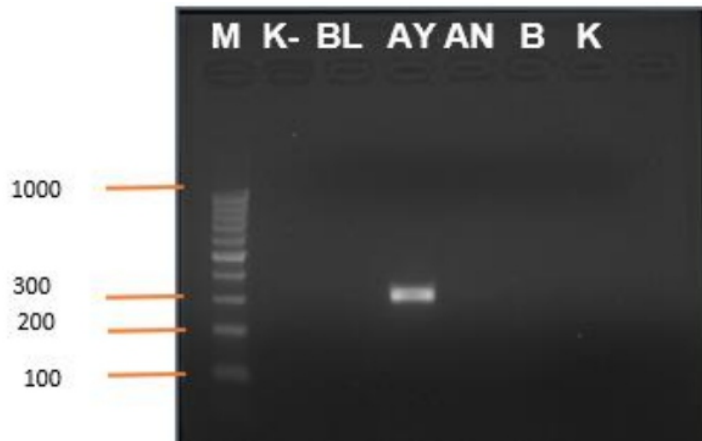
Figure 2. Visualization of Single PCR Method Specificity Test. M: 100 bp marker, K-: Negative control, BL: Blank, AY: chicken DNA, AN: dog DNA, B: pig DNA, K: horse DNA

The results of visualization using 2% agarose gel (Figure 2) showed that DNA bands were only formed in the column with the code AY which was the target DNA, and the target DNA was chicken DNA with a target length of 306 bp. The absence of the formation of DNA bands other than in the target DNA column indicates that the primers that have been designed have properties that are specific to the target DNA.

3.4. Primer Specificity Test for Multiplex PCR Method

In the specificity test using the multiplex PCR four types of animal DNA and different primers were used in the same reaction. The primers consisted of the Co1 gene primer to amplify chicken DNA 306 bp, the Cyt b gene primer to amplify dog DNA 103 bp, the gene D primer -loop to amplify 835 bp pig DNA and the CO1 gene primer to amplify 113 bp horse DNA.
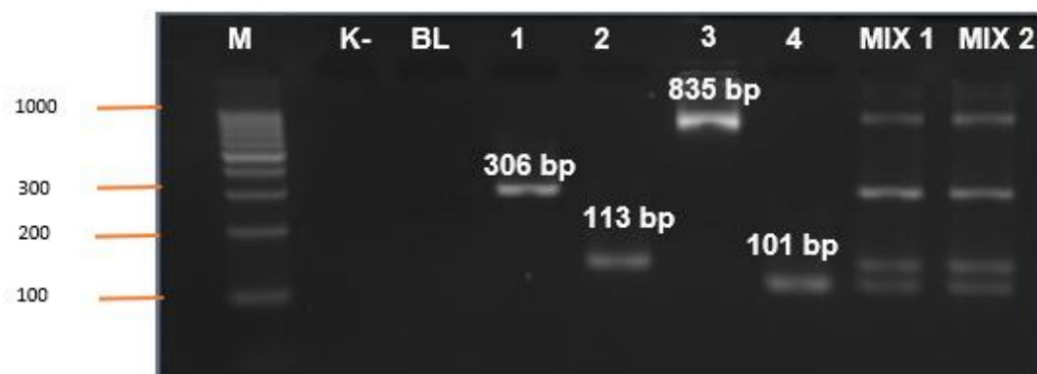
Figure 3. Visualization of the Multiplex PCR Method Specificity Test. M: Marker 100 bp, K-: Negative Control, BL: Blank, 1: Chicken DNA, 2: Horse DNA, 3: Pig DNA, 4: Dog DNA, Mix 1&2: Mixed DNA (chicken, dog, pig and horse)
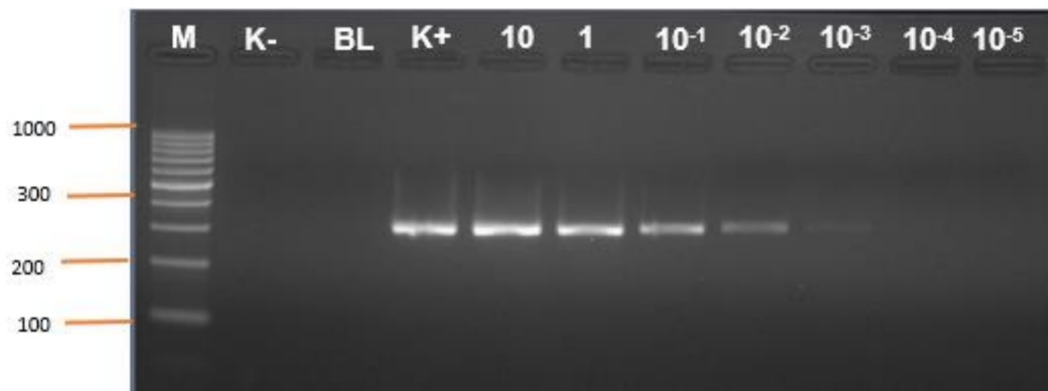
Figure 4. Visualization of Sensitivity Test of Single PCRM Method: Marker, K-: Negative control, BL: Blank, K+: Positive Control, 10-10 -5: Chicken DNA dilutions

