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The Effect of Neem Leaves Powder (*Azadirachta Indica A. Juss*) and Storage Time to Rejected Corn Quality

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Abstract— This research has aimed to determine the level of neem leaves powder (*Azadirachta indica A. Juss*) and the optimal storage time of rejected corn to decrease aflatoxin content, percentage of water content, and percentage of moldy seeds. This research uses a completely randomized experimental design (CRD) 6 x 4 factorial with three replications. Where factor A is the level of neem leaves powder (0, 0.5%, 1%, 1.5%, 2%, 2.5%) and factor B is the storage time (2, 4, 6 and 8 weeks). The analysis showed that there was no interaction ($P > 0.05$) between factor A and factor B to the content of aflatoxin, percentage of water content and percentage of moldy seeds, but respectively for factor A and factor B had a very significant effect ($P < 0.01$) against aflatoxin content and percentage of water content. For a percentage of moldy seed, only factor B had a very significant effect ($P < 0.01$). The results of the study can be concluding that the selected treatment found on neem leaves powder level of 1% with four weeks of storage time (A3B2). In this condition, a decrease in the aflatoxin content of rejected corn from 170 ppb to 53 ppb (a decrease of 68.43%) with a water content of 14.47% and 0% moldy seeds.

Keywords— Aflatoxin, Neem leaves, Rejected corn, Storage time.

I. INTRODUCTION

Corn is one of the most widely used agricultural commodities as raw material for animal feed, especially poultry. In 2018 Indonesia's corn production was estimated to reach 30,056 million tons of dry corn; this is higher than the production in 2017, only around 28,924 million tons of dry corn (ARAM I, BPS 2018). An increase in corn production from year to year.

Often the increase in corn production is not matched by proper post-harvest handling and the length of the distribution chain from farmers to collectors resulting in corn being easily damaged. Storage is one of the most critical post-harvest handling chains, with storage of corn availability being continuous. Storage must pay attention to factors such as corn water content, the relative humidity of the air, the temperature in the storage warehouse and the layout of the pile to avoid warehouse pests and fungus contamination

This contamination opportunity is quite significant because the tropical climate in Indonesia, which has high humidity and environmental temperature, is very supportive of the growth and development of mycotoxin-producing fungi (Rachmawati *et al.*, 2004). Damage at this storage level will cause a decrease in the quality of

corn, both qualitatively and quantitatively; this will affect the selling power of the corn.

Fungi that often contaminate corn during storage are fungi *Aspergillus sp.*, specifically *Aspergillus flavus* and *Aspergillus parasiticus*. Both these fungi produce mycotoxins in the form of aflatoxins. Aflatoxin is a secondary metabolite of *Aspergillus flavus* and *Aspergillus parasiticus*. A survey conducted by the UGM Faculty of Agricultural Technology in collaboration with the East Java Food Security Agency by taking 84 corn samples at the farmer level and 55 at the collector level found that 30% of corn at the farm level was contaminated by aflatoxin above two ppb and 10% was contamination with aflatoxin above 100 ppb with the highest value of 470 ppb. At the level of traders, 45% of aflatoxin polluted corn is above 20 ppb, while those above 100 ppb are 18% (Rahayu, 2010). Aflatoxin contamination in feed can reduce the bodyweight of broiler chickens (Al-Shawabkeh *et al.*, 2009).

Aflatoxins consist of 4 types, namely aflatoxin B1, B2 G1, and G2. Research conducted by Bahri *et al.*, (1994), on broiler feed, states that the most prevalent mycotoxin is aflatoxin, especially aflatoxin B1. Aflatoxin B1 is the most dangerous toxic compound and is

categorization as an IA group carcinogenic compound. Chronic effects of AFB1 poisoning can cause a significant decrease in the body weight of broilers in feeding containing AFB1 200 ng / g for eight weeks. Supported by Muthiah *et al.*, (1998) that AFB1 causes livestock health problems such as stunted growth and death so that livestock production decreases.

