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COMPARATIVE STUDY OF SAWDUST MEDIA AND FERMENTED MIXTURE OF SAWDUST-COW DUNG FOR REARING *Oryctes rhinoceros* (COLEOPTERA: SCARABAEIDAE) LARVAE

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ABSTRACT

Newly emerged *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) adults are required to test the virulence isolates of *Alphanudivirus oryrhinocerotis* (Lefavirales: Nudiviridae). *Alphanudivirus oryrhinocerotis* was formerly known as *Oryctes rhinoceros nudivirus* (OrNV). The tested emerging adults were all in healthy condition and uniform in age. This study aimed to analyze two types of breeding media for *O. rhinoceros* larvae: sawdust media (SM) and a mixture of sawdust and cow dung in a 1:1 ratio (MSC), the latter media had been fermented for three months. For the experiment, second- and third-instar *O. rhinoceros* larvae were maintained in 2-liter containers containing moist SM until they metamorphosed into adults. Male and female adults emerging from the SM were then placed together in a container with some sawdust, fed with sugar cane, and their lifespans were observed. The MSC medium was used to maintain third-instar *O. rhinoceros* larvae until they developed into adults. The emerged adults from the MSC were individually placed in 2-liter containers with some sawdust and sugar cane as food, and their lifespans were observed. Various parameters were measured, including the number of days it took for larvae to develop from third instar into adults, the percentage of adult emergence, sex ratio, infection rate of *Metarhizium anisopliae* fungi, the mean lifespan of adults in the laboratory, and the longest individual survival time. The research showed that SM and MSC media did not make a significant difference in the percentage of adult emergence, sex ratio, and *M. anisopliae* infection rate. Group maintenance of *O. rhinoceros* imagoes results in longer age compared to single maintenance. Females kept in groups have a longer lifespan than males, and males kept singly have a longer lifespan than

females. Adults with a length of 4 cm were produced from third-instar larvae reared in both SM and MSC media. Based on this finding, the sawdust media is suggested to be used as breeding media for *O. rhinoceros* larvae for economical and practical reasons.

Keywords: Cow dung, light bulb, rearing media, sawdust, sugarcane

ABSTRAK

Imago *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) yang baru muncul diperlukan untuk menguji virulen isolasi dari *Alphanudivirus oryrhinocerotis* (Lefavirales: Nudiviridae). Virus ini sebelumnya dikenali sebagai *Oryctes rhinoceros nudivirus* (OrNV). Imago yang diuji kesemuanya dalam keadaan sihat dan umurnya seragam. Kajian ini bertujuan untuk menganalisis dua jenis media pembiakan larva *O. rhinoceros* iaitu media habuk papan (SM) dan campuran habuk papan dengan najis lembu dalam nisbah 1:1 yang telah difermentasi selama tiga bulan (MSC). Untuk eksperimen, larva *O. rhinoceros* instar kedua dan ketiga dikekalkan dalam bekas 2 liter yang mengandungi SM lembap sehingga ia bermetamorfosis menjadi imago. Imago jantan dan betina yang muncul dari SM kemudiannya diletakkan bersama dalam bekas dengan beberapa habuk papan, diberi makan dengan tebu, dan jangka hayat ia diperhatikan. Media MSC digunakan untuk memelihara larva instar tiga hingga muncul menjadi imago. Imago dari media MSC dipelihara secara tunggal dalam bekas 2-liter yang diberi sedikit serbuk habuk papan dan tebu sebagai makanan, dan jangka hayat ia diperhatikan. Pelbagai parameter diukur termasuk bilangan hari yang diambil untuk larva berkembang daripada instar ketiga kepada imago, peratusan kemunculan imago, nisbah jantina, kadar jangkitan kulat *Metarhizium anisopliae*, purata jangka hayat imago di makmal dan tempoh masa paling lama kelangsungan hidup individu. Kajian menunjukkan bahawa media SM dan MSC tidak menunjukkan perbezaan signifikan dalam peratusan kemunculan dewasa, nisbah jantina dan kadar jangkitan kulat *M. anisopliae.* Imago yang dipelihara berkelompok memiliki umur lebih panjang dibandingkan imago yang dipelihara secara tunggal. Betina yang dipelihara berkelompok memiliki jangka hayat lebih panjang daripada jantan,dan jantan yang dipelihara tunggal mempunyai jangka hayat yang lebih lama daripada betina. Imago berukuran 4 cm dihasilkan dari larva instar ketiga yang dipelihara baik dalam media SM mahupun MSC. Berdasarkan hasil ini, disarankan menggunakan SM sebagai media untuk memelihara larva *O. rhinoceros* atas sebab lebih murah dan mudah diperolehi.