Based on the results of the primer specificity test using the multiplex PCR method (Figure 3), it can be seen that the primers used during the test have specific properties for the targeted DNA fragment. This is indicated by the formation of four DNA bands in the mix 1 and mix 2 columns. DNA bands that are formed in the mix 1 and mix 2 columns have different product sizes according to their respective target lengths. After the visualization process using the electrophoresis method, the target DNA bands will separate specifically which can be seen based on the thickness of the target DNA, and the thickness of the target DNA formed is based on the weight and amount of the targeted DNA [22].

3.5. Test the Sensitivity of the Single PCR Method

Primer sensitivity test is a test carried out to determine the smallest concentration limit that can be detected by a primer that has been designed. After the sensitivity test, the LOD (Limit of Detection) will be known or how sensitive the primer has been designed to detect the smallest concentration of the targeted template DNA [23]. PCR primer sensitivity testing was carried out using DNA from chicken meat, where chicken DNA with an initial concentration of 50 ng/µl was made in several dilution series, namely 10 ng/µl, 1 ng/µl, 10 -1 ng/µl, 10 -2 ng/ µl, 10 -3 ng/µl, 10 -4 ng/µl and 10 -5 ng/µl.

Based on the visualization results of the chicken Co1 primer sensitivity test (Figure 4), it can be seen that a single band of chicken DNA is formed in the DNA column with a concentration of 10 ng/µl, 1 ng/µl, 10 -1 ng/µl, 10 -2 ng/µl, and 10 -3 ng /µl, so it can be stated that the chicken CO1 primer has good sensitivity because it is able to detect sample DNA up to a concentration of 10 -3 ng/µl, where the positive control used is chicken DNA with a concentration of 50 ng/µl and the negative control is ddH2O. The single band formed is getting thinner or its intensity is getting lower as the DNA concentration decreases. The number of bands produced by each primer depends on the distribution of homologous sites in the genome [24].
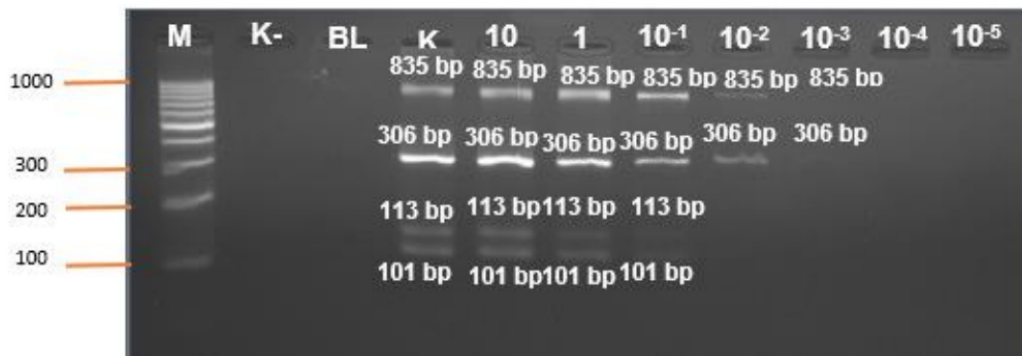
3.6. Sensitivity test of multiplex PCR method

The sensitivity test of the multiplex PCR method was carried out using several series of dilutions with concentrations of 10 ng/µl, 1 ng/µl, 10 -1 ng/µl, 10 -2 ng/µl, 10 -3 ng/µl, 10 -4 ng/µl and 10 -5 ng/µl. Based on the visualization results of the sensitivity test using the multiplex PCR method (Figure 5), it can be seen that each primer used has a different sensitivity. In the chicken primer from the C01 gene with the target of 306 bp and the pig primer from the D-loop gene with the target DNA being 835 bp, a single band of DNA was formed in the DNA column with a concentration of 10 ng/µl, 1 ng/µl, 10 -1 ng/µl, 10 -2 ng/µl, and 10 -3 ng/µl, while for dog primers from the Cyt b gene with a target of 101 bp and horse primers from the CO1 gene with a target DNA of 113 bp. Both types of primers were only able to detect sample DNA up to a concentration of 10 -2 ng/µl. There are several factors that can affect the success of sensitivity testing with the multiplex PCR method including the number of primers used during the reaction, competition between primers during the reaction, the amount of DNA template used, the concentration and purity of the template DNA to be used in the reaction and the ingredients. Others are used during the reaction so that the reactions that occur in multiplex PCR are more complex [25].

3.7. Meatball Sample Sampling Test

The meatball samples consisted of four types of meat from different animal species, namely chicken, dog, pork and horse, which were processed with a composition of 50% animal meat (chicken, dog, pork and horse) and meatballs which were the result of a mixture of several types of meat (chicken, dogs, horses and pork) which were processed with a composition of 20% on each meat.