The results of mycotoxin experiment on local corn (Java, North Sumatra, Lampung, and South Sulawesi) and imports (USA and Argentina) from various feed mills in Indonesia that were tested by ELISA showed that AFB1 was detected in the concentration range of 19.1 - 87.4ng / g (Tangendjaja *et al.*, 2008). Thus, the above conditions conflict with the SNI regarding the required aflatoxin content limit of 50 ng/g for feed (SNI, 2000). Aflatoxin will accumulate into the body tissues of livestock that consume, resulting in the presence of residues in livestock products that will endanger the health of consumers. Paulin *et al.* (2011) state that aflatoxin is also dangerous for the body because it can cause aflatoxin, which can be the forerunner to hepatitis B and liver cancer. Considering the effects that can be caused by contamination of food by aflatoxins are quite detrimental; thus, it is necessary to inhibit the biosynthesis of aflatoxin in corn in order to create food safety from animal origin.

One alternative in inhibiting aflatoxin biosynthesis is by giving neem leaves (*Azadirachta indica A. Juss*). Neem leaves (*Azadirachta indica A. Juss*) are known as natural anti-fungi and biopesticides. The use of natural biopesticides derived from plants is very safe to use because it can biodegrade, does not damage the environment with minimal side effects. Neem leaves extract reported to contain active ingredients azadirachtin, solanine, melantriol, and nimbin, which function as pesticides (Tjahjani and Rahayu, 2003). Compounds contained in neem leaves such as azadirachtin, solanine, nimbin, and nimbidin, where these compounds function as cell growth intruders that can result in fungal cell death (Syamsudin, 2007).

Bhatnagar and McCormick, (1988) reported that a mixture of neem leaf extract with a buffer of 0.01 potassium phosphate had shown to inhibit biosynthesis and aflatoxin production by *Aspergillus flavus* in vitro without affecting fungal growth. Research conducted by Bhuiyan *et al.*, (2013) reported that the addition of neem leaf powder (*Azadirachta indica A. Juss*) by 20 g in 1 kg each of rice, corn, and wheat which were then stored for six months did not show aflatoxin contamination except in maize. However, the level of contamination in corn is also deficient at 0.8-3.2 ug/ kg. Extracts obtained from neem leaf and seed extracts can inhibit aflatoxin

biosynthesis and inhibit fungal growth (Abyaneh *et al.*, 2005; Allameh *et al.*, 2002). Apart from being an antifungal and natural biopesticide, the addition of neem leaf powder (*Azadirachta indica A. Juss*) to broiler feed by 1, 2, 3 g/kg can increase the average body weight (Wankar *et al.*, 2009).

With this problem, many corn farmers have sorted/rejected by feed mills. This resulted in maize not being utilized, and farmers suffered losses. Based on the above problems, it is necessary to do a research to find out the effectiveness of giving neem leaves to the rejected corn/mill sort in reducing the level of aflatoxin contamination so that the rejected corn can be reuse.

II. MATERIALS AND METHODS

2.1. Materials

The materials used in this study were rejected/sorted corn from PT. Japfa Comfeed Indonesia Padang with 15% water content and 170 ppb aflatoxin content of 57.600 g and neem leaves powder as much as 720 g. The equipment used is an oven, a sifter, a blender, an analytical balance, a plastic bag, and a woven bag.

2.2. Method

The design used is a Completely Randomized Design (CRD) Factorial 6x4 with three replications. Factor A is the level of neem leaves powder consisting of 6 (six) levels, namely:

A1 : control (without neem leaves powder)

A2 : neem leaves powder 0,5 %

A3 : neem leaves powder 1 %

A4 : neem leaves powder 1,5%

A5 : neem leaves powder 2%

A6 : neem leaves powder 2,5%

Factor B is the storage time of corn consists of 4 (four) levels, namely:

B1 : 2 weeks

B2 : 4 weeks

B3 : 6 weeks

B4 : 8 weeks

Data analysis uses statistical analysis with an analysis of variance by the Completely Randomized Design (CRD) 6x4 factorial with three replications. If there are treatment differences, then the differences between treatments are tested with Duncan's Multiple Range Test / DMRT (Steel and Torrie, 1995).

