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MORTALITY RATE OF *SITOPHILUS ZEAMAIS* IN LOW TEMPERATURE STORAGE

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ABSTRACT

Maize is one of the important commodities contributed as food consumption and other bioproducts to the world. The high demand of maize in global must be followed its availability in markets. Decline the post-harvest losses along maize distribution will keep the availability in markets and solve food security. One of the post-harvest losses caused is a pest, especially in maize storage. The temperature treatment to control Sitophilus zeamais, a major insect found in maize storage, becomes potential action to reduce the post-harvest losses. This study aimed to investigate the Sitophilus zeamais mortality rate and physical changes of maize kernel under low-temperature conditions. The Sitophilus zeamais was cultured within four months before being used for experiments. The two different low-temperature conditions and one control treatment were used in this study. The results showed that the temperature of maize kernel was changed, indicating the movement of maize from room temperature to low-temperature storage. Furthermore, in the low-temperature treatments, the maize moisture content was slightly changed compared to the control treatment. In addition, there is no chilling injury issue to maize kernel. However, the unknown mechanism of Sitophilus zeamais protection to the low temperature was found in the Low Temperature I treatment through the finding of whole grain. In the mortality rate, the highest mortality rate was found at Low Temperature II treatment (95.30 ±11.54%). These findings suggest that low-temperature treatment could be the action for maize pest management, especially in storage.

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INTRODUCTION

Maize provided carbohydrate sources for living organisms, not only for food consumption but also used as bioproducts, such as biofuel. The high demand for maize (*Zea mays*) as food and other bioproducts in industrial is expected to increase time by time. Over the years, global maize production has shown a steady increase, but human consumption of maize grain has remained steady (Ranum et al., 2014). Meanwhile, in the biofuel sector, maize is the predominant raw material for bioethanol production and the predominant raw material in biogas production (Skoufogianni et al., 2019). Many utilization of maize as raw material for several bioproducts, have to be followed by its availability in the market.

Improvement of productivity or production value is unable to solve the availability issue of maize in the market. After the maize is harvested from the farm, the maize will then be distributed through supply chain activity. During distribution, maize will have both quantitative and qualitative losses with variably causes occurred during post-harvest stages, from land harvesting to the consumers (Palzer et al. 2012; Marpaung et al. 2020a; Marpaung et al. 2020b). During the rainy season, which potentially increases the maize moisture content, the post-harvest quality reduction occurred around ± 57% of total maize products in Indonesia. Meanwhile, the post-harvest losses are between 5.3-15.2% at the farm level during rainy and dry seasons (Purwadaria 1988). Thus, proper post-harvest management would help the actors in the maize supply chain to reduce the post-harvest losses of quality and quantity.

Post-harvest food loss reduction is critical for the sustainable improvement of food and nutrition security. One of the critical problems of reducing post-harvest loss is the lack of analytical evidence on losses and their determinants along distribution chains (Affognon et al., 2015). The example of a 5 % loss at the marketing point in quantities may be calculated differently from a comparable amount of loss at the harvest level. For instance, at the marketing point, a 5 % loss in quantities may be calculated differently from a comparable loss at the harvest level. The prices usually increase as products move from one point of the distribution chain to another downstream level. The cost of losses will increase at a later point in the distribution chain. Therefore, avoiding post-harvest losses at the later point in the distribution chain will result in more excellent overall value than reducing losses at the earlier point.

The insect pests were one of the significant causes of maize post-harvest losses. They are appeared along the distribution chain, from land to storage. Most insects, which affect the post-harvest losses, are caused by a single population of pests, depending on the region and the agroecosystems (López-Castillo et al., 2018). Several species are involved in maize losses during the distribution chain, such as *Sitophilus zeamais, Prostephanus truncatus, Sitotroga cerealella, Rhyzopertha dominica, and Tribolium castaneum,* which are considered as significant pests that cause severe problems in agriculture globally. Maize weevil (*Sitophilus zeamais*) causes post-harvest losses during storage up to 40% of total production, many found in developing countries (López-Castillo et al. 2018). Proper post-harvest management through *Sitophilus zeamais* controlling will reduce the losses.

The maize weevil (*Sitophilus zeamais*) is a common pest found in maize storage. The life cycle of *Sitophilus zeamais* is started from egg to larva, from larva to pupa, and from pupa to adult maize weevil (Fahad et al., 2018). Their life cycle period will also be correlated to the maize storage. In the optimum environment condition, the maize weevil will also be growing well by consuming the maize grain, breaking the grain by chewing the hole, resulting in the losses of maize. Besides causing post-harvest losses, *Sitophilus zeamais* is also caused to reduce nutritional quality, weight, and germination rates of seeds in developing countries (Caneppele et al., 2003). In the study on fungal abundance on stored corn, *Sitophilus zeamais* was also involved as the carrier from outside to storage (Barney et al. 1995), which is gain food safety issue. Pest management of *Sitophilus zeamais* in maize storage must become the primary concern by actors who play in each point during the distribution chain.

