Resistance response of five maize grain varieties to *Sitophilus zeamais* and *Sitophilus oryzae* (Coleoptera: Curculionidae)

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ABSTRACT

Warehouse pests that have the potential to damage corn kernels are *Sitophilus zeamais* and *Sitophilus oryzae*. Information on the resistance of corn kernels of several varieties is still little reported. Research on the resistance response of five varieties of corn kernels (Madura-3, Lokal Madura, Pioneer P32 Singa, P27 Gajah, and NK 212) to *S. zeamais* and *S. oryzae* pests was conducted at the Plant Health Laboratory, Agrotechnology Study Program, Faculty of Agriculture, National Development University "Veteran" East Java. This study aims to determine the response of feed type resistance in the form of five varieties of corn seeds to the attack of *S. zeamais* and *S. oryzae* pests. The results showed that the highest percentage of mortality of *S. zeamais* and *S. oryzae* imago was in corn seeds of Madura-3 (17.15%) and NK 212 (37.22%) varieties. Fecundity tests on *S. zeamais* and *S. oryzae* pests with the lowest mean were on corn seeds of P27 Gajah (6.33 grains) and NK 212 (4.33 grains). The lowest percentage of seed weight loss in *S. zeamais* and *S. oryzae* pests was in the varieties P27 Gajah (1.34%) and NK 212 (1.05%). The highest percentage of sex ratio of *S. zeamais* and *S. oryzae* imago was in the varieties P27 Gajah (1.57%) and NK 212 (1.23%). The conclusion of this study is that the maize seeds that are resistant to *S. zeamais* attack are the P27 Gajah variety, while the maize seeds that are resistant to *S. oryzae* attack are the NK 212 variety.

Keywords: maize grain; resistance response; *Sitophilus zeamais*; *Sitophilus oryzae*.

INTRODUCTION

Maize (*Zea mays* L.) is one of the food crop commodities that has an important role in the development of the agricultural sector, especially food security (Ningsih *et al.*, 2017). One of the efforts to increase corn production is by paying attention to post-harvest handling. Proper post-harvest handling is needed to obtain high quality corn and reduce yield loss. Storage is the final stage of post-harvest handling that can affect the quality and quantity of maize kernels. Maize is stored in the form of kernels with a moisture content ranging from 11-13%. Damage to corn kernels in storage is generally caused by post-harvest pests or warehouse pests. Damage is caused by a decrease in the quantity and quality of maize kernels (Lapinangga *et al.*, 2019).

Warehouse pests that potentially attack corn kernels are *Sitophilus zeamais* and *Sitophilus oryzae*. According to Manueke *et al.* (2015) *S. zeamais* is the main pest of corn kernels and is known as corn weevil or corn powder pest. Symptoms of *S. zeamais* pest attack are hollow seeds, then over time the seeds become broken and crushed into flour or powder (Suriani *et al.*, 2019). *S. oryzae* is the main pest of rice and grain, so it is known as rice weevil or rice powder pest. *S. oryzae* is reported to be able to breed in maize media even though its main host is grain or rice. Symptoms of *S. oryzae* attack on corn kernels begin with the formation of several irregular holes on the surface of the corn kernels. This pest can reduce the nutritional quality, seed weight, and germination percentage of corn kernels (Saenong, 2016).

The warehouse pests *S. zeamais* and *S. oryzae* undergo perfect metamorphosis (holometabola) because in their development through 4 stages: egg, larva, pupa and imago phases in the seed (Manueke *et al.*, 2015). Female imago lay eggs on corn kernels before harvesting and in storage. The eggs hatch into larvae and feed on the inside of the corn kernels. The larvae complete their life cycle inside the kernels, causing the kernels to be damaged. Imago damage the kernels with its snout-like mouthparts (roustrum). Affected kernels will develop small holes that accelerate the destruction of the kernels, which then become powdery. Imago makes exit holes in the pericarp so that the seeds have larger holes (Hasnah *et al.*, 2014).