2.3. Research Implementation

The neem leaves are dried in the sun or oven at a temperature of 600 C so that the water content is reduced, and then the neem leaves are grinding with a blender. Then filtering is done using a sieve.

Each treatment consisted of 800 grams of corn added with various levels of administration of neem leaf powder (0.5%, 1%, 1.5% 2%, 2.5%). The rejected corn is put into a container and then sprinkled with neem leaves powder according to the treatment until both are homogeneous. The rejected corn is put into a woven bag measuring 30 x 40 cm — storage at room temperature for 2, 4, 6, and 8 weeks.

2.4. Observed variables

1. Qualitative Calculation of Corn Contaminated Aflatoxin (UV light).

Observation of corn is done visually using a device equipped with UV light with a wavelength of 360 nanometers. As much as 800 grams of corn mashed with a grinding machine and placed in the tray then leveled. For the calculation of aflatoxin content, corn is brought to the darkroom then the tool is turned on. The tray containing refined corn is place under UV light, and the aflatoxin content is calculated.

2. Percentage of water content

To calculate the water content in corn can use the Moisture Tester Kett PM410 tool. Prepare a sample of corn each treatment as much as 100 g/replicate, then put in the tool evenly. The tool will work automatically, and on the monitor screen, the results show the moisture content in corn, and three repetitions are done.

3. Percentage of mouldy seeds

Moldy seed observations made visually with the sense of sight. The formula can calculate the percentage of moldy seeds::

$$\frac{\text{Weight of corn (week)} - \text{weight of whole seeds}}{\text{weight of corn during storage (week)}} \times 100\%$$

III. RESULT AND DISCUSSION

3.1. The effect neem leaves powder (*Azadirachta indica* A. Juss) and storage time to the aflatoxin content (ppb) of rejected corn.

The average aflatoxin (ppb) content of the rejected corn added by neem leaves powder and different storage times can be seen in Table 1.

Statistical analysis showed that there was no interaction between factor A (level of neem leaves powder) and factor B (storage time) to the content of aflatoxin (ppb) on rejected corn, but respectively for factor A and factor B had a very significant effect (P <0.01) of the aflatoxin content of rejected corn where the

initial content of aflatoxin before the addition of neem leaves powder is 170 ppb.

Table 1. The average of aflatoxin content (ppb).

Level (%)	Storage time (week)				Averag e
	B1 (2)	B2 (4)	B3 (6)	B4 (8)	
A1 (0)	172,00	188,00	191,00	225,67	194,17 ^a
A2 (0,5)	150,00	137,00	156,67	170,00	153,42 ^b
A3 (1)	138,67	53,67	133,33	136,67	115,58 ^c
A4 (1,5)	148,00	105,67	129,67	143,67	131,75 ^c
A5 (2)	137,33	89,33	146,00	145,00	129,42 ^c
A6 (2,5)	159,33	109,00	129,33	132,67	132,58 ^c
Averag e	150,89	113,78	147,67	158,94	
e	a	b	a	a	

Based on the results of the DMRT test (Duncan Multiple Range Test) on factor A showed that corn without neem leaves powder (control) the aflatoxin content was significantly (P <0.05) higher than all treatments, while the addition of neem leaves powder level is 0.5% (A2) the aflatoxin content was significantly (P<0.05) lower than adding 0% (control) but significantly (P <0.05) higher than adding 1-2.5% level of neem leaves powder (A3, A4, A5 A6). The addition of level 1- 2.5% (A3, A4, A5 A6) had a significantly different effect (P> 0.05) on the aflatoxin content. In factor B, the storage duration of 2, 6, and 8 weeks (B1, B3, and B4) were significantly (P <0.05) higher than the aflatoxin content of 4 weeks (B2).

In factor A, the highest aflatoxin content is found in corn without neem leaves powder (control / A1), which is then followed by a level of 0.5% (A2) neem leaves powder. Where there is no inhibition in the growth of *Aspergillus flavus* in producing aflatoxin in corn without neem leaves powder (control). Another case with the addition of 0.5% neem leaves powder (A2), in this treatment the level of neem leaves powder is relatively smaller among other levels, this causes no maximum inhibition of aflatoxin production.