Pest management is known as practice for the control of pests. The standard method applied in most maize storage is fumigation, using methyl bromide and phosphine as the fumigants. However, there is an environmental health issue with methyl bromide and phosphine usage at a specific limit (Budnik et al., 2012). Several strategies have been applied to control the maize in storage, including chemical treatment (Yang et al. 2020; Parwada et al. 2018), biological treatment (Adarkwah et al. 2012; Mbata et al. 2018), and physical control (Gvozdenac et al. 2019; Nakakita and Ikenaga, 1997; Lu and Jhang, 2016). One of the promising pest management methods is high or low temperatures storage.

A previous study (Nakakita and Ikenaga 1997) showed that low-temperature storage is successfully controlled insect pests. The decline of respiration rate, oxygen consumption, and locomotory activity was shown in low-temperature storage. However, the lowest temperature was only until 5 ^{o}C, and the mortality rate was not investigated. Another study (Gvozdenac et al. 2019) in low-temperature tolerance of *Sitophilus zeamais* showed that the 100% mortality rate was found started from -15 ^{o}C within 180 min incubation. They suggested having a high mortality rate of *Sitophilus zeamais*, prolonging the time incubation, and decreasing the storage temperature could be the way. However, the grain was absent during incubation time, unrepresented the actual situation in the maize storage. In this study, the mortality rate of *Sitophilus zeamais* under low temperature storage was investigated. In addition, the physical properties of the stored maize were also identified here.

METHODS

Insect Collection

Sitophilus zeamais was cultured in the Laboratory of Biosystems Engineering, Institute of Technology Sumatera, Indonesia (25.7±0.1°C and 82.66±0.57% relative humidity) without exposure to any insecticide (Fig 1). The maize with about 14-15% moisture content was used as food media for the insect. Every month, the insect was sub-cultured by moving 1-3 healthy adults to 300-gram maize in a plastic container (Diameter 10 cm; Height 8 cm). Before experimental treatment was applied, 5 *Sitophilus zeamais* adults were randomly moved to a plastic jar containing 300-gram maize and incubated for 3 hours to ensure the S.zeamais were in health condition.

Experimental Protocol and Condition

The five *Sitophilus zeamais* adults were randomly selected and put into a plastic container containing 300 grams of maize. Each treatment had three plastic containers as their replications. The low-temperature condition was carried out in the separate freezers and refrigerators. In order to minimize the loss of cold air when opening the door, the freezing chamber had been sealed with a wrapped plastic and left enough space to fit with the thermohygrometer cable for temperature recording. The low-temperature treatments were conducted within 3 hours of storage. After the given exposure period, the samples were removed from the freezer or refrigerator and incubated to a dish at room temperature condition within 30 min to make sure the *Sitophilus zeamais* were still alive or not.

The environmental conditions are shown in Table 1. The maize moisture content and temperature were measured by a grain moisture tester (Graigar LDS-1G). The following equation calculated the mortality rate:

$$Mortality\ Rate = (\frac{Final\ Population}{Initial\ Population} X100\%$$

Figure 1 The *Sitophilus Zeamais* Culture In Maize

Table 1 Environmental Storage Conditions

Condition		Mean	SD
Temperature Storage (°C)	Control	25.7	±0.1
	Low Temperature I	-0.96	±-0.25
	Low Temperature II	-18.13	±-0.05
Relative Humidity (%)	Control	82.66	±0.57
	Low Temperature I	Undetected	Undetected
	Low Temperature II	72.33	±2.08

Table 2 Temperature Changes Of Maize Kernel

Temperature of Maize	Pre-Treatment (°C)	Post-Treatment (°C)
Control	27.03±0.06	27.63±0.15
Low Temperature I	27.10±0.00	23.20±2.50
Low Temperature II	27.07±0.06	19.87±2.40

Statistical Analysis

The *Sitophilus zeamais* mortality data after exposure to low temperatures were calculated as percentages. Treatment percentage number of mortalities was analyzed by analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT), with insect mortality as the response variable and low-temperature treatment as a model, to know the impact low-temperature storage on mortality rate.