The pests *S. zeamais* and *S. oryzae* are reported to damage grains in storage such as maize, rice, wheat, sorghum, soybean, peanut, and others. Both pests are able to damage and develop well in commodities that are still intact and can complete their life cycle in seeds so that they can cause real damage. According to Harahap & Rakhmasdiah (2016) warehouse pest insects can cause damage to 5-10% of the material stored in the warehouse. The level of damage caused by warehouse pests can be influenced by the variety of corn kernels. The corn kernel varieties used in this study include Madura 3, Lokal Madura, Pioneer P32 Singa, P27 Gajah, and NK 212. Research on the resistance test of five varieties of corn kernels to the development of *S. zeamais* and *S. oryzae* pests has never been conducted. This study aims to determine the resistance response of feed types in the form of five varieties of corn seeds to *S. zeamais* and *S. oryzae* pests.

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MATERIALS AND METHODS

Time and Place

This research was conducted from January 2024 to April 2024 at the Plant Health Laboratory, Agrotechnology Study Program, Faculty of Agriculture, National Development University “Veteran” East Java.

Tools and Materials

The tools used in this study include plastic jars, digital scales, digital microscopes, mobile phone cameras, label paper and stationery. Materials used in this study include *S. zeamais* imago and *S. oryzae* imago obtained from SEAMEO BIOTROP, as well as corn seeds of Madura 3, Lokal Madura, Pioneer P32 Singa, P27 Gajah, and NK 212 varieties obtained from local farmers.

Research Implementation

a. Identification of Warehouse Pests

Identification of warehouse pests is done to ensure that the pest species used in propagation are *Sitophilus zeamais* and *Sitophilus oryzae*. Identification is done by observing the morphology of warehouse pests. Morphological observations of *S. zeamais* and *S. oryzae* were made based on their morphological characteristics. These characteristics include color, body shape, antennae, body size, wing shape, and number of tarsi. Morphological observations of *S. zeamais* and *S. oryzae* were made at the level of imago stadia using the Key to the Identification of Postharvest Insect Pests: Coleoptera and Lepidoptera by Astuti *et al.*, 2022.

b. Propagation of *S. zeamais* and *S. oryzae*

Insect propagation was carried out at the Plant Health Laboratory, Agrotechnology Study Program, Faculty of Agriculture, National Development University “Veteran” East Java with an average temperature of 27 °C and 75% humidity. Insect pests were obtained from the Entomology Laboratory, Seameo Biotrop. The propagation of *S. zeamais* and *S. oryzae* was done by keeping one pair of imago in a plastic jar (12 cm high and 10 cm in diameter) fed with 100 grains of corn seed.

c. Treatments

The treatment combinations used were 5 maize grain varieties (Madura-3, Lokal Madura, Pioneer P32 Singa, P27 Gajah, and NK 212) and 2 warehouse pest species (*S. zeamais* and *S. oryzae*). Each treatment contained 3 replicates, each replicate containing 100 maize grains and 1 pair of *S. zeamais* or 1 pair of *S. oryzae* insects.

Observation Parameters

a. Imago Mortality (%)

Observations of *S. zeamais* and *S. oryzae* imago mortality were made 45 days after infestation. All infested imago were removed from the jar and the mortality rate was calculated. According to Nurhayati (2011) the mortality rate was calculated using the following formula:

$$Z = \frac{a}{b}\ x\ 100\ \%$$

Notes: Z = mortality rate, a = number of dead insects, b = total number of insects.

b. Fecundity

Fecundity observations were made once a week by counting the number of corn grains that had been stabbed by *S. zeamais* and *S. oryzae* imago. Each treatment was given one pair of test pest imago. The characteristics of corn kernels that had been burrowed by imago were egg marks around the entrance hole and physically damaged kernels. Observations were made until the female imago stopped laying eggs (Manueke *et al.*, 2015).