Based on the visualization results (Figure 6), in column A containing a sample of chicken meatballs a DNA band is formed which has a size of 306 bp; in column B which contains a sample of dog meatballs a DNA band is formed with a size of 101 bp; in column C which contains a sample of pork balls a DNA band is formed with a size of 835 bp; in column D which contains a sample of horse meatballs, one DNA band with a size of 113 bp is formed. So, it can be concluded that the primer is able to amplify the DNA of chickens, dogs, pigs, and horses specifically with the multiplex PCR method on meatball samples. While in column E which contains samples of meatballs made from a mixture of chicken, dog, pork and horse meat, four DNA bands of different sizes are formed which indicate that the four types of primers used during the sampling test with the multiplex PCR method were able to amplify the targeted DNA simultaneously. Specifically, the four DNA bands formed were chicken DNA with a size of 306 bp, dog DNA with a size of 101 bp, pig DNA with a size of 835 bp and horse DNA with a size of 113 bp. After visualization with agarose each sample DNA band will be formed according to the size of the intended product [26].

Figure 5. Visualization of the Multiplex PCR Method Sensitivity Test. M: Marker 100 bp, K-: Negative Control, BL: Blank, 10-10 -5: DNA dilutions (chicken, dog, pig and horse)

Figure 6. Visualization of Meatball Sampling Test with Multiplex PCR Method. M: Marker 100 bp, K-: Negative Control, BL: Blank, K+: DNA of chicken, dog, pork and horse, A: Chicken Meatballs, B: Horse meatballs, C: Pork meatballs, D: Dog meatballs, E: Meatballs mixed meats (chicken, dog, pork and horse)

Figure 7. Visualization of commercial bovine meatball sampling test. M: Marker 100 bp, K-: Negative Control, BL: Blank, K+: DNA of chicken, pork,and bovine, 1: bovine meatball brand A, 2: bovine meatball brand B, 3: bovine and chicken meatball brand C, 4: bovine meatball of stall A, 5: bovine meatball of stall C

3.8. Commercial Meatball Sample Sampling Test

Samples of commercial meatballs were taken based on 3 brands of bovine meatballs sold in supermarkets and 2 samples of meatballs sold by meatball traders in Malang, East Java, Indonesia.

Four of the five samples of commercial meatballs used were meatballs which claimed to be made from bovine meatballs, while one of five commercial meatballs used was meatballs which are claimed to be made from a mixture of bovine and chicken meatballs. Sampling of commercial meatballs which are claimed to be bovine meatballs is carried out to authenticate the truth of the claims of these commercially sold bovine meatballs. In the positive control commercial bovine meatball sampling test used consisting of pork, chicken and bovine DNA, based on the visualization results in the positive control column three DNA bands of different sizes were formed. These bands represent chicken DNA (306 bp), pig DNA (835 bp) and bovine DNA (155 bp).

Based on the visualization results (Figure 7) in columns 1 and 2 containing bovine meatballs from brands A and B, a DNA band with the same size as the positive control bovine DNA was formed, which was 155 bp, which indicated that the sample brand A bovine meatballs contained bovine DNA, while in column 3 containing samples of brand C meatballs, two DNA bands with different sizes were formed, namely chicken DNA 306 bp and bovine DNA 155 bp which indicated that this meatball contained two DNA from different animal species, namely chicken and bovine and in columns 4 and 5 which contained bovine meatball samples from stalls A and B did not form bovine DNA bands with a size of 155 bp but formed 306 bp chicken DNA bands, so the claim that the meatballs were made from bovine was incorrect or inappropriate, because the meatball samples did not detect bovine DNA. The success of DNA identification in samples by the multiplex PCR method is influenced by the primers used during the sampling test, where the primers used function as a barrier to the amplified DNA fragments. Besides that the primer also gives a hydroxyl group (-OH) at the 3' end which will be required during the extension process [18].

4. Conclusions

CO1 primer designed to detect chicken DNA (Gallus gallus) has specific properties and is sensitive to target DNA based on testing with the Single PCR and PCR multiplex methods. Based on the sampling test on samples of meatballs using the multiplex PCR method using five types of meatballs, it shows that, in the meatball samples, animal DNA was detected that corresponded to the raw material for making it, while in the sampling test on commercial bovine meatballs from five samples of bovine meatballs, two of them did not contain bovine DNA and were falsified with chicken.