At the level of neem leaves powder of 1-2.5% (A3, A4, A5, A6) low aflatoxin content can be caused by the ability of neem leaves to inhibit aflatoxin biosynthesis where the more doses are given, the higher the percentage of inhibitory power to aflatoxin production which was marked by a decrease in aflatoxin levels during the study. This is caused by the presence of active compounds contained in neem leaves. The active compounds in neem leaves extract do not inhibit the ability of fungal growth

but interfere with the production of aflatoxin, wherein inhibition of aflatoxin production is associated with morphological changes in fungi (Abyaneh et al., 2005).

The addition of neem leaves powder in this study is believed to reduce the aflatoxin content in corn. This is supported by previous studies, among others conducted by Abyaneh *et al.*, (2005) showing that the addition of neem leaves extract solution above 10% can effectively inhibit the production of aflatoxins from *Aspergillus flavus*. Furthermore, Ariwardhani's research (2008) showed that the influence of neem leaves extract was able to inhibit the production of aflatoxin as evidenced by the decrease in the content of aflatoxin on GAN (Glucose Ammonium Nitrate) media that had been inoculated with *Aspergillus flavus*. The effect of neem leaves extract on aflatoxin biosynthesis is related to morphological changes in the mycelium of the fungus *Aspergillus flavus*. Furthermore, the results of research by Zeringue Jr. and Bhatnagar (1994) showed that after incubation for three days, the production of aflatoxin decreased by 90% and the fungus biomass of *Aspergillus parasiticus* decreased by 51% compared with cultures without volatile compounds of neem leaves. In other words, by inhibiting fungal growth by neem leaves activity, aflatoxin production is also inhibited because aflatoxin is a secondary metabolite compound produced by the fungus *Aspergillus flavus*.

Neem leaves produce terpenoid compounds and phenolic compounds which function as antifungal. The terpenoid compounds in neem leaves such as azadirachtin, nimbidin, nimbin, nimbolide, and nimbidic acid are known to suppress the growth of pathogenic fungi by disrupting cell walls or inhibiting cell wall permeability so that essential components needed by pathogenic fungi such as proteins emerge from cell walls, cells then slowly die (Biswas, 2002., Koul *et al.*, 2008 in Sekarsari *et al.*, 2013). Terpenoid compounds can bind to protein and lipid molecules so that they can affect the physiological function of cell membrane proteins and protein enzymes (Utami, 2005). This is supported by Agustini *et al.*, (2016) where nimbin and nimbidin compounds as antifungal substances in neem leaves extract can inhibit the growth of mycelium fungus *A. porri*.

Phenolic compounds, which are included in phenolic compounds are flavonoids and tannins. Phenolic compounds can inhibit aflatoxin production, this was stated by Kim *et al.* (2006) in which mitochondria play a role in the supply of acetyl-CoA which is a major precursor in aflatoxin biosynthesis. Damage to the mitochondrial respiration chain caused by the phenolic

component is part of the inhibition of aflatoxin production. Phenolic compounds interact with cell membrane proteins that cause precipitation and denaturation of cell membrane proteins (Manitto, 1992). Damage to the cell membrane causes changes in the permeability of the membrane, resulting in fungal cell membrane lysis (Parwata and Dewi, 2008).

The mechanism of action of flavonoids in inhibiting fungal growth is by disrupting fungal cell membrane permeability. Hydroxyl groups found in flavonoid compounds cause changes in organic components and nutrient transport which will eventually lead to toxic effects on fungi (Jupriadi, 2011 in Nuryani 2016). Furthermore, Harboune (1987) states that flavonoid compounds enter fungal cells through holes in the cell membrane formed because phenol compounds have denatured cell membrane lipids. These protein compounds will be denatured by flavonoids through their hydrogen bonds. The ability of flavonoids to bind to proteins causes inhibition of cell wall formation so that hyphal growth is also inhibited because the cell wall composition needed is not met.