c. Seed Weight Loss

Observations of seed weight loss were made 45 days after infestation by taking 50 seeds randomly from each treatment. Weight loss was calculated by Gwinner *et al.* (1996). According to the form of the formula put forward is as follows:

$$\text{Weight loss (\%)} = \frac{(\text{Wu x Nd}) - (\text{Wd x Nu})}{\text{Wu x (Nd + Nu)}}\ \text{x 100 \%}$$

Notes: Wu = weight of undamaged seeds, Nu = number of undamaged seeds, Wd = weight of damaged seeds, and Nd = number of damaged seeds.

d. Sex Ratio

Sex ratio observations were based on the imago that emerged from the infestation. All imago that emerged were observed for their sex. Calculation of sex ratio is done by comparing the number of male imago with female imago (Manueke *et al.*, 2015). The sex ratio was calculated using the formula according to Saenong (2009) as follows:

$$\text{P males (\%)} = \frac{\text{Sum. of male insect}}{\text{Total insect}}\text{x100 \%}$$

$$\text{P females (\%)} = 100\ \% - \text{P male (\%)}$$

e. Calculation of Sensitivity Index (IK)

Sensitivity index value is a measure used to evaluate the level of resistance of feed to pest attack. The sensitivity index value is used to determine the level of sensitivity of each type of feed to pest infestation (Table 1) (Antika *et al.*, 2014).

Table 1. Criteria for sensitivity index to *S. zeamais* and *S. oryzae* pests

Sensitivity Index Value	Category
0-3	Resistant
4-7	Somewhat resistant
8-10	Susceptible
≥ 11	Very susceptible

Source: Antika, S.R.V., L.P. Astuti, & R. Rachmawati. (2014). Development of *Sitophilus oryzae* Linnaeus (Coleoptera: Curculionidae) on various types of feed. *Journal of HPT*, 2(4), 77-84.

The sensitivity index of each type of food was calculated using the Dobie method (1977 in Astuti *et al.*, 2013) with the formula:

$$IK = 100\ x\ [\log e\ (\frac{F}{D})]$$

Notes: F = number of new imago and D = median developmental time calculated daily from the middle of the oviposition period until the emergence of 50% new imago (Dobie, 1977 in Astuti *et al.*, 2013).

Data Analysis

The data obtained were statistically analyzed using Analysis of Varience (ANOVA). If there are differences between treatments, then further tests are carried out using Duncan's Multiple Range Test (DMRT) at the 5% level.

RESULTS AND DISCUSSION

Imago Mortality

Mortality is one of the important factors in the growth and development of insect pests (Manueke *et al.*, 2015). Observations of imago mortality were made to determine the level of death of *S. zeamais* and *S. oryzae* imago at 45 days after infestation. Feeding treatments in the form of five varieties of corn seeds showed significant differences in the mortality of *S. zeamais* and *S. oryzae* (Table 2).

The results showed that the highest percentage of mortality of *S. zeamais* imago was found in Madura-3 corn seeds with an average of 17.15% (Table 2). The percentage of mortality of *S. zeamais* imago on Madura-3 corn kernels showed significantly different results from the Lokal Madura, Pioneer P32 Singa, and P27 Gajah varieties. Madura-3 corn kernels are among the varieties that are resistant to *S. zeamais* attack because it has the highest percentage of imago mortality. *S. zeamais* pests cannot develop on Madura-3 corn kernels because Madura-3 contains secondary metabolite compounds that are antifeedant for *S. zeamais* pests. Tryptophan and lysine are known as secondary metabolite compounds that are antifeedant and play a role in corn seed resistance to *S. zeamais* attack (Keba & Sori, 2013).

The results showed that the highest percentage of mortality of *S. oryzae* imago was found in the NK 212 variety with an average of 37.22% (Table 2). The percentage of mortality of *S. oryzae* imago on corn seeds of NK 212 variety showed significantly different results from the Lokal Madura, Pioneer P32 Singa, and P27 Gajah varieties. NK 212 maize seeds are among the varieties that are resistant to *S. oryzae* infestation because it has the highest percentage of imago mortality. The high mortality is a major limiting factor in controlling the development of very high *S. oryzae* populations in nature (Manueke *et al.*, 2015). *S. oryzae* cannot develop on NK 212 maize seeds because the pericarp of NK 212 tends to be thick and hard. According to Antika *et al.* (2014) feed mismatches such as feed grains that are too hard can cause high mortality of *S. oryzae* imago.