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Settlement Preference of the Lobster Larvae on the Different Shelter Materials in the Larvae Shelter Device (LSD)

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A B S T R A C T

The lobster life phase is eggs, phyllosoma, puerulus, juvenile, and young lobster. The metamorphose coincides with its inshore movement and ends up into adult lobsters that inhabit the continental shelf area. The wild population of lobsters is determined by the survival rate of the puerulus. Fish larvae aggregating devices have been given significant results as a shelter or refuge area for several fish species. This device is then referred to as the Larva Shelter Device (LSD). The question is whether there is a preference for lobster larvae on attractor or shelter materials. The innovation of shelter materials in LSD is cement sack paper, waring nets and gunny sacks. The number of lobster larvae in different materials was analyzed. The research was carried out in February-May 2021 at Mutiara Beach Karanggongso of Prigi Bay, Tasikmadu, Watulimo, Trenggalek, East Java, Indonesia. The identification method of puerulus species is based on taxonomic and morphological characteristics. Initial identification is done by recording and photographing species, then adjusting to the experiences of fishermen compared to Jones and Dao 2010. The study found there were 3 types of puerulus species collected including puerulus of Panulirus ornatus, P. homarus, and P. versicolor. The highest species presentation in the three shelter materials was P. homarus. The data indicate that different shelter materials had no significant effect on the species composition of the spiny lobster puerulus.

Keywords Lobster, Prigi, Puerulus, Shelter, Refuge,

1. Introduction

Lobster (Panulirus sp.) is a species that belongs to the crustacean subphylum with spiny lobster type. The Indo-West Pacific region has 11 species of lobster from the genus Panulirus with 6 of them found in Indonesian waters. In Indonesia, 6 types of lobsters can be found from the Palinuridae family including Scalloped spiny lobster (Panulirus homarus), Ornate spiny lobster (Panulirus ornatus), Painted spiny lobster (Panulirus versicolor), Longlegged spiny lobster (Panulirus longipes), Pronghorn spiny lobster (Panulirus penicillatus), and Mud spiny lobster (Panulirus polyphagus). Those six lobster species can be found in the South Java waters. (Panulirus homarus and P. penicillatus) are the dominant lobster species in the South Java waters. Lobster (Panulirus sp.) has a local name as udang barong or udang karang [1–3].

The lobster life cycle after the eggs hatch consists of several phases, starting from the phyllosoma phase which moves planktonically or swims against the current so that in this phase, it struggles to reach offshore or shallow waters. Then it continues to puerulus phase whose movements are already nectonic or actively swimming, which can be seen from moving towards the coast and settling in shallow coastal

waters. In the next phase, the larvae (puerulus) will change their skin or moult to become juvenile which begins to look pigmented. The time spent from egg to juvenile ranges from 10-18 months [4,5]. Lobster (Panulirus sp.) is a species that has potential and economic value in Indonesia. The economic value is known from the high demand in the domestic and export markets [6]. The potential for puerulus of lobster that lives in the Indonesian seas is estimated to reach 20 billion fish each year. This number is estimated based on the adult phase when breeding female lobsters can lay up to 460,000 eggs. The social value of catching lobster pueruli has become the livelihood of small-scale fishers. Lobster particularly puerulus fishing can improve the welfare of coastal fishing households [7–9].Lobsters have been utilized not only at the adult life phase but also at the early life stage or puerulus phase. Since national lobster production still depends on the wild stock, the puerulus utilization has become the potential pressure on the sustainability of the wild stock of lobster. The high economic value of lobster has merely come from the demand from the international market. The largest utilization is only through fishing or collecting from the wild as the cultivation is absent and still in research development. It has a major concern about the availability of lobsters in nature [10,11]. Therefore, in accordance with the regulations from FAO which were revealed to the Minister of Marine Affairs and Fisheries Regulation (PERMEN KP No. 17 of 2021) to create sustainable fishery activities, this research is related to the composition of the species to different shelter attractors materials or shelters. It is necessary to do to determine the wild lobster population enhancement as well as to support national lobster culture development program. Shelter attractors set in the larvae shelter device (LSD) that serve as a refuge for lobster larvae from natural mortality or low survival rate during their early life history can be considered in making a policy in the scope of the ecosystem approach to fisheries management. The study was aiming to find out the best material for larvae shelter device as a larvae refugia [12].

2. Materials and Methods

This research was conducted at Mutiara beach in the waters of Prigi Bay Karanggoso, Tasikmadu village, Watulimo sub-district, Trenggalek district, East Java by collecting data on the catch of lobster pueruli species in each treatment and interviews to determine the identification of lobster clear seed species. The treatment in this study used 3 types of shelter materials, namely cement paper, burlap/gunny sacks, and waring/monofilament (Figure 1). The data obtained were processed with Microsoft Excel and SPSS software to determine the difference in catches.

The identification method is carried out by recording and photographing species, then adjusting to the habits and experiences of fishermen in identifying lobster clear seed species. The reference used as a key to identify the characteristics is the book by Clive Jones entitled "Identification of tropical palinurid lobster puerulus and juveniles" [13,14]. The analysis used to test species composition between shelter materials were Chi-square, One Way ANOVA or Analysis of Variance is commonly

referred to as the F test, and Tukey test for further analysis.

