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Effect of Addition of Mimba Leaf Extract (Azadirachta Indica A. Juss) to Corn with Different Types of Packaging on Water Content, Percentage of Moldy Seeds, and Aflatoxin Levels

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Abstract *— This study was conducted to determine the effect of mimba leaf extract and the type of packaging on corn stored at 4 weeks. This study used an experimental method with Completely Randomized Design (CRD) consisting of 4 treatments, and each treatment was replicated with 4 times. The treatment was the types of packaging, consist of cartoon without cover, plastic sacks, paperboard, and vacuum plastic. The measured variables were water content, moldy seed percentage, and aflatoxin content in corn after stored. Results of this study showed that the effect of adding mimba leaf extract on all types of packaging significantly different (P<0.05) on moisture content, the percentage of moldy seeds, and the aflatoxin content. The conclusion was the adding of mimba leaf extract on all types of packaging can maintain moisture content, percentage of moldy seeds, and reduce the aflatoxin content.*

Keywords*— *Aflatoxin, Corn, Mimba leaf, Type of packaging.

I. INTRODUCTION

Corn is the main raw material for poultry feed. Corn storage and processing greatly affect the quality of the corn. Corn is very easily contaminated if it is not stored properly. To reduce damage to corn, the storage method must be considered and the humidity temperature is considered so that people are competent in producing good quality corn.

Corn is a source of energy, with a carbohydrate / starch content of 64%. Domestic maize production has not been able to meet domestic needs, so it must import. In the period 1990 - 2001, the use of imported maize as raw material for the feed industry increased sharply at a rate of around 11.81% per year. Starting in 1994, the dependence of feed factories on imported maize was very high, around 40.29% and in 2000 it reached 47.04%, while the remaining 52.96% came from domestically produced corn (Deptan 2005).

Corn is one of the plants at risk of contamination by Aspergillus sp. especially Aspergillus flavus and Aspergillus parasiticus which produce aflatoxins that are carcinogenic and harmful to humans and animals. Aspergillus sp. can contaminate corn plants while they are still in the garden or during storage. These molds are naturally occurring molds that can grow in the soil so that parts of the corn plant are often affected by Aspergillus sp. These are the roots, then the stems, leaves, fruit of the corn, and then propagate to the deeper parts (Somantri 2005). According to Rahayu (2012), Aspergillus flavus is a type of fungus that often contaminates food, this type of mold can cause Aspergillosis infection and is also the mold that produces the most aflatoxins. If someone consumes food that is contaminated with low concentrations of aflatoxin continuously, it can damage the liver and reduce the immune system.

Mimba plant is a medicinal plant that has various uses to be developed as a basic material for making vegetable pesticides. Mimba leaves contain active ingredients called azadirachtin and salanin (Balfas, 1994). Mimba can produce more than 20 different types of

secondary metabolites. The leaves and seeds contain several secondary metabolites which are active as vegetable pesticides including azadirachtin salanine, meliontriol, and nimbin. Azadirachtin is used as an active ingredient in botanical fungicides that can inhibit the growth of fungi that cause plant disease (Mirin, 1997). Syamsudin (2007) reports that the compounds contained in mimba leaves are azadirachtin salanin, nimbin and nimbidin, where these compounds function as a disruptor to cell growth which can lead to fungal cell death.

Mimba leaf extract contains the main active compound azadiractin. Azadirachtin acts as an ecdysone blocker or a substance that can inhibit the work of the ecdysone hormone, a hormone that functions in the metamorphosis process of insects. Insects will be disturbed in the molting process, or the process of changing from eggs to larvae, or from larvae to cocoons, or from cocoons to adults. Usually, failure in this process often results in death (Wiwin, 2008).

The packaging used to store feed ingredients can affect how long it can be stored. The packaging is one way to protect or preserve the product. The packaging is an important material in various industries. The damage caused by the environment can be controlled by packaging. The packaging must keep the product clean and provide protection against contamination and other contaminants. Packaging must provide protection for food against physical damage, water, air, and light and must function properly, efficiently, and economically in the packaging process, and must have size, shape, weight, and facilitate further processing and must provide information and appeal to consumers. (Syarief, et al., 1989). One of them is from the type of packaging in the form of a plastic sack.

Plastic bags have smaller pores than other types of packaging. Plastic bags are rapidly being used because they are strong, waterproof, inert, transparent, can be formed, filled, and sealed by machine. Plastic bags can be used as packaging materials because they protect products from light, air, heat transfer, contamination, and contact with chemicals. The flow of gas and water vapor through plastics is influenced by plastic pores, plastic thickness, and molecular size that diffuses the product (Syarief and Irawati, 1988).