Kata kunci: Najis lembu, mentol, media pembiakan, serbuk gergaji, batang tebu

INTRODUCTION

The primary breeding material for *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) consists of organic matter that has not fully decomposed. This organic matter includes coconut leaves, sago, sugar cane, *Oncosperma tigillarium*, oil palm, dead palm trunks (still standing or fallen), empty oil palm bunches, organic waste, bran heaps, cocoa pods, bagasse, haystacks, sawdust, manure from various livestock such as cows, buffalos, and horses, sago flour remnants, coconut husks, cocoa trunks, kapok, sugar cane and remaining mill residues (Cumber 1957; Gressitt 1953; Kalshoven 1980; Pallipparambil 2015; Wikardi 1980). Once organic material has completed its decomposition, it becomes unsuitable for larvae breeding sites (Kalshoven 1980). Adult *O. rhinoceros* bore into the tops of host plants to access liquid sap. The primary host plants for these imagoes are coconut (*Cocos nucifera*), oil palm (*Elaeis guineensis*), and other *Elaeis* spp. as the family of Arecaceae (Gressitt 1953; Molet 2013). In addition, many other Arecaceae families, some members of Asparagaceae, Bromeliaceae, Casuarinaceae,

Cyatheaceae, Pandanaceae and Poaceae families have also been reported suffer to *O. rhinoceros* attacks with less effect (Gressitt 1953; Molet 2013). The distribution of *O. rhinoceros* as the main pest follows the distribution of coconut and oil palm plantation areas (Pallipparambil 2015). Rafie and Amit (2024) have reported chemical insecticide usage in controlling the imagoes of *O. rhinoceros* burrow within oil palms.

Oryctes rhinoceros larvae must be reared in laboratory settings to produce healthy emerged adults of the same age for assessing the virulence of an *Oryctes rhinoceros nudivirus* (OrNV) isolate. The recent species name of OrNV is *Alphanudivirus oryrhinocerotis* that follows the binomial system (van Oers et al. 2023). *Alphanudivirus oryrhinocerotis* classified as a Baculovirus without occlusion bodies within the *Nudiviridae* family, genus *Alphanudivirus* (Jehle et al. 2013), infects both larval and imago stages of *O. rhinoceros* in the mesenteric epithelial cells and fat bodies (Huger 2005; Jehle et al. 2013). The International Committee on Taxonomy of Viruses (ICTV) has determined 15 levels of the virus hierarchy, namely: Realm; Subrealm; Kingdom; Subkingdom; Phylum; Subphylum; Class; Subclass; Orders; Suborders; Family; Subfamily; Genus; Subgenus; and Species. The divergence spectrum of genetic virosphere is accommodated by the 15 virus rankings (Gorbalenya et al. 2020).

A higher taxonomic level of Nudiviridae family are Naldaviricetes class and Lefavirales order (van Oers et al. 2023). The Naldaviricetes class is an abbreviation of the words Nuclear Arthropod Large DNA Viruses or large DNA viruses that infect arthropods. The Naldaviricetes grouping is based on the *pif* gene, the gene that regulates the mechanism of infection in the host. The Lefavirales order is grouped based on homologous genes for late phase transcription for the formation of RNA polymerase (*lef*-4, *lef*-8, *lef*-9). The name Lefavirales is taken from the word last-expression-factor (Schopenhaver 2022).

