INTRODUCTION

Rice (Oryza sativa) is an important cereal food and a good source of carbohydrate for tropical people (Ashamo, 2009). In Indonesia, rice is the main ingredient that supports the population. The process of storing rice can be damaged by infestations of pests and impact to economic losses, among these are Sitophilus oryzae, S. zeamais, Sitotroga cerealella, Rhizopertha dominica, Oryzaephilus surinamensis (Ashamo, 2009; Astuti et al., 2013; Astuti, Mario, & Widjayanti, 2018).

Among the insects that cause damage to rice grains in storage, the rice weevil, Sitophilus oryzae is the most important species (Nwaubani, Opit, Otitodun, & Adesida, 2014). Sitophilus oryzae is a major cosmopolitan pest affecting stored rice in the world (Rees, 2004). The damage to the grain is caused by both larva and adult (Rees, 2004). The outbreak of this pest make the stored rice unfit for human consumption (Rashid et al., 2009). One of the ways to reduce postharvest losses of grains is breeding for varietal resistance (Throne, Baker, Messina, Kramer, & Howard, 2000). The factors that confer resistance to the grains against infestation by a variety of storage insects are varied. Resistance to pest in stored grains can be manifested as antibiosis (Rahardjo, Astuti, Sugianto, & Rizali, 2017). Some properties of the grain affects the insect’s biology such as reducing longevity and increasing mortality, reproduction and damage (Costa et al., 2016). Alternatively, the grain may display antixenosis, where in the behaviors of the insects are affected and resulting in reduced feeding and oviposition (Costa et al., 2016).

Various types of rice such as milled, brown, red, and black rice are the main food ingredients currently consumed by many people in Indonesia. However, efforts to avoid these foods from infestation of postharvest pests have not been widely known. One effort that can be done is by selecting varieties or types of rice that are resistant to postharvest pest attacks. This study aimed to examine the susceptibility of brown, milled, red, and black rice to S. oryzae.

MATERIALS AND METHODS

This research was conducted at Plant Pest Laboratory, Department of Plant Pests and Diseases.
Faculty of Agriculture, Universitas Brawijaya. This study was conducted from May to September 2017.

Brown rice (Diamond rice variety from Singojuru Village, Sumberbaru Subdistrict, Banyuwangi Regency), milled rice (Ciherang rice variety from Kanigoro Village, Pagelaran Subdistrict, Malang Regency), black rice (Watudodol rice variety from Singojuru Village, Sumber Baru Subdistrict, Banyuwangi Regency), and red rice (BWI-1 rice variety from Singojuru Village, Sumberbaru Subdistrict, Banyuwangi Regency) were examined in this research. Feed propagation used milled rice (Mentikwangi rice variety from Sumber Ngepoh Village, Lawang Subdistrict, Malang Regency).

Sterilization of Rice
Sterilization of feed used an icebox cooler at ca. -15 °C for a week (Heinrichs, Medrano, & Rapusas, 1984). The feed was transferred from the icebox cooler at 5 °C for a week to avoid re-infestation by insects or mites. The feed was placed at 27 ± 2 °C for as long as two weeks before using.

Rearing of Sitophilus oryzae
S. oryzae adults reared in this experiment (Heinrichs et al., 1984), obtained from a collection in the Pest Laboratory, Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Brawijaya. Rearing was started by infesting 100 pairs of S. oryzae adults into a glass tube (t = 23 cm and d = 14 cm) which contained 500 g of milled rice (Mentikwangi rice variety). The glass tube was covered with white cloth and labeled. The glass was stored in the multiplication room with a temperature of 27 ± 2 °C and humidity of 65 ± 5 %. The adult of S. oryzae was released after being left for a week. The separation of male and female S. oryzae adults was carried out based on the shape of the rostrum or muzzle and the shape of the tip of the abdomen.

Feed Hardness Test
Feed hardness test was conducted in Laboratory of Agricultural Food Technology, Faculty of Agricultural Technology, Gadjah Mada University. The hardness test aimed to determine the level of hardnes of rice grains used in the study by calculating the amount of force needed to destroy each grain. Universal Testing Machine (BDO-FB0.5TS) tool was used in this research.