Figure 1. Types of shelter materials, left-right: cement paper, burlap/gunny sacks, and waring/monofilamennet

3. Result and Discussion

3.1. Puerulus Species Identification

The results of the identification of pueruli collected from Mutiara beach in the waters of Prigi Bay Karanggoso, Tasikmadu village, Watulimo sub-district, Trenggalek district as many as 4 species, namely Panulirus ornatus, Panulirus homarus, Panulirus versicolor, and Panulirus polyphagus.

The morphological characteristics of the puerulie species are as follows:

1. Lobster Mutiara (Panulirus ornatus)

The physical color is transparent, the eye color is brownish-black, and there is a bulge at the tip of the antenna that can light up. The antennas are characterized by having a black ring in the middle and the area after the ring and the tip before the protrusion is milky white (Figure 2).

Figure 2. Puerulus of Ornate spiny lobster (Panulirus ornatus)

2. Lobster Pasir (Panulirus homarus)

The physical color is transparent, the eye color is brownish-black. The antennas are transparent and characterized by having a black band in the middle (Figure 3).

Figure 3. Puerulus of Scallopped spiny lobster (Panulirus homarus)

3. Lobster Bambu (Panulirus versicolor)

The physical color is transparent, the eye color is brownish-black. The antennas are characterized by milky white color (Figure 4).

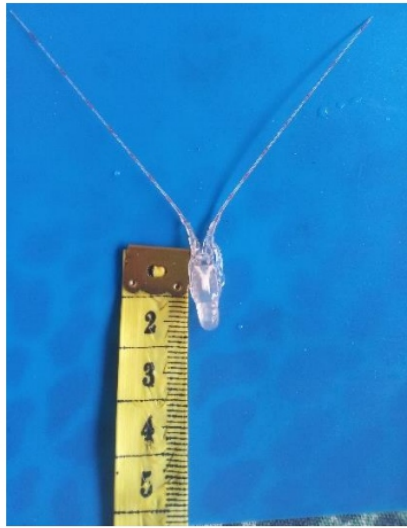**Figure 4.** Puerulus of Painted spiny lobster (Panulirus versicolor)

4. Lobster Pakistan (Panulirus polyphagus)

The physical color is white, the eye color is black. The antennas are characterized by many red bands

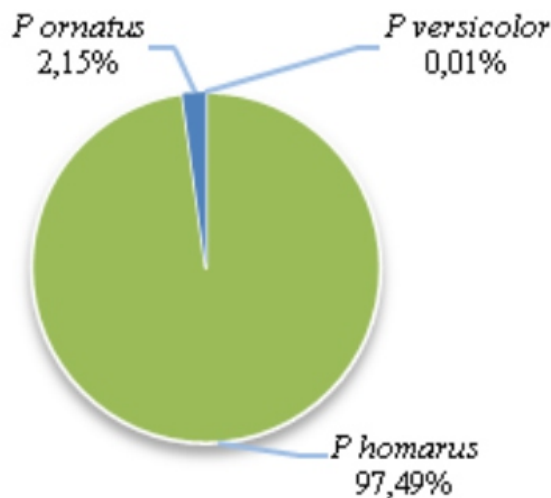along with the antenna (Figure 5).

Figure 5. Puerulus of Mud spiny lobster (Panulirus polyphagus)

3.2. Puerulus Species Compositions on Each Shelter Materials
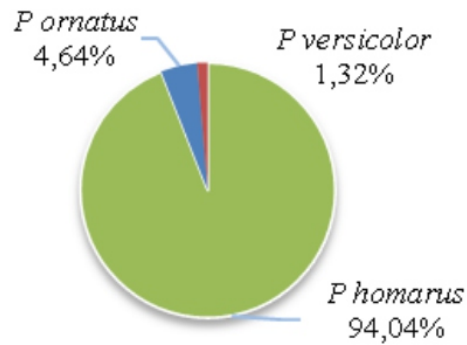
The graph of the percentage of catch of puerulus in each treatment of different shelter materials is as follows:

Figure 6. Proportion (percentage) of the number of individuals of puerulus species of Cement Paper Shelter

The results that nener nets with shelter attractors made of cement paper obtained the proportion of P. ornatus(lobster mutiara) of 2.15%, P. homarus (lobster pasir) of 97.49% and P. versicolor (lobster bambu) by 0.01% (Figure 6)
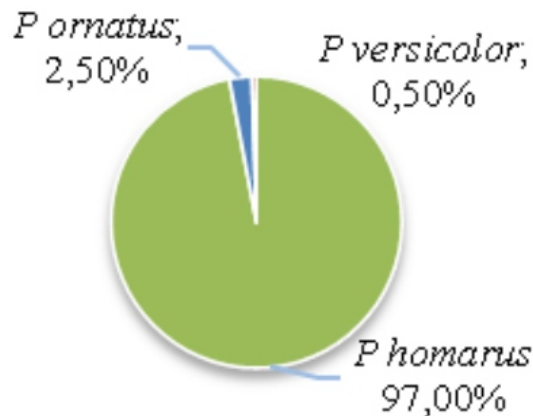
Figure 7. Proportion (percentage) of the number of individuals of puerulus species of Burlap Sack Shelter

Shelter attractors made from jute/burlap/guny sacks caught the proportion of P. ornatus (lobster mutiara) of 4.64%, P. homarus (lobster pasir) of 94.04% and P. versicolor (lobster bambu) by 1.32% (Figure 7).