It can be concluded that the influence of neem leaves can cause damage to the fungal cell wall or lysis where this effect resembles some other antifungal compounds. Fungal cell walls are essential to protect cells from osmotic pressure changes, where if there is interference with the osmotic pressure it can increase cell vacuolation or even cause death in organisms due to osmotic shock. Chitin and glucan contained in the cell walls of *Aspergillus* species are molecules that are sensitive to antifungal compounds (Joklik, 1980 in Abyaneh, 2005). Lysis of *Aspergillus flavus* mycelium will cause deactivation or inhibition of aflatoxin production. Pitt (1993) states that the inhibition of aflatoxin production may be caused by enzymes released during lysis of the fungus mycelium. The same thing was said by Namazi *et al.* (2002) where damage to mycelium and conidium molds is one of the characteristics of the aflatoxin deactivation process.

In factor B (storage time), the aflatoxin content in corn with a storage period of 4 weeks (B2) is lower than other treatments (B1, B3, and B4), this is likely during four weeks storage is the best time of effectiveness of neem leaves in inhibiting the production of aflatoxin. Although it is not known exactly how many effective time intervals required by neem leaves to inhibit aflatoxin production due to the lack of research on the effect of neem leaves and storage time on the aflatoxin content of corn. According to Jay (1996) that the ability of mold to accumulate and aflatoxin depends on several factors,

namely the genetic potential of the mold, environmental requirements (substrate, humidity, temperature, pH), and the length of contact between the mold and the substrate. This is what causes the longer storage time the higher the aflatoxin content, as seen in corn with eight weeks storage (B4). According to Susanto (2008), long-time storage will increase the content of aflatoxin, because with increasing time will provide opportunities for the fungus producing aflatoxin to produce secondary metabolites of aflatoxin.

Although the neem leaves are known for its anti-fungi properties which can inhibit the growth of fungus but the nature of the neem leaves in inhibiting the growth of this fungus by suppressing growth is not by stopping or killing the fungus. It can be interpreted that with long storage time, fungus spores can continue to grow and produce aflatoxin.

Furthermore, other factors that can affect the increase in aflatoxin content in corn during storage are temperature, humidity, and substrate. During the research, corn storage temperature ranged from 26-35°C with humidity of 70-80%. This is supported by the opinion of Syarif *et al.*, (2003) that the optimum temperature for producing aflatoxin is 25-35°C and production yield varies based on the composition of the substrate. Below 12°C no metabolism produces aflatoxin. Even if there are tiny numbers, especially at a temperature of 15°C.

The results of Maryam's research (2006) that storage temperatures between 25-32°C cause the growth of aflatoxin-producing fungi will increase by tens to thousands of ppb after being stored 28 days supported if storage techniques are not heeded. Aflatoxin-producing fungi can grow on a substrate that has fat content. According to Pater and Bullerman (1988), in general, the content of fat, protein, trace elements, amino acids and fatty acids in a material can encourage the production of aflatoxins by *Aspergillus flavus*.

The selected treatment based on the lowest aflatoxin content was in the A3B2 treatment (1% neem leaves powder level with 4 weeks storage time) which was 53.67 ppb (a decrease of 68.43% from the original aflatoxin content of 170 ppb fell to 53.67 ppb).

3.2. The effect neem leaves powder (*Azadirachta indica* A. Juss) and storage time to the percentage of water content (%) of rejected corn.

The average percentage of water content (%) of the rejected corn added by neem leaves powder and different storage times can be seen in Table 2.

Statistical analysis showed that there was no interaction between factor A (level of neem leaves powder) and factor B (storage time) to percentage of water content on rejected corn, but respectively for factor

A and factor B had a very significant effect ($P < 0.01$) on the percentage of water content of rejected corn.