Proximate Feed Analysis
Proximate analysis was conducted in the Food Quality and Safety Testing Laboratory, Agricultural Product Technology Department, Faculty of Agricultural Technology, Universitas Brawijaya. This analysis aimed to determine the nutritional content of each type of rice, including carbohydrate, protein, fat, water and ash content. The proximate and protein contents were analyzed using the method as described by (Pendl, Bauer, Caviezel, & Schulthess, 1998) and the Kjeldahl method respectively.

Phenol Content Analysis
Analysis of phenol content was conducted at UPT Service Analysis and Measurement of Chemistry Department, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya. This analysis aimed to determine the phenol content of each type of rice and using the spectrophotometric apparatus method.

Each type of rice as much as 30 g was mixed and inserted into a glass tube (d = 6.5 cm and t = 9 cm) then infested 30 pairs of S. oryzae aged one to two weeks. Sitophilus oryzae adults was released a week after infestation. Then each type of rice was separated and placed in a glass tube (d = 6.5 cm and t = 9 cm) and placed in the experimental room. The study was arranged in a Completely Randomized Design which was repeated 6 times. The observed variables were:
1. Mortality of S. oryzae adult infestation
   An observation was conducted to determine the number of S. oryzae adult mortality after infestation for seven days. This method was used to remove S. oryzae which has been infested in each treatment for seven days and then counted the number of mortality and living male and female adults.
2. Number of S. oryzae eggs
   An observation was conducted a week after infestation by observed each rice grain using stereo microscope to count the number of eggs. Each rice grain was put back into a glass tube and maintained until it becomes larvae.
3. Number of S. oryzae larvae
   A stereo microscope was used to count the number of larvae in each rice type. After hatching eggs, larvae were marked by the presence of larval cavity inside the rice grains. The larvae were put into glass tube in the laboratory conditions and maintained until pupae.
4. Number of S. oryzae pupae
   The pupae were observed and then counted when the layer of rice become thin and the presence
of frass on the surface of the rice. The observation was conducted by stereo microscope for each rice grain. Then each rice type was returned into a glass tube and kept in the laboratory until F1 imago.

5. Number of *S. oryzae* F1 imago

The number of F1 imago were counted for ca. 56 days (Abebe, Tefera, Mugo, Beyene, & Vidal, 2009). The emerged F1 imago were separated between males and females by looking at the shape of the rostrum and shape of the abdomen tip.

6. Loss of feed weight

The loss of feed weight was calculated by the following method of (Gwinner, Harnisch, & Muck, 1990):

\[
\text{Loss weight} \% = \left( \frac{W_u \times N_d - (W_d \times N_u)}{W_u \times (N_d + N_u)} \right) \times 100 \text{ ...........1}
\]

Remarks:

Wu : Weight of unbroken rice; Nu : Amount of undamaged rice; Wd : Weight of broken and unbroken rice

7. Susceptibility Index

The susceptibility index was used to determine the susceptibility level of each type of rice. Susceptibility index was calculated by using the formula (Dobie & Kilminster, 1978) with the equation:

\[
\text{Susceptibility index} = \left[ \frac{\log F}{D} \right] \times 100 \text{ ..................2}
\]

Remarks:

F : Number of F1 imago D : Median time of development, which is estimated as the time starting from the middle of the oviposition time to the 50 % of F1 imago appear

Susceptibility level was categorized based on the Dobie’s susceptibility index value of each rice types that proposed by Dobie (1974). It has been modified as described on Table 1.

| Table 1. Susceptibility’s Categories based on Susceptibility Index (Dobie, 1974) |
|---------------------------------------------|
| Index of susceptibility | Susceptibility’s Categories |
|--------------------------|----------------------------|
| 0 – < 4   | Resistant           |
| 4 – < 8   | Moderate Resistant  |
| 8 – < 11  | Susceptibility     |
| ≥ 11      | Highly Susceptibility|

Statistical Analysis

Analysis of variance was used to compare the differences between four rice types at α < 0.05. Tukey HSD was adopted as Post Hoc test for the significant one.