Shelter made of waring got (P. ornatus) of 2.5%, (P. homarus) of 97.0 % and (P. versicolor) by 0.5% respectively (Figure 8).

The results of the proportions can be concluded descriptively that puerulus of P. homarus caught from Mutiara Beach has the highest value for any type of treatment of the three different shelter attractor materials. While the lowest proportion value is lobster bamboo (P. versicolor).

Figure 8. Proportion (percentage) of the number of individu of puerulus species of Waring or monofilament net Shelter

3.3. Puerulus Species Composition Analisis between Shelter Materials

The analysis of the composition of the lobster pueruli species using chi-square test aims to identify and prove the three treatments of shelter materials (cement paper, burlap sacks, and waring/monofilament) in a statistical approach whether there is a significant difference or not. The software used to perform this analysis was SPSS (Statistical Package for Social Science).

The results of the chi-square analysis in Table 1 can be seen in the Asymp value. Sig is 0.481, so because of the Asymp value. Sig 0.481 > 0.05, it can be concluded that accept H0 and reject H1. The conclusion is that the difference in shelter material has no significant effect on the composition of the number of individuals of each species caught.

Table 1. Chi-square test Effect of Shelter Attractor Material and Clear Lobster Seed Species

Shelter materials		Puerulus species		
		P ornatus	*P. homarus*	*P. versicolor*
Cement sack	*Observed*	6	272	1
	Expected	8	269	1
Guny sacki	*Observed*	7	143	2
	Expected	4	145	1
Waring/ monofila ment	*Observed*	5	194	1
	Expected	5	193	1
Chi-square = 3.480, df = 4, Asimp. Sig = 0.481				

The results of this study are different from the results according to Priyambodo et al. [15], the type of shelter materials attractor and depth influence the number of lobsters pueruli species. The reason for the absence of differences is that the waters used as a place to test the treatment are homogeneous. This statement is reinforced by the results of research which, according to Erlania et al. [16], the highest distribution of pueruli was found in the characteristics of shallow waters, high turbidity levels and muddy sand bottoms. According to Mujiyanto et al. [17], the Karanggonggso location in Prigi Bay has a mixed type of bottom substrate which was about 50% of fine sand and muddy, the rest is a mixture of broken coral and shells. This opinion is in line with what happened to fishermen on Mutiara beach, where fishermen lobster pueruli in waters with mud-sand bottom substrate.

3.4. Statistical Analysis of Puerulus Species Composition on Each Shelter Materials

Data analysis with One Way ANOVA serves to see whether there are differences in the composition of the number of individuals of each species of puerulus in each shelter attractor. The software used to perform this analysis was SPSS.

3.4.1. Puerulus Species Composition on Cement Sack Analysis of the treatment of cement paper shelter attractor material with One Way ANOVA test through SPSS software obtained the following results
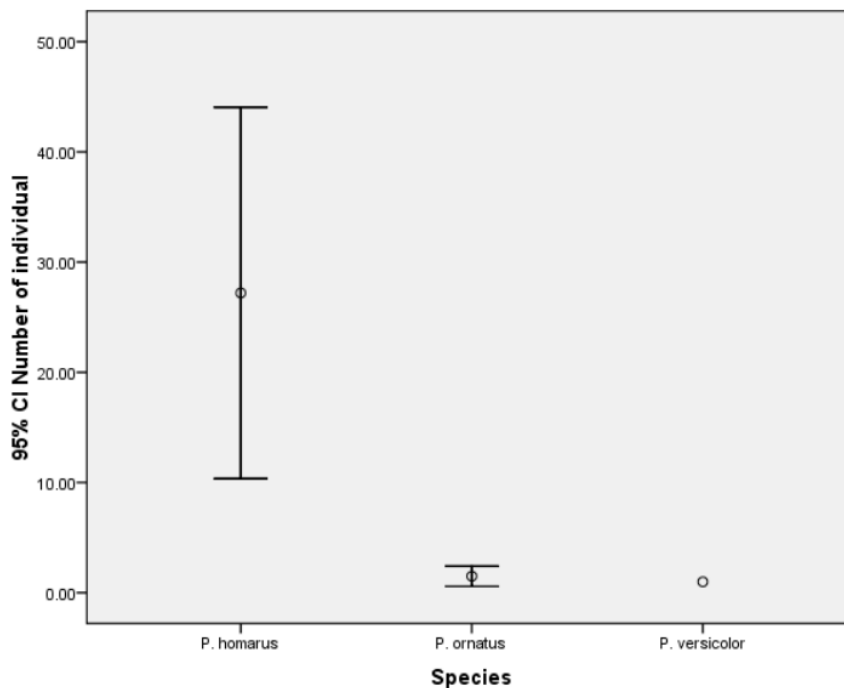
(Table 2).