Various *Oryctes rhinoceros*-rearing media have been reported. Schipper (1976) reported using a mixture of dry cow dung and rotting kapok wood (in a 1:1 ratio) without prior fermentation. Twenty healthy larvae from the field were placed in a 23 cm x 32 cm x 53 cm plastic container filled with this dry cow dung and rotting kapok medium, which was replaced every six weeks until the larvae metamorphosed into pupae. Schipper (1976) also added 200 larvae to a drum filled with cow dung and decaying kapok wood, replenishing these materials as needed. The pupae were then separated into 400 ml cans and kept until adult emergence. Moslim et al. (2010) utilized decomposing oil palm fruit bunches to rear *O. rhinoceros* from larvae until adulthood. Kalshoven (1980) reared *O. rhinoceros* using a mixture of sawdust and cow dung (MSC) that had been sterilized. Wikardi (1980) used sawdust plus cow dung in a 1:1 ratio without sterilization but through fermentation.

Sawdust media (SM) is more readily available in Java than oil palm fruit bunches (Moslim et al. 2010) or kapok wood (Schipper 1976). Additionally, using sawdust as a larval breeding medium is more manageable compared to the process of fermenting sawdust with cow dung. This study aimed to compare the efficacy of two laboratory-rearing media for *O. rhinoceros*: sawdust alone (SM – sawdust media) and MSC in a 1:1 ratio that had been fermented for three months, in producing metamorphosed emergent adults.

MATERIALS AND METHODS

A comparison study between sawdust (SM) and a fermented mixture of sawdust with cow dung (MSC) for rearing *O. rhinoceros* larvae was conducted at the Insect Pathology Laboratory, IPB University, Bogor, West Java, Indonesia.

Larvae Rearing in Sawdust Media (SM)

Second- and third-instar *O. rhinoceros* larvae were collected from a sawdust heap in the Parung Kuda sub-district, Sukabumi Regency, West Java, Indonesia (GPS coordinates: -6.820678, 106.764761). A total of 130 larvae were brought to the laboratory and identified as *O. rhinoceros* using the Scarabaeidae larvae identification key (Beaudoin-ollivier et al. 2000). Larvae were reared in the laboratory from March until December 2017, with each individual recorded its data including: average days of larvae metamorphosed into adult, number of dead larvae, number of emergent adults, sex ratio, lifespans of adult, survival ability of adults, and incident of death larvae due to external causes such as the fungus *Metarhizium anisopliae* and death from other causes.

Sawdust from Jabon wood (*Neolamarckia cadamba*) obtained from sawmills in the Jasinga sub-district, Bogor Regency, West Java, Indonesia was used. Plastic containers measuring 20 cm in diameter and 20 cm in height, filled with moist sawdust, were utilized for rearing the larvae. The sawdust was replaced with new material if consumed by the larvae. Larvae were reared until they developed into pupa and adults. Emergent adults were housed in plastic containers with sawdust at the bottom and fed with sugarcane. For mating conditions, one male and three female imagoes were placed in each container and observed until death. The rearing room was equipped with a lamp covered with green plastic containers to provide light resembling moonlight.

Larvae Rearing in Fermented Mixture of Sawdust with Cow Dung (1:1) (MSC)

Third-instar *O. rhinoceros* larvae were taken from rotting coconut trunks in the Tayu sub-district, Pati Regency, Central Java, Indonesia (GPS coordinates: -6.538083, 111.056032). A total of 100 larvae were collected and brought to the laboratory.The larvae identification and rearing methods were conducted similarly to Larvae Rearing in SM**,** with the only difference being the replacement of the media with MSC media. MSC was prepared by combining 7-day-old cow dung with Jabon wood sawdust in a 1:1 ratio. The mixture was stirred thoroughly and covered with plastic sheets for 3 months. During the fermentation process, the MSC media maintained a warm temperature, and after the 3 months, the media reached room temperature.Emerged adults were individually housed in plastic containers with a small amount of sawdust at the bottom and fed with sugarcane. The rearing room was equipped with a lamp covered with a green plastic container to simulate moonlight. The observations were also conducted with similar variables as rearing in Sawdust Media (SM) from Februari 2019 until Januari 2020.

Data Analysis

The observational data of *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) including: average days of larvae metamorphosed into adult, number of dead larvae, number of emergent adults, sex ratio, lifespans of adult, survival ability of adults were recorded and analyzed. Comparative analysis of data from culture media SM and MSC using the RStudio integrated development environment (IDE) for R, version 1.4.1564 by Posit PBC 2011.