Table 2. The average percentage of water content (%)

Level (%)	Storage time (week)				Average e
	B1	B2	B3	B4	
A1 (0)	14,70	14,50	14,37	14,43	14,50 ^a
A2 (0,5)	14,57	14,40	14,13	14,17	14,32 ^b
A3 (1)	14,70	14,47	14,27	14,01	14,36 ^b
A4 (1,5)	14,50	14,43	14,10	14,04	14,27 ^b
A5 (2)	14,50	14,40	14,17	14,07	14,28 ^b
A6(2,5)	14,47	14,63	14,03	14,13	14,32 ^b
		14,47	14,18	14,14	
Average	14,57 ^a	^b	^c	^c	

Based on the results of the DMRT test (Duncan Multiple Range Test) on factor A showed that corn without neem leaves powder (control) the water content was significantly ($P < 0.05$) higher than all treatments, while the level of 0.5 - 2.5% (A2, A3, A4, A5 A6) neem leaves powder had no significant effect ($P > 0.05$) on water content during the study. For factor B, the water content at 2 weeks storage time was significantly different ($P < 0.01$) higher than 4, 6 and 8 weeks storage time and 4 weeks storage time was very significantly different ($P < 0.01$) higher than storage time of 6 and 8 weeks while storage time of 6 weeks was not significantly different ($P > 0.05$) with storage time of 8 weeks.

In factor A, the percentage of the water content of corn without neem leaves powder (control / A1) is higher than that of corn given with neem leaves powder with various levels. This can be caused by corn which is not given neem leaves, inhibition of the growth of the fungus *Aspergillus flavus* does not occur so that water levels increase because the fungus will undergo metabolic processes that will ultimately produce CO₂, H₂O, and energy. In contrast to controls, all treatments with the addition of neem leaves powder of various levels ie, from 0.5 - 2.5% (A2, A3, A4, A5, and A6) had the same effect when viewed from the results of the DMRT analysis. This can be caused by the neem leaves particles used in this study in the form of powder so that it can absorb water contained in corn.

The water content of corn rejected during storage in this study decreased from 2 weeks of storage (B2) to 8 weeks of storage (B4) as shown in Table 2. Where the initial storage water content ranges from 15%. Same to the results of research Widianingrum *et al.*, (2010) in the first 4 weeks of corn storage the water content decreased from the water content at the beginning of storage. Decreased water content in corn during storage can be

caused by the material still undergoing the process of respiration, transpiration, air concentration and the activity of microorganisms. The process of respiration is an overhaul of organic materials from food ingredients such as carbohydrates, proteins and fats to produce CO₂ and H₂O with a higher respiration rate and the longer the respiration rate increases, the process of reshuffle will increase which causes a decrease in water content (Ahmad, 2013).

The rate of respiration can be influenced by the availability of the substrate and the concentration of air (the availability of O₂ and CO₂). According to Setyadjit and Syaifullah (1994), high respiration can reduce the water content of food during storage. Higher concentrations of O₂ in the storage space than CO₂ concentrations cause the rate of respiration to be faster so that a decrease in the water content of the material, and vice versa if higher CO₂ concentrations and O₂ concentrations limit the rate of respiration of the material can be inhibited so that a decrease in water content can be prevented. It can be assumed that storage with average air concentrations causes the process of respiration to run generally at a rapid rate so that it will lose more water content.

The decrease in water content during storage in this study was also caused by transpiration. Transpiration is a process of losing water in the form of gas from living tissue. The transpiration occurs because of differences in temperature and relative humidity of corn stacks with the environment. From the temperature and Rh differences, the results of corn respiration will evaporate. Water in corn tends to move to areas where the humidity is smaller. Water that evaporates from corn is the result of respiration in which carbohydrates into simple sugars are then converted into water and carbon dioxide (Sutardi and Tranggono, 1990). The storage temperature in this study ranges from 26-35°C. According to Suparjo (2010) at a high enough temperature which is around 25-27°C, evaporation of the water content of the material will take place fairly quickly. This will encourage the material to release the free water it contains to retain moisture and prevent more significant water loss. The change in material water into the vapor phase is driven by an increase in temperature.