RESULTS AND DISCUSSION

For the average number of eggs, larvae, pupae and F1 imago of *S. oryzae*, there were differences between rice types (Fig. 1). The average number of eggs in the milled rice was significantly lower compared to the brown, the red and the black rice. While the average number of eggs in the red rice was the highest. The red rice is preferred by *S. oryzae* as suitable environment to lay their eggs (Fig. 1). The average number of larvae, pupae and F1 imago were also significantly lower compared to the rest of rice types. Growth and development of larvae, pupae and F1 imago were related to the nutrient contents in the rice. The significant differences on the number of larvae, pupae and F1 imago should be related to nutrient contents on each rice. There was a positive correlation between nutrients in rice and development of adult especially for fat (r = 0.928) and ash (r = 0.939) contents. (Demissie, Swaminathan, Ameta, Jain, & Saharan, 2015) reported that corn varieties with low ash content will be more resistant to the storage pests, *Sitotroga cerealella*. A strong negative correlation was observed between development period and kernel weight, moisture and protein contents. The low number of adult emergences was the resistance indicator of rice types. Gerema, Bogalo, Mangitsu, & Lule (2017) stated that the adult of *S. oryzae* on resistant varieties produce a low number of progenies.

The average of eggs and larvae periods was significantly different compared to preadult’s period of *S. oryzae* (Fig. 2). In contrast, the type of rice was no significantly different to pupae period (Fig. 2). The type of rice was significantly different to developmental pre adult stage of *S. oryzae* (Fig. 2).

The long duration of developmental preadult’s period of *S. oryzae* in milled rice compared to other rice was caused by the imbalance of nutrition in feed. This was influenced by carbohydrate, fat and ash content. There was a positive correlation of developmental preadult’s to carbohydrate (r = 0.914), lipid (r = -0.987), and ash (r = -0.992) contents in each types of rice. Ojo & Omoloye (2012) stated that the development period (from egg to adult) and the number of adult’s progenies were influenced by the nutrient’s balance in feed such as protein, starch, sugar, fat, fiber, vitamins and minerals.
Fig. 1. Average number of each stage (egg, larva, pupa and F1 Imago) of *S. oryzae* on four rice types (the same letter shows there are no significant differences between four rice types for each stage by Tukey HSD at $\alpha < 0.05$)

Fig. 2. Average developmental time of each stage (eggs, larvae, pupae and pre-adults) of *S. oryzae* on four rice types (the same letter shows there are no significant difference between four rice types for each stage by Tukey HSD at $\alpha < 0.05$)
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For the average of preoviposition and life cycle periods showed a significant difference in rice types (Table 2). In contrast, the percentage of feed weight loss showed no significant difference. The preoviposition period of *S. oryzae* in brown rice (6.70 days) was shorter than black rice (7.50 days), brown rice (7.56 days), and milled rice (8.20 days). The life cycle of *S. oryzae* in brown rice (38.63 days), black rice (38.73 days), and brown rice (39.40) was shorter than milled rice (43.33 days) (Table 2).

The results showed that the index of rice susceptibility in black rice (5.41), red rice (5.41) and brown rice (5.61) were higher than milled rice (0.79). The percentage of loss of feed weight was positively correlated to contents of fat (r = 0.977) and ash (r = 0.983). The higher of fat and ash contents in the feed would increase the feeding activity in larvae period. There was a negative correlation between the percentage loss of feed weight and the duration of preadult development (r = -0.998). (Rizwana, Hamed, Naheed, & Afghan, 2014) reported that there is a negative correlation between the duration of development and the loss of feed weight and feed damage percentages.