Table 2. Mean Mortality Percentage of *S. zeamais* and *S. oryzae* Imago

Treatment	Mean Percentage of Imago Mortality±SD	
	S. zeamais	*S. oryzae*
Madura-3	17.15 ± 14.95 b	24.44 ± 15.84 ab
Lokal Madura	0.00 ± 0.00 a	6.08 ± 5.63 a
Pioneer P32 Singa	0.00 ± 0.00 a	0.00 ± 0.00 a
P27 Gajah	0.00 ± 0.00 a	1.77 ± 1.53 a
NK 212	5.21 ± 0.59 ab	37.22 ± 25.62 b

Notes: Numbers accompanied by the same letter in the same column indicate not significantly different based on 5% DMRT test.

Table 3. Fecundity testing results of *S. zeamais* and *S. oryzae*

Treatment	Mean Fecundity±SD (grains)	
	S. zeamais	*S. oryzae*
Madura-3	13.00 ± 8.72 a	7.67 ± 2.08 a
Lokal Madura	18.67 ± 4.16 a	13.67 ± 0.57 a
Pioneer P32 Singa	35.33 ± 32.00 a	39.67 ± 28.54 a
P27 Gajah	6.33 ± 3.21 a	40.67 ± 31.66 a
NK 212	22.66 ± 4.04 a	4.33 ± 3.21 a
Rerata Total	19.20 ± 10.43	21.20 ± 13.21

Notes: Numbers accompanied by the same letter in the same column show no significant difference based on 5% DMRT test.

Fecundity

Insect fecundity is the ability of insects to reproduce or produce eggs (Herlinda *et al.*, 2021). Fecundity observations were made to determine the number of eggs produced by *S. zeamais* and *S. oryzae* pests during infestation. The fecundity test results of *S. zeamais* and *S. oryzae* pests on five varieties of corn seeds showed no significant difference (Table 3).

The results showed that fecundity testing on *S. zeamais* pests with the lowest average was found in corn seeds of the P27 Gajah variety with an average of 6.33 grains (Table 3). The fecundity test results with the lowest average indicate that the P27 Gajah corn kernels are not suitable as a medium for the development of *S. zeamais* pests, meaning that the P27 Gajah variety is a good and resistant seed in storage. Corn seeds of the P27 Gajah variety are known to contain chemical compounds such as tannins, phenols, and alkaloids that are anti-pest, so they can inhibit the growth and reproduction of *S. zeamais* pests on corn seeds of the P27 Gajah variety.

The results showed that fecundity testing on *S. oryzae* pests with the lowest average was found in corn seeds of NK 212 variety with an average of 4.33 grains (Table 3). The fecundity test results with the lowest average indicate that NK 212 corn kernels are not suitable as a medium for the development of *S. oryzae* pests, meaning that the NK 212 variety is a good and resistant seed in storage. NK 212 maize seeds are known to contain chemical compounds such as phenols, alkaloids, or flavonoids that are anti-pest, so they can inhibit the growth and development of *S. oryzae* pests on NK 212 maize seeds.

Seed Weight Loss

The percentage of seed weight loss is a parameter used to see the level of damage to stored grains (Hendrival & Melinda, 2017). The results of the percentage decrease in seed weight showed that different varieties of corn seeds had a significantly different effect on *S. zeamais* and *S. oryzae* pests (Table 4). According to Laili and Suharto (2019), the decrease in weight in each treatment is caused by the large number of seeds that are gnawed and perforated, causing the weight of the seeds used to decrease or shrink.