Correlation Between Categorical Variables

The X-Squared test using the RStudio program is used to determine whether there is a correlation between two categorical variables (McHugh 2013). If the P-Value is smaller than the significance level (0.05), there is a significant correlation between two variables. The X-squared test was conducted to identify correlations between: (i) type of media and metamorphosis success rate, (ii) type of media and sex ratio; and (iii) type of media and the infection rate of *M. anisopliae*.

The X-Squared test formula is:

$$\chi^2 = \sum \frac{(O_{ij} - E_{ij})^2}{E_{ij}}$$

χ^2: Chi Squared value
O_{ij}: The frequency observed in the-ij.
E_{ij}: The expected frequency in the-ij, calculated with

$$E_{ij} = \frac{\text{Sum of Total Row}_i \times \text{Sum of total Column}_j}{\text{Sum of Total}ij}$$

Comparation Between the Means of Two Independent Samples
The t-test is used to compare the means of two independent samples (Walpole et al, 2016). If the P-Value is smaller than the significance level (0.05), there is a significant effect between two samples. The statistical T-test is used to determine whether there is an effect in the method of maintaining imago in groups and individually on their survival as well as for the average of lifespan of imago. If the P-Value is smaller than the significance level (*0.05*), there is a significant effect the method of maintaining imago and its average age.

T-Test Formula is:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

$\bar{X}_1, \bar{X}_2$: The average value of sample 1 and 2.
s_1^2, s_2^2: Variance of sample 1 and 2.
n_1, n_2: Size of sample 1 and 2.
t: t values.

RESULTS AND DISCUSSION

Rearing Media of *Oryctes rhinoceros*
Larvae reared on SM (67%) and MSC media (66%) successfully metamorphosed into adults (Table 1). The results of the x-squared test of differences in the types of larval maintenance media obtained a P-value of *0.9951>0.05* (significance level). This means that there is no significant difference in the success of larvae metamorphosing into imago between those maintained in media (SM) and media (MSC). *O. rhinoceros* larvae maintained in both SM and MSC media were able to produce the same number of imagoes.

Table 1. Comparison of Sawdust Media (SM) and fermented Mixture of Sawdust and Cow Dung (MSC) for rearing *Oryctes rhinoceros* larvae in laboratory

No.	Criteria	Sawdust Media (SM)	Fermented Mixture of Sawdust and Cow Dung (MSC)
1	Total reared larva (individual)	130	100
2	Emergent adults (%)	67[a]*	66[a]
3	Dead larvae (%)	33	34
4	Larvae infected by *Metarhizium anisopliae* fungus (%)	65[a]	62[a]
5	Dead larvae with rot characteristics (%)	35	38
6	Male: female ratio (%)	45:55[a]	55:45[a]
7	Average of third-instar larvae Metamorphosed into adults (days)	57	57
8	Average male lifespans (days)	141[b]	153[a]
9	Average female lifespans (days)	198[a]	139[b]
10	Longest male lifespan (days)	208	273
11	Longest female lifespan (days)	247	236

*The same letters indicate no significant differences. Different letters indicate significant differences.

The emerged adults exhibited a male-to-female ratio of approximately 1 (0.81 for SM and 1.22 for MSC media). The results of the sex ratio using x-squared test obtained a P-value of $0.3041 > 0.05$ (significance level). There was no statistically significant difference in the male-female ratio between larvae maintained in SM media and MSC media. Although there were variations in proportions (SM had more females, MSC tended to produce more males), these differences could have occurred by chance. Both media type treatments produced a relatively balanced male-female ratio. *O. rhinoceros* follows a type K animal reproduction pattern, where the ratio of males to females is approximately 1 (Price et al. 2011).

When male and female adults were kept together (SM treatment), they tended to have a longer lifespan compared to being kept singly (MSC treatment): the lifespan on SM treatment was 170 days (5.67 months), while on MSC media it was 146 days (4.87 months) (Table 1). This is supported by t-test results, where the P-value ($9.124e\text{-}16$) < 0.05. This means that there is a very significant difference between the average age of the imago in the two maintenance methods. Group maintenance of *O. rhinoceros* imago results in a longer age compared to single maintenance, with a difference of almost 1 month (25.7 days). The effect of group maintenance on extending the life of this imago is consistent and statistically significant.