RESULTS AND DISCUSSION

Physical Properties Changes of Maize Kernel

Table 2. shows that the temperature of the maize kernel was changed. In the low temperature, I and low temperature II storage, the temperature of maize kernel was decreased from 27.10±0.00 °C to 23.20±2.50 °C and from 27.07±0.06 °C to 19.87±2.40 °C respectively, compared to the control, which is stored in the room temperature condition, the temperature of maize kernel was increased. The temperature changes indicated that the movement of maize from room temperature to low-temperature storage has occurred.

Environmental conditions of storage will affect the quality of maize kernel. In certain RH conditions, the maize kernel will absorb water from the environment and accelerate deterioration (Aqil 2020). The temperature changes of the maize kernel are shown in Table 2. In this study, the RH of control and low temperature II treatment were 82.66±0.57% and 72.33±2.08%, respectively. Unfortunately, the RH in low temperature I was undetectable due to the limitation of the thermohygrometer instrument. In the previous study, in 23 °C with 60% RH, maize's physical properties and physicochemical quality were reduced under six months of storage (Coradi et al. 2020). However, in 10 °C with 40% RH, maize's physical properties and physicochemical quality were maintained under six months of storage. The degradation rate is directly proportional to the period of seed storage in a high RH environment (Barton 1943). The low RH was found in the low-temperature storage in this study. A variety of empiric experiments have been performed concerning temperature and relative humidity on grain viability.

In most cases, lower temperature and lower relative humidity have been found to extend it (Volenik et al., 2007). Fewer experiments have looked at the effect of oxygen on grain viability. However, it applies to specific commodities. Apart from that, several researchers have reported exceptions to such rules. In the maize, it was reported that the viability change occurred at a specific temperature and RH (Volenik et al., 2007).

Table 3 Moisture Content Changes Of Maize Kernel

Moisture Content of Maize	Pre-Treatment (%)	Post-Treatment (%)
Control	15.8±0.20	16.23±0.12
Low Temperature I	15.73±0.05	15.8±0.00
Low Temperature II	15.66±0.05	15.93±0.32

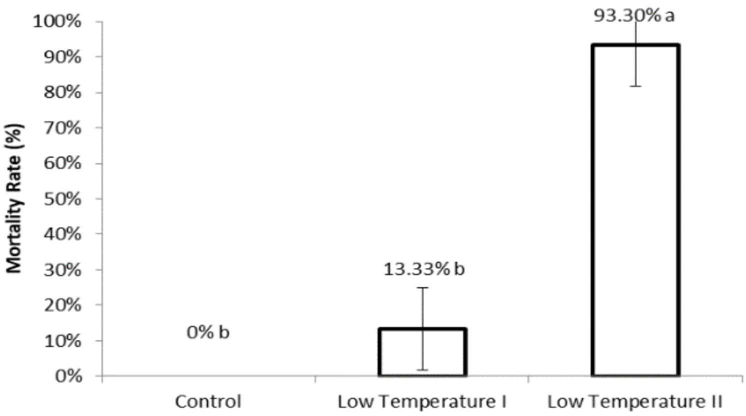

Figure 2 The Mortality Rate Of *Sitophilus Zeamais* Under The Low-Temperature Condition

In the low-temperature treatment, agriculture products will also affect the moisture content changes, which becomes an essential parameter in physical preferences from consumers. Several factories have their own rules for the moisture content of maize products. Therefore, the effort to optimize the maize quality in storage must consider the moisture content changes. The moisture content changes of maize kernel were shown in Table 3. In this study, the low-temperature storage of maize with *Sitophilus zeamais* resulted in the moisture content were increased from 15.8±0.20% to 16.23±0.12% in the control treatment, from 15.73±0.05% to 15.8±0.00% in the Low Temperature I treatment, and from 15.66±0.05% to 15.93±0.32% in Low Temperature II treatment. The low-temperature treatment has less amount moisture content changes compared to the control treatment. This suggests that low temperatures are effective in improving grain resistance to grain deterioration. Several studies showed that specific temperature and RH conditions would affect moisture content (Volenik et al., 2007; Sawant et al., 2012; Coradi et al., 2020). The germination energy and capacity of maize with 15% moisture content remain even after a ten-day storage period in low-temperature storage, -25 ^{o}C.

Meanwhile, maize with above 15% moisture content showed decreased germination energy and ability under -20 ^{o}C and 25 ^{o}C for more than three days of storage (Domin et al. 2020). These findings suggest low-temperature storage as a potential treatment for pest control in maize seeds storage. Another factor for low-temperature storage as a potential treatment to maintain the maize quality is low respiration rate. Respiration is a metabolic process which is occurred in living organisms, and water is one of the products. High temperatures can intensify respiration rate rapidly and accelerate improvements in seed storage that affect the decline in the productivity and vigor of seeds. Meanwhile, the low temperature and RH condition would minimize the rise of grain moisture content and the rate of grain respiration during the storage (Aqil 2020). Therefore, further investigation on the storage of maize with *Sitophilus zeamais* under low-temperature conditions will give interesting information.