The lowest percentage of seed weight loss in the *S. zeamais* pest treatment was found in the P27 Gajah variety with an average of 1.34% (Table 4). The percentage decrease in corn seed weight of the P27 Gajah variety is significantly different from the Lokal Madura variety. It is suspected that *S. zeamais* cannot develop well on corn kernels of the P27 Gajah variety because the variety contains nutrients that may not support optimal growth for *S. zeamais*. P27 Gajah corn kernels have a low protein content of 3.2 g, whereas according to Askanovi (2011) the protein content of corn kernels affects the level of seed weight loss after infestation. Good nutritional content can affect the development of *S. zeamais* pests (Hendrival & Mayasari, 2017).

The lowest percentage of seed weight loss in the treatment of *S. oryzae* pests was found in the NK 212 variety with an average of 1.05% (Table 4). The percentage of seed weight loss of NK 212 was significantly different from the Pioneer P32 Singa and P27 Gajah varieties. *S. oryzae* pests are thought to be unable to develop properly in NK 212 corn kernels because this variety has a harder physical structure and thick seed coat, making it difficult for *S. oryzae* pests to penetrate. NK 212 corn kernels are known to contain 3.2 g of protein and 1.2 g of fat, which may be less than optimal for the growth and development of *S. oryzae* pests. According to Hendrival *et al*. (2019) *S. oryzae* pests are insects that require nutrients during their life cycle.

Sex Ratio

Male and female *S. zeamais* imago have clear differences in characteristics (Figure 1). The difference between male and female imago can be seen from the size of the snout and the tip of the abdomen. Male imago have a shorter snout, thicker and rougher surface, while female imago have a longer snout, rather thin and smoother surface. The tip of the abdomen of male imago curves downward, while the tip of the abdomen of female imago is straight backward.

Male and female *S. oryzae* imago also have clear differences (Figure 2). The difference between male and female imago can be seen from the size of the roustrum (snout) and the tip of the abdomen. According to Manueke *et al.* (2015) male imago have a shorter and slender roustrum, while female imago have a longer and larger roustrum. The tip of the male imago abdomen is curved and tapered, while the tip of the female imago abdomen is not curved or straight back, slightly enlarged and blunt. Female imago are larger than male imago.

Table 4. Average percentage of weight loss in five maize grain varieties against *S. zeamais* and *S. oryzae* attack

Treatment	Mean Percentage Weight Loss±SD	
	S. zeamais	*S. oryzae*
Madura-3	1.86 ± 1.12 a	1.06 ± 0.72 a
Lokal Madura	5.06 ± 1.81 b	3.36 ± 2.24 ab
Pioneer P32 Singa	3.29 ± 1.20 ab	5.51 ± 2.02 b
P27 Gajah	1.34 ± 0.89 a	5.01 ± 1.60 b
NK 212	2.94 ± 1.05 ab	1.05 ± 0.77 a

Notes: Numbers accompanied by the same letter in the same column indicate not significantly different based on 5% DMRT test.

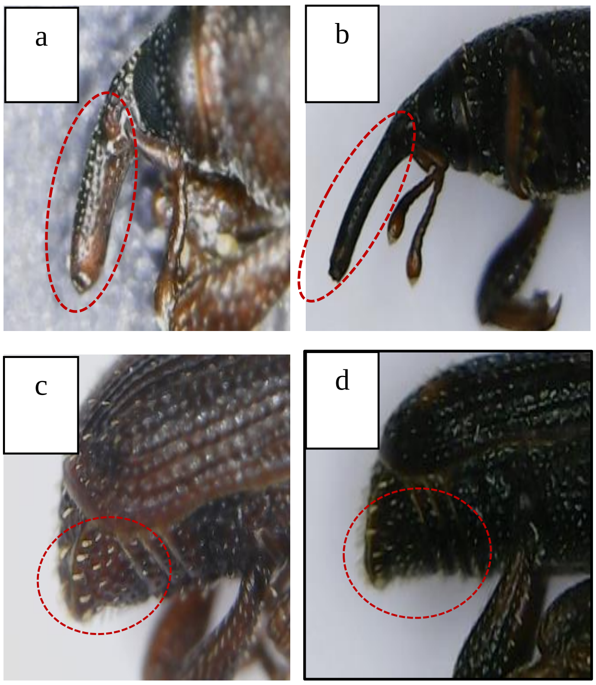

Figure 1. Roustrum and abdominal end of *S. zeamais* taken using digital microscope with 500x magnification. (a) Roustrum of male imago; (b) Roustrum of female imago; (c) Abdominal end of male imago; (d) Abdominal end of female imago.