Korb and Heinze (2021) stated that sociality is associated with longevity and a larger pace of ageing. Small groups of animals (3 to 20 individuals) could increase the survival rate of the breeders. Korb and Heinze (2021) gave an example for group-living of birds and mammals that they are live significantly longer than their non-social relatives. *O. rhinoceros* adults have gregarious behaviour that they are using semiochemical communication by a male-produced aggregation pheromone and a female-produced sex pheromone (Hallett et al. 1995). It is supposed that imagoes kept alone have more stress experiences compared to imagoes that live with another imagoes. Slow aging of insects received by avoidance the stress. Excessive stress could shorten lifespan and stress resistance is correlated with longevity. For example, honey bees and other social insects have advantages over solitary species by individuals can directly help each other (Walton et al. 2024).

There is a significant difference in the average age of male and female O. rhinoceros kept in single and in groups. O. rhinoceros imago kept singly has a higher average male age (153 days) than those kept in groups (141 days), with significant support for a significant difference P-value (*0.0165*) *<0.05*. Ethyl 4-methyloctanoate (E4-MO) is the main component of the aggregation pheromone released by male imago. This pheromone is released by males at night when insects are active, which causes both other males and females to come to it (Bedford 2013). Male *O. rhinoceros* are like pioneers who will find the right place to eat, mate and breed material alone (Paudel et al. 2023)

The average life expectancy of females who were kept in groups (the average was 198 days) was significantly higher than their solitary counterparts (the average was 139 days). There is a difference of 59 days on average. The average lifespan of females kept in groups was also longer with the P-Value (*4.469e-13*) which is much less than the value of 0.05. Out of the two, O. rhinoceros imago is the one who would rather live in social groups (Paudel et al 2023) which is expected to favor increased longevity among female imago. Bedford (1980) reported that *O. rhinoceros* adults in France lived for around 5 months, while in India, they lived for approximately 4.7 months, consistent with the findings of this research.Individual adults reared on SM survived for up to 247 days or 7.6 months (Figure 1), whereas those reared on MSC media lived up to 273 days or 8.5 months (Figure 2).

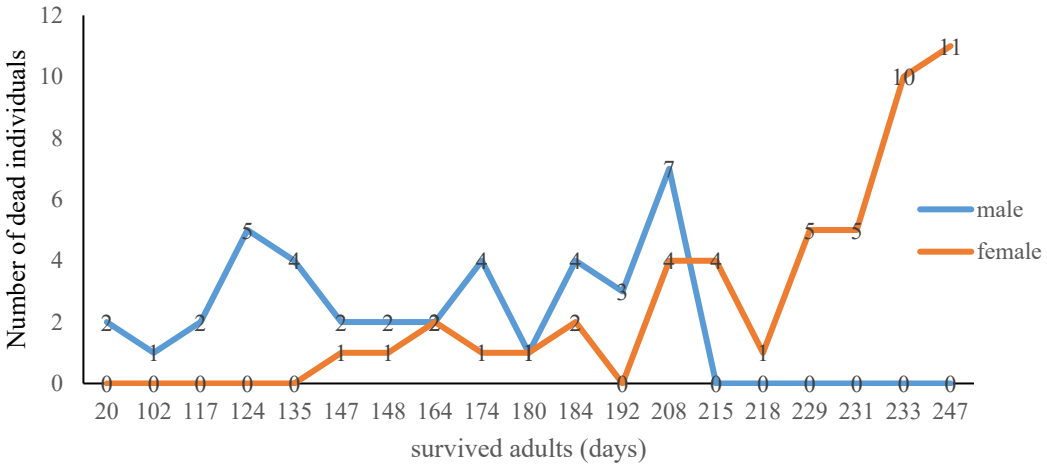

Figure 1. Survival ability of 87 *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) adults in laboratory conditions: males and females housed together in a container and reared on Sawdust Media (SM)