### Table 3. Average of Preoviposition and Life cycle’s periods and losses of feed weight on four different rice types

| Rice Types    | Preoviposition (days) (Mean ± SE) | Life Cycle (days) (Mean ± SE) | Loss of feed weight (%) (Mean ± SE) |
|---------------|-----------------------------------|------------------------------|-----------------------------------|
| Brown Rice    | 6.70 ± 0.24 a                      | 39.40 ± 1.08 a               | 3.87 ± 0.94                       |
| Milled Rice   | 8.20 ± 0.09 c                      | 43.33 ± 0.98 b               | 0.46 ± 0.30                       |
| Red Rice      | 7.56 ± 0.14 b                      | 38.63 ± 0.67 a               | 5.65 ± 3.02                       |
| Black Rice    | 7.50 ± 0.05 b                      | 38.73 ± 0.47 a               | 5.43 ± 2.14                       |
| Tukey HSD (5 %) | 0.45                              | 2.51                         | -                                 |

Remarks: The same letter shows there are no significant difference between four rice types for each column by Tukey HSD at α < 0.05.

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The susceptibility index for milled rice (0.79) was lower compared to other types of rice (Table 3). This showed that milled rice was categorized as in resistant category (SI = 0-3) to *S. oryzae* attack. Whereas brown rice, red rice and black rice were categorized as in moderate resistant with 5.61, 5.41, and 5.41, respectively (Table 3). The susceptibility index of a variety was influenced by the nutrient content in the feed, including ash and fat. There was a positive correlation between the susceptibility index of rice types with ash content (r = 0.854) and fat (r = 0.840). Astuti et al. (2013) stated that there is positive correlation between the ash content and the number of eggs laid, the number of new adult (F1), the median time of development, and the susceptibility index of several milled rice varieties to *Rhyzopertha dominica*. The fat content was positively correlated to the loss of feed weight (r = 0.977) and indicated that there was more insect feeding activity due to the higher fat content in the feed. The susceptibility of a rice variety can be indicated by the low insect growth and the longer insect development. There was a positive correlation between the susceptibility index and the number of new adult (r = 0.980) and the loss of feed weight percentage (r = 0.930). The abundance of emerged new adult indicated the susceptibility was high and it increased the feed weight loss percentage due to the increased eating activity. It is also described by researchers that there was a positive correlation between the susceptibility index and the number of new adults and the percentage of feed weight loss (Abebe, Tefera, Mugo, Beyene, & Vidal, 2009; Mebarkia et al., 2010; Rahardjo, Astuti, Sugiarto, & Rizali, 2017)

### Table 3. Index of Susceptibility and Susceptibility’s Categories for Four Different Rice Types to *S. oryzae* Attacks Based on Dobie Susceptibility Index

| Rice Types | Index of Susceptibility | Susceptibility’s Categories |
|------------|-------------------------|----------------------------|
| Brown Rice | 5.61                    | Moderate Resistant          |
| Milled Rice| 0.79                    | Resistant                  |
| Red Rice   | 5.41                    | Moderate Resistant          |
| Black Rice | 5.41                    | Moderate Resistant          |

Remarks: Based on Dobie susceptibility index Categories; 1 - 5 is resistant, 6 - 10 is moderate resistant, 11 - 15 is susceptible, and 16 - 21 is highly susceptible.

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CONCLUSION

The susceptibility index for milled rice (0.79) was lower compared to the other types of rice. The susceptibility index for brown, red and black rice were 5.61, 5.41 and 5.41 respectively. Based on the sustainability index values, milled rice was categorized as in rice resistant to S. oryzae, while black rice, brown rice, and red rice were moderate resistant. The resistance of rice varieties to pests S. oryzae in this study was influenced by the feed quality factors i.e. the adequacy and balance of nutrients, especially ash and fat.

ACKNOWLEDGEMENT

Author would like to thank to Laboratory of Plant Pest, Department of Pests and Diseases, Faculty of Agriculture, University of Brawijaya for the facilities and technical support. Related to financial support, authors thank to Faculty of Agriculture by PNBP Research Scheme Project 2017. Author would also like to thank Muhammad Bayu Mario and Dewi Fajarwati for helping during laboratory activities.

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