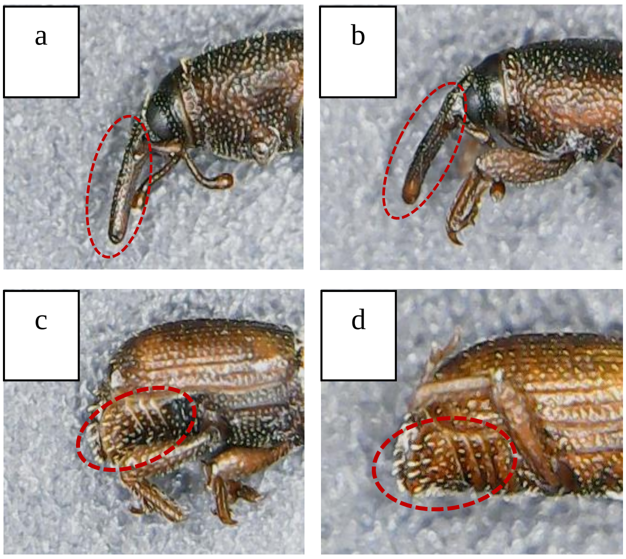

Figure 2. Roustrum and abdominal end of *S. oryzae* taken using digital microscope with 500x magnification. (a) Roustrum of male imago; (b) Roustrum of female imago; (c) Abdominal end of male imago; (d) Abdominal end of female imago.

Table 5. Mean percentage sex ratio of *S. zeamais* and *S. oryzae*

Treatment	Mean Percentage of Sex Ratio					
	S. zeamais			*S. oryzae*		
	♂	♀	♂/♀	♂	♀	♂/♀
Madura-3	48.54	51.46	0.94	53.33	46.67	1.14
Lokal Madura	35.75	64.25	0.56	45.37	54.63	0.83
Pioneer P32 Singa	49.12	50.88	0.97	42.96	57.03	0.75
P27 Gajah	61.11	38.89	1.57	44.43	55.57	0.80
NK 212	48.22	51.78	0.93	55.19	44.81	1.23

Sex ratio is the ratio between the number of males and females produced by the female parent in one reproduction. Insect pests generally have the instinct to produce more female offspring than male offspring (Marques *et al.*, 2016). The results showed that the percentage of sex ratio of *S. zeamais* and *S. oryzae* had a significant difference (Table 5).

Based on the results of the study, corn seed variety P27 Gajah has the highest sex ratio value and > 1 compared to varieties Madura-3, Lokal Madura, Pioneer P32 Singa, and NK 212. The average percentage of sex ratio of *S. zeamais* imago in the P27 Gajah variety is 1.57%. The percentage of sex ratio of *S. zeamais* with a value > 1 is due to the number of male imago is greater than the number of female imago, so the number of individuals produced will be less. The small number of individuals affects the resistance of corn kernels. The fewer the number of individuals produced, the smaller the percentage of corn seed resistance. The results showed that *S. zeamais* pests have a high potential to breed in corn kernels of Madura-3, Lokal Madura, Pioneer P32 Singa, and NK 212 varieties because they have a sex ratio value <1, which means that the number of female imago is greater than the number of male imago, so the potential for breeding *S. zeamais* is also quite high. The results of the research on the percentage of sex ratio of *S. oryzae* imago showed that corn seed variety NK 212 had the highest sex ratio value and > 1 compared to varieties Madura-3, Lokal Madura, Pioneer P32 Singa, and P27 Gajah. The average percentage of sex ratio of *S. oryzae* imago in NK 212 variety is 1.23%.