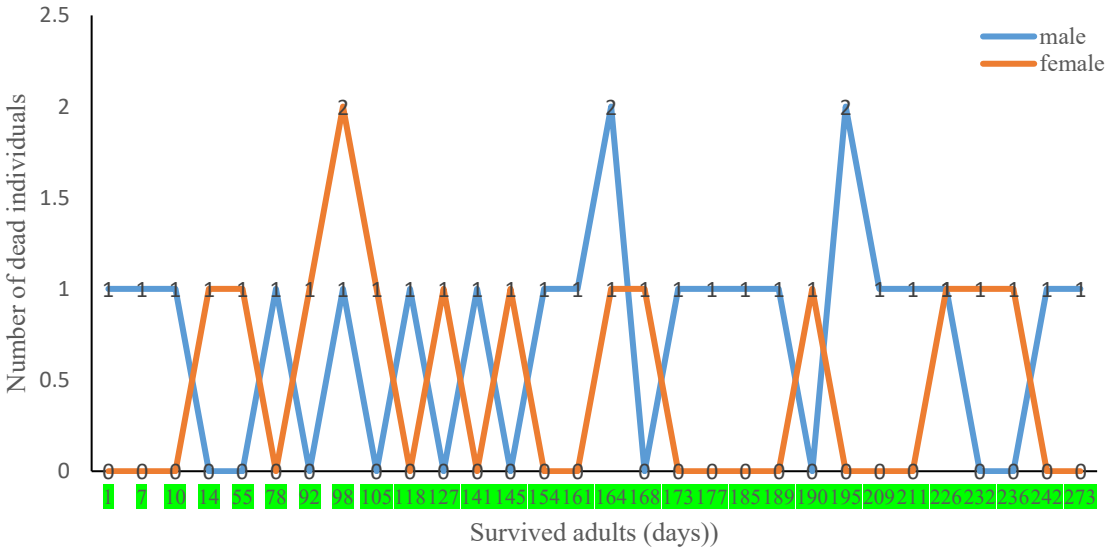

Figure 2. Survival ability of 36 *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) adults in laboratory conditions: segregation by gender in separate containers (a further 30 adults were dissected to obtain their mid gut for molecular testing)

Number of Days for Larvae to Metamorphose into Emerging Adults and Their Length

The larvae reared in SM originated from second and third instar stages and underwent metamorphosis into emergent adults at three different levels, each with varying lengths: 1) Larvae reared in the laboratory for 30–57 days produced emergent males with a length of 4.12 cm and females with a length of 4.24 cm. 2) Larvae reared in the laboratory for 58–106 days resulted in males with a length of 3.58 cm and females with a length of 3.65 cm. 3)Larvae reared in the laboratory up to 107–195 days yielded males and females with a length of 3.1 cm. Some second-instar larvae reared in SM resulted in smaller-sized adults (3 cm) compared to those from the field (4 cm). This discrepancy may be attributed to differences in nutritional quality during the larval phase (Pallipparambil 2015). Nutrition from Jabon wood (*Neolamarckia cadamba*) sawdust may be less supportive for *O. rhinoceros* larval growth and development. Although male adults typically exhibit larger body sizes and longer horns than females (Gressitt 1953; Kalshoven 1980), both female and male *O. rhinoceros* adults reared in SM had relatively short horns.

Larvae reared on MSC media metamorphosed into emergent adults at two different intervals, each with specific lengths: 1) Larvae reared in the laboratory for 30–57 days resulted in emergent males with a length of 4.03 cm and females with a length of 3.94 cm. 2) Larvae reared in the laboratory for 58–106 days produced males with a length of 4.15 cm and females with a length of 4.06 cm. The addition of cow dung to Jabon wood sawdust serves as a nutritional boost for the growth and development of *O. rhinoceros* larvae, resulting in emergent adults with lengths of approximately 4.0 cm.

Media Selected for Obtaining Emergent Adults

Emergent *Oryctes rhinoceros* adults are needed to test the virulence level of an *Alphanudivirus oryrhinocerotis* isolate. For a valid determination, the adults tested must be healthy and of the same age. While rearing *O. rhinoceros* in the laboratory presents challenges such as requiring

large amounts of food, a long lifespan, and significant living space (Schipper 1976), it is a relatively feasible option if the laboratory lacks cell culture facilities.