The Mortality Rate of *Sitophilus zeamais* in Low-Temperature Condition

In order to solve pest problems, the mortality rate of *Sitophilus zeamais* was investigated in this study. The mortality rate of *Sitophilus zeamais* was showed in Figure 2. The mortality rate of *Sitophilus zeamais* was found 0% in the control treatment, 13.33±11.55% in the Low Temperature I treatment, and 95.30 ±11.54% in the Low Temperature II treatment. According to statistical analysis, the low-temperature treatment significantly affected the mortality of *Sitophilus* *zeamais* at $p<0.05$ level. The highest mortality rate was found in Low Temperature II treatment.

In the previous study, the maize weevil without any maize grain incubation showed the 100% mortality rate began from -15 ^{o}C with 180 min incubation. Furthermore, the mortality rate in the -4 ^{o}C reached 32.5% with 180 min (Gvozdenac et al. 2019). Both Low Temperature I and Low Temperature II have a similar mortality rate to the previous study. The incubation with maize kernel will increase the tolerance of *Sitophilus zeamais* to the low temperature, resulting in the decrease of mortality rate. Another study conducted by Salha et al. (Salha et al.) showed that the mortality rate reached 100% in the 6 ^{o}C with 43 days incubation. Prolonging the incubation time and lowering the temperature storage may increase the mortality rate in maize storage. However, within weeks or months, prolonged exposure to suboptimal temperatures could provide substantial control levels (Beckett 2011).

In this study, the *Sitophilus zeamais* was considered susceptible to low temperatures, particularly in -18 ^{o}C storage conditions. Compared to the high-temperature treatment storage, the acclimation to a sublethal high temperature significantly enhanced the heat tolerance level of *Sitophilus zeamais* adults, causing the reduction of their subsequent susceptibility to lethal high temperatures (Lü and Zhang 2016). Meanwhile, in extremely low-temperature storage, they are known to be non-acclimatized in hours and days. Extreme temperatures are effortlessly efficient, but they can be economically and technologically demanding (Gvozdenac et al., 2019).

Self-Protection of *Sitophilus zeamais* Under Low-Temperature Condition

As shown in Fig 3., the holes were found in the several amounts of maize kernel in the Low Temperature I treatment, but nothing in Low-Temperature II and control treatment. Our pre-assumption of such phenomena came from *Sitophilus zeamais*. The mortality rate result showed that the mortality rate in Low Temperature I (around 0 oC) was meager, the *Sitophilus zeamais* still chunk the hole. Another previous study showed that in a lower temperature, the locomotory activity of *Sitophilus zeamais* will be decreased, similar to their respiratory rate. At 5 ^{o}C, *Sitophilus zeamais* could still walk, although the movement was slowed down, while the *S. oryzae* were paralyzed (Nakakita and Ikenaga 1997). In the cases of incubation of maize with *Sitophilus zeamais* in 6 ^{o}C within 43 days storage, it has a prolonged reduction of the population (Salha et al. 2010). However, there is no further investigation about maize kernel characteristics after low-temperature treatment. In order to know the mechanism and survival way of *Sitophilus zeamais* under low-temperature storage, a further experiment is necessary to be conducted.

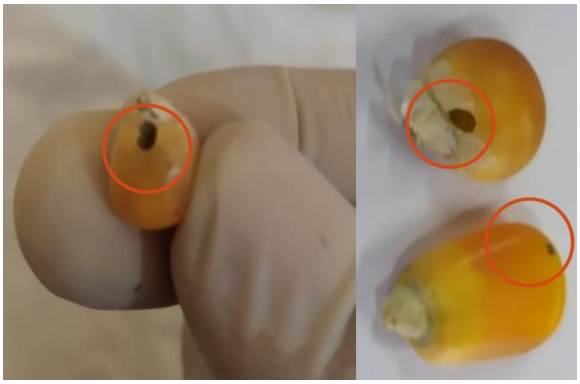

Figure 3 The Hole As *Sitophilus Zeamais* Protective Action In Low-Temperature Condition

CONCLUSION

This study assessed the effect of low-temperature storage on the mortality rate of maize pests. Based on the results, the *Sitophilus zeamais* has the highest mortality rate at -18 ^{o}C, with the mortality rate almost 100%. It is followed by the small amount of moisture content changes, and the physical appearance of maize remains compared to the control treatment. These findings suggest, the Low-Temperature II treatment could work as pest management for maize instead of chemical usage, which is known as a standard treatment.

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