Table 6. Mean sensitivity index of five maize grain varieties to *S. zeamais* and *S. oryzae* attack

Seed Variety	*S. zeamais*		*S. oryzae*	
	Rerata IK	Category	Rerata IK	Category
Madura-3	10.68	Susceptible	4.41	Somewhat resistant
Lokal Madura	29.06	Very susceptible	24.47	Very susceptible
Pioneer P32 Singa	22.75	Very susceptible	35.98	Very susceptible
P27 Gajah	1.03	Resistant	44.67	Very susceptible
NK 212	20.54	Very susceptible	1.03	Resistant

The percentage of *S. oryzae* sex ratio with a value > 1 indicates that the number of male imago is greater than the number of female imago, so the number of individuals produced will be less. The small number of individuals affects the resistance of corn kernels. The fewer the number of individuals produced, the smaller the percentage of corn seed resistance. The results showed that *S. oryzae* pests have a high potential to breed in corn kernels of Lokal Madura, Pioneer P32 Singa, and P27 Gajah varieties because they have a sex ratio value < 1. According to Manueke *et al.* (2015) the ideal sex ratio for each creature is 1 ≤ 1, meaning that the smaller the sex ratio, the more female individuals so that the number of individuals producing offspring will be greater, thus the breeding potential of *S. oryzae* is quite high.

Calculation of Sensitivity Index

Calculation of the sensitivity index is used to determine the suitability of pests with feed media. The value of the sensitivity index indicates that the type of feed is increasingly sensitive to the attack of *S. zeamais* and *S. oryzae* pests (Antika *et al.*, 2014). The sensitivity index of five varieties of corn kernels against *S. zeamais* and *S. oryzae* is in different categories, because these types of feed have different nutritional content and seed hardness levels. The mean sensitivity index of the five varieties of maize grain to *S. zeamais* and *S. oryzae* attack showed significantly different results (Table 6).

The results showed that the lowest mean value of the sensitivity index in five varieties of corn kernels against *S. zeamais* was found in the P27 Gajah variety with a value of 1.03 (Table 6). Based on the results, P27 Gajah maize kernels are categorized as resistant or insensitive to *S. zeamais* infestation. The level of resistance of the P27 Gajah variety can be seen based on the results of the lowest percentage of fecundity testing which is 6.33% (Table 3), the lowest percentage of seed weight loss which is 1.34% (Table 4), and the highest average percentage of *S. zeamais* imago sex ratio which is 1.57% (Table 5).

The results showed that the lowest mean value of sensitivity index in five varieties of corn kernels against *S. oryzae* attack was found in NK 212 with a value of 1.03 (Table 6). Based on the results of the study, NK 212 corn kernels are categorized as resistant or not sensitive to *S. oryzae* pest attack. The level of resistance of NK 212 corn kernels can be seen based on the results of the highest percentage of mortality of *S. oryzae* imago at 37.22% (Table 2), the lowest percentage of fecundity testing at 4.33% (Table 3), the lowest percentage of seed weight loss at 1.05% (Table 4), and the highest average percentage of sex ratio of *S. oryzae* imago at 1.23% (Table 5). The resistance of maize kernels to pest attack during storage is thought to be influenced by endosperm hardness, protein content, amylose, fat and starch granule size. Shell density and seed hardness are also related to seed resistance to pests during storage (Lapinangga *et al.*, 2019).

CONCLUSION

The resistance response of the type of feed in the form of five varieties of corn seeds has a significant effect on *S. zeamais* and *S. oryzae* pests. The results showed that the corn seed variety that is resistant to *S. zeamais* pests is the P27 Gajah variety because it has the lowest sensitivity index value of 1.03. The corn seed variety that is resistant to *S. oryzae* pests is the NK 212 variety because it has the lowest sensitivity index value of 1.03.

AUTHORS CONTRIBUTIONS

DF and DM consider and plan research methods. DF conducted the research, collected data and analyzed the data. DM and ET interpreted the data and provided feedback or comments on the research flow and data analysis. All authors have read and approved the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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