In Java Island, SM is readily available, making it a practical choice for rearing media compared to MSC. Both SM and MSC media yielded similar results in terms of the percentage of emergent adults and mortality rates during rearing. Thus, SM should be selected as the preferred media for rearing *O. rhinoceros* larvae. It is essential to ensure that larvae are in the third-instar stage to obtain emergent adults of standard size (±4 cm). Larvae should be sourced from areas with low levels of *Alphanudivirus oryrhinocerotis* infection, such as Central Java, West Java, Central Sulawesi, and West Sulawesi (Rahayuwati et al. 2020), to minimize the likelihood of emerging adults being infected with *A. oryrhinocerotis*. The Nudivirus family possesses the ability to act as an EVE (Endogenous Viral Element) (Feschotte & Gilbert 2012). EVEs represent DNA sequences from a virus that has become integrated into the genome of MSC media yielded comparable results to SM across various parameters, including the number of emerging adults, infection rate of *Metarhizium anisopliae* fungi, adult length, and other relevant factors (Table 1). Wikardi (1980) utilized an MSC in a 1:1 ratio without sterilization but subjected to one month of fermentation. A fermentation duration of one to three months still proves effective for rearing *O. rhinoceros* larvae. Rearing using MSC media is advisable in scenarios where only second-instar *O. rhinoceros* larvae are available.

***Metarhizium anisopliae* Fungi**

Rearing *Oryctes rhinoceros* larvae on both SM and MSC media posed challenges related to *Metarhizium anisopliae* infection. Despite previous findings suggesting that biological control of *O. rhinoceros* with *M. anisopliae* has no significant impact on population decline (Young 1974), the opposite outcome was found in laboratory-rearing conditions. In this study, 28 out of 130 larvae in the SM treatment and 21 out of 100 larvae in the MSC media treatment died, with *M. anisopliae* infection observed in the larvae, pupae, and adult stages of *O. rhinoceros*. The X-Squared test showed a P-value (0.9481) > 0.05 (significance level), where the mortality rate of *O. rhinoceros* by the *M. anisopliae* between the two SM and MSC media was not significantly different. Both media showed very similar patterns in terms of causes of death. The fungus *M. anisopliae* was the main cause of death in SM and MSC media (> 60% of total deaths). Sawdust is therefore suggested as a suitable medium for research aiming to discover new isolates of *M. anisopliae*.

Detecting the initial stage of *M. anisopliae* infection poses a challenge as the infection develops inside the larvae with no noticeable odour changes. However, later stages reveal small dots of white mycelium on the body surface of larvae, which gradually widen and transform into green mycelium over time. *M. anisopliae* is commonly known as the green muscardine fungus. Based on the length of conidia, the characteristic conidia of *M. anisopliae* found on *O. rhinoceros* in our study indicated the presence of *Metarhizium anisopliae var major* (Figure 3).

Figure 3. Infection of *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) larvae by *Metarhizium anisopliae var major* conidia (100x magnification)

CONCLUSION

Both SM and the 3-month fermented mixture of sawdust and cow dung in a ratio of 1:1 (MSC) yielded identical results in terms of the percentage of number for third-instar larvae to metamorphose into adults, the infection rate of *Metarhizium anisopliae* fungus, male-to-female ratio. Group maintenance of imagoes results in a longer age compared with single maintenance. O. rhinoceros imago kept singly has a higher average male age, while females who were kept in groups was significantly higher lifespan. Based on this finding, the sawdust media is suggested to be used as breeding media for *O. rhinoceros* larvae for economical and practical reasons.

AUTHORS DECLARATION

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Conflict of Interest
The authors declare that they have no conflict of interest.

Ethics Declarations
No ethical issue is required for this research.

Data Availability Statement
This is a Final Year Project (FYP) and the data are currently in the FYP dissertation of Rahayuwati (2021).

Authors' Contributions
Sat Rahayuwati (SR) and Yayi Munara Kusumah (YMK) conceptualized this research and designed experiments; SR, YMK, Nabil Naufal (NN) participated in the interpretation of the data; SR wrote the paper and YMK participated in the revisions of it. All authors read and approved the manuscript.

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