The inductive statistics showed that there is a difference in the number of clear lobster seed catches in the shelter attractor made of cement paper (ANOVA, F=12.988, df=2, p<0.001) (Table 2), so further tests were carried out. The Tukey test was performed which looks more detail on the differences in species composition. The test found that P. homarus had significant differences compared to P. ornatus and P. versicolor (Table 3, Figure 1).

Table 2. One Way ANOVA of puerulus species on cement sack

Number of species	Sum of Squares	df	Mean Square	F	Sig.
Species	4,807.400	2	2,403.700	12.988	.000
Galat	4,996.900	27	185.070		
Total	9,804.300	29			

Table 3. Tukey test of puerulus species on cement sack Shelter.

Puerulus species	N	Subset for alpha = 0.05	
		1	2
P. versicolor	10	.1000	
P. ornatus	10	.6000	
P. homarus	10		27.2000

Figure 9. Puerulus species composition on cement sack shelter material

3.4.2. Puerulus Species Composition on Gunny Sack

One Way ANOVA on the number of each species collected from the gunny sack shelter materials are shown in Table 4.

Table 4. One Way ANOVA of puerulus species on guny sack

Species Number	Sum of Squares	df	Mean Square	F	Sig.
Species type	1,261.667	2	630.833	7.962	.002
Galat	2,139.300	27	79.233		
Total	3,400.967	29			

The test shows that there is a significant difference in the number of puerulus catches in the shelter attractor made of guny/burlap sack (ANOVA, F=7.962, df=2, p<0.05) (Table 4). the Tukey test found that P. homarushad significant differences compared to the P. ornatus and P. versicolor (Table 5, Figure 10).

Table 5. Tukey test of puerulus species on guny sack shelter

Puerulus Species	N	Subset for alpha = 0.05	
		1	2
P versicolor	10	.2000	
P ornatus	10	.7000	
P homarus	10		14.2000
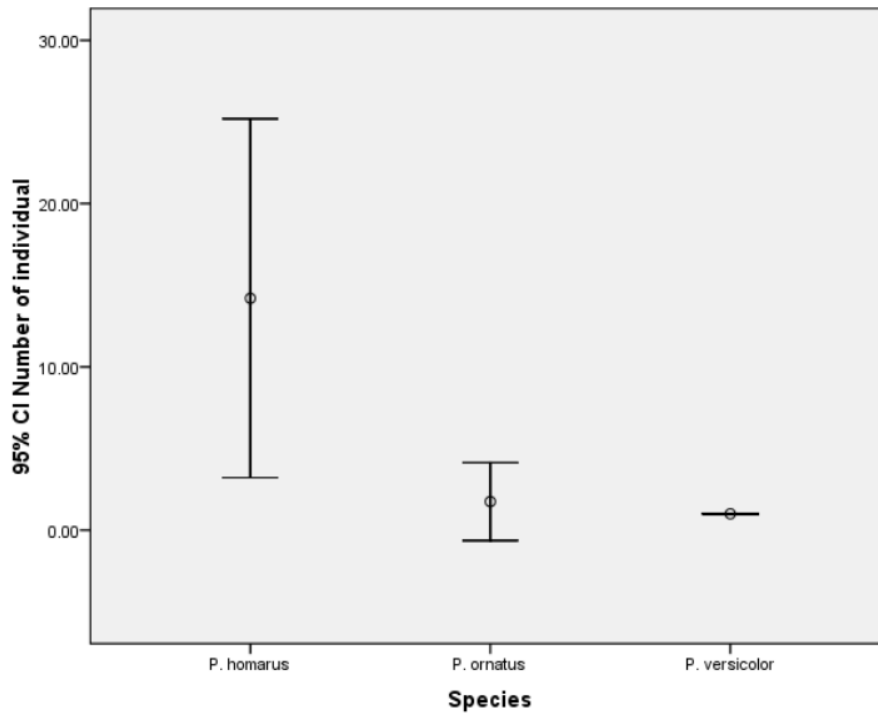
Figure 10. Puerulus species composition on guny sack shelter material

3.4.3. Puerulus Species Composition on Waring/Monofilament Net

Analysis of treatment of waring shelter materials with One Way ANOVA test through SPSS software got the following results.

**Table 6.** One Way ANOVA of puerulus species on waring/monofilament materials

Number Species	Sum of Squares	df	Mean Square	F	Sig.
Species type	2,432.867	2	1,216.433	7.465	.003
Galat	4,399.800	27	162.956		
Total	6,832.667	29			

Table 7. Tukey test of puerulus species on monofilament shelter

Puerulus Species	N	Subset for alpha = 0.05	
		1	2
P versicolor	10	.1000	
P ornatus	10	.5000	
P homarus	10		19.4000

The test shows that there is a significant difference in the number of puerulus catches in the shelter attractor made of waring/monofilament net (ANOVA, F=7.465, df=2, p<0.05) (Table 6). The Tukey test found that P. homarus had significant differences compared to P. ornatus and P. versicolor (Table 7, Figure 11).