Another opinion, where the decrease in water content during storage can be caused by the growth and metabolic activity of microorganisms need water to transport nutrients or waste materials into and out of cells. All of these activities require liquid water. Water that undergoes crystallization and forms ice or chemically bound water in a solution of sugar or salt cannot be used microorganisms

(Syarif *et al.*, 2003). Woven bag has hygroscopic properties and can absorb water up to 34% (Hartanto, 2003).

3.3. The effect neem leaves powder (*Azadirachta indica* A. Juss) and storage time to the percentage of mouldy seeds (%) of rejected corn.

The average percentage of moldy seeds (%) of the rejected corn added by neem leaves powder and different storage times can be seen in Table 3.

Table 3. The average percentage of mouldy seeds (%)

Level (%)	Storage time (week)				Average
	B1	B2	B3	B4	
A1 (0%)	0,00	0,00	0,00	1,33	0,33
A2 (0,5%)	0,00	0,00	0,00	2,00	0,50
A3 (1%)	0,00	0,00	0,00	2,00	0,50
A4 (1,5%)	0,00	0,00	0,00	2,00	0,50
A5 (2%)	0,00	0,00	0,00	1,00	0,25
A6(2,5%)	0,00	0,00	0,00	2,00	0,50
Average	0,00 ^b	0,00 ^b	0,00 ^b	1,72 ^a	

Statistical analysis showed that there was no interaction between factor A (level of neem leaves powder) and factor B (storage time) to the percentage of moldy seeds on rejected corn. However, factor B had a very significant effect ($P < 0.01$) on the reject moldy corn seeds.

Based on the results of the DMRT test (Duncan Multiple Range Test) on factor B, moldy seeds at 8 weeks storage time were significantly different ($P < 0.01$) from 2, 4 and 6 weeks storage time. At the 8-week storage period (B4) the growth of the fungus looks very visually significant. There is a real correlation between storage time and fungus growth, so the longer the storage time the more mold spores are formed.

This is supported by opinion of Miller and Trenholm (1994) where the number of spores that infect and the storage time are one of the factors that influence the resistance of corn during storage. Although neem leaves contain active compounds known as antifungal with extended storage time, the effectiveness of neem leaves in inhibiting the growth of fungus spores decreases. Furthermore, the neem leaves are inhibiting by suppressing growth not by stopping or killing the fungus.

It can be interpreted that the fungus spores of *Aspergillus flavus* continue to grow with increasing storage time. Storage time that is too long will cause more significant damage due to the growth and proliferation of fungus contaminants. Research conducted by Nafiah

(2009) shows that the storage of corn for 6 weeks has a marked increase in the total changes in damaged corn kernels when compared to the previous week. This can also be one of the factors causing the growth of fungus/mold. Where the factors that influence the cracking of seeds are: changes in water content due to changes in weather, improper stripped and warehouse attacks. Damage to feed ingredients due to changes in water content is the most common case, making it easier for the growth of microorganisms, especially fungus. Microorganisms take and eat food substances from seeds or other raw materials that cause damage to the protective layer of material. In addition to causing physical damage due to its transient nature, microorganism paves the way for contaminating contaminants such as mycotoxin-producing molds which can increase damage to feed ingredients such as seed holes, crushed and broken.

The packaging method also affects the growth of the fungus during storage. The corn was packed in a woven bag measuring 40 x 25 cm during the study. Aprianie's research results (2009) show that the storage of material by packaging it in packaging material will be able to withstand the expenditure of heat energy produced by respiration from microbes from the packaging environment to the environment outside the packaging material. Respiratory energy that is retained by the packaging material will turn into heat and moisture that will accumulate during storage. After a few days, the condition inside the gunny sack is more humid than the outside air. Higher humidity than conditions outside the packaging material is also caused by a decrease in the water content of the material in the burlap sack so that *Aspergillus flavus* can grow and reproduce accurately.

IV. CONCLUSION

The use of neem leaves powder by 1% with 4 weeks storage time can reduce the aflatoxin content of rejected corn from 170 ppb to 53 ppb (a decrease of 68.43%) with a water content of 14.47% and moldy seeds 0%.

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