The treatment of three different shelter materials (cement paper, gunny sacks, waring) revealed that the dominance of the puerulus species overall is scalloped spiny lobster (Panulirus homarus) followed by the other two species. The dominance of the clear seed species of sand lobster is reinforced by the opinion of Haryono et al. [18], in which the waters in the southern districts of Central Java are dominated by the scalloped spiny lobster. The results of this study are reinforced by the location of the installation of nener nets which are covered with mud which is the habitat of sand lobsters. This result is corroborated by Holthuis [19], in which the sand lobster is very fond of sand and mud habitats, hiding under rocks and areas close to river mouths. Shelter attractors were made of logs, waring nets, rice sacks and cement paper, the largest catch was obtained by clear seed species of sand lobster with shelter attractors made of cement paper which were operated at the bottom of the water. It was predicted that the dominant inhabitant of the South Java Sea and the Indian Ocean, in general, is P. homarus [2, 3].
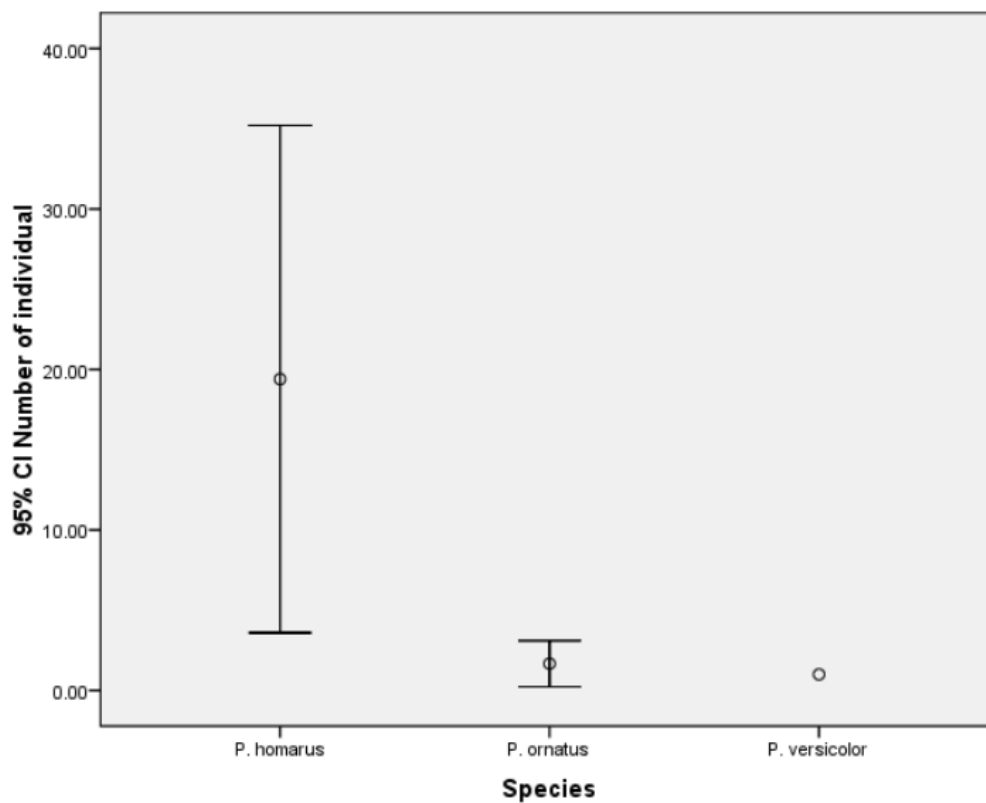
Figure 11. Puerulus species composition on monofilament shelter material

4. Conclusion

There were four species lobsters' pueruli found during the study. The species are Panulirus ornatus, P. homarus, P. versicolor and P. polyphagus. The most prominent feature is the pattern on the antenna. The ornate lobster puerulus (P. ornatus) has the characteristics of having a black ring, the antenna area after the ring to the tip before the head is milky white, and the tip of the antenna head can glow orange-black. The puerulus of scalloped spiny lobster (P. homarus) have a characteristic antenna with a black ring in the middle and a clear antenna at the tip. The painted spiny lobster (P. versicolor) has completely clear antennae. The puerulus of mud spiny lobster (P. polyphagus) have antenna that have many red rings arranged sequentially to the tip. Different types of shelter attractor materials (cement sack paper, burlap/guny sacks, and waring/monofilament) did not affect the species composition of lobster larvae. Overall P. homarus is the dominant inhabitant in those three different shelter materials followed by P. versicolor and P. ornatus. The need for further research on the effect of the combination of environmental factors and shelter attractor materials on the puerulus species composition.

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