II. MATERIAL AND METHOD

2.1. Materials Research

The materials used are mimba leaf flour, Aquades solvent, corn from PT Japfa comfeed with an aflatoxin content of 200 ppb with a moisture content of 15%, and the type of packaging (open container, plastic sack, paperboard, vacuum plastic). The tools used are evaporators, analytical scales, filter paper, and glassware.

2.2. Research Implementation

2.2.1. Experimental Design

This study was performed by using a completely randomized design with 4 types of packaging treatments and 4 replications. Treatment A (Plastic Sack), treatment B (Cardboard Paper), treatment C (Open Container), and treatment D (Vacuum Plastic). The mathematical model used is according to Steel and Torrie (1995). The data obtained were analyzed by ANOVA, if there is an influence on the measured variables, it will be followed by the DMRT test.

2.2.2. Mimba Leaf Extraction

Mimba leaves (Azadirachta Indica A. Juss) are dried first in the sun. After the ingredients become dry and then blended into flour, the extraction process begins by mixing 50 grams of mimba leaf flour (Azadirachta Indica A. Juss) with 250 ml of Aquades solvent then soaking for 24 hours so that the active substances contained in the mimba leaf flour dissolve in solvent. Furthermore, filtering is done with filter paper. The filtrate obtained is collected, and then evaporated with a vacuum evaporator at a temperature of 550C to obtain a concentration that resembles oil. Each extraction result is put into a basin of 20 ml or a dose of 2.5% of the weight of corn weighing 800 grams each. Because based on the research of Sonyarati (2006) the 2.5% dose has had an effect on the attack of warehouse pests sitophilus zeamir matsh on corn that has been given mimba leaf extract, then the corn that has been filled with extract is homogenized until evenly distributed, then the corn is dried and air dried for about 1-2 hours. or it can also be oven at 600C for 60 minutes so that the extract that has been mixed is absorbed by the corn. Furthermore, storage is carried out in different containers for 4 weeks using plastic sacks, paperboard, open containers, and vacuum plastic. Each treatment, a pile of corn is placed on a board to prevent evaporation. Samples were viewed weekly. Sampling was carried out after 4 weeks of age.

2.3. Observed variables

2.3.1. Percentage of Water Content of Corn

To calculate the moisture content in corn, it can be calculated using a tool commonly used by the company PT. Japfa Comfeed Indonesia Tbk, Padang branch, namely the PM 410 kett tester tool, can be seen in the picture below.

Fig.1 : PM-410 Moisture Tester

Prepare the corn that has been stored for 4 weeks (100 grams per treatment), put it in a stainless glass measuring 100 grams of corn, then put it in the circle of this tool evenly, this tool works automatically and on the monitor screen the moisture content of the corn is read.

2.3.2. Determination Percentage of Visually Contaminated Corn with Fungus / Mold

This test is carried out visually with the sense of sight, corn that has been given a known weight treatment is initially separated then weighed and the percentage is calculated.

$$\% \text{ of corn contaminated with fungus / mold} = \frac{weight\ of\ polluted\ corn}{weight\ of\ corn\ sample}\ x\ 100\%$$

2.3.3. Determination of Percentage Qualitative Aflatoxin Contaminated Corn (UV Light)

Corn that we have observed in the previous stage and its weight has been known to be followed by visual observation under UV light, after being observed under UV light, then we calculate the percentage of corn contaminated by Aspergillus sp.

$$\% \text{ Tainted Corn} = \frac{weight\ of\ aflatoxin-contaminated\ corn}{weight\ of\ corn\ contaminated\ with\ mold}\ x\ 100$$

III. RESULT AND DISCUSSION

3.1. Percentage of Water Content of Corn

Corn from PT Japfa Comfeed has a moisture content of 15%. The average percentage of mimba leaf extract on the packaging type to moisture content during storage for 4 weeks can be seen in Table 1.

Table 1. The effect of giving mimba leaf extract on the type of packaging on moisture content in maize with a storage time of 4 weeks.

Treatment	Percentage of Water Content of Corn (%)
Plastic Bag (PB)	14,63[a]
Paperboard (P)	14,58[a]
Open Container (OC)	14,65[a]
Vacuum Plastic (VP)	11,85[b]
SE	0,15

Note: Different superscripts in the same column show a very significant difference (P <0.01)

Table 1 shows that the average water content has a very significant effect (P <0.01) at 4 weeks of storage. Based on the DMRT further test, it showed that the average types of packaging in the form of plastic bags, paperboard, and open containers were not significantly different (P> 0.05) but significantly different (P <0.05) with vacuum plastic. In all types of packaging, the water content decreases after storage, this is due to the growth and metabolic activity of microorganisms requiring water to transport nutrients or waste materials into and out of cells, all these activities require water in liquid form. Water that crystallizes and forms ice or water that is chemically bound in a solution of sugar or salt cannot be used by microorganisms.

This is in accordance with the research of Widianingrum, et al. (2010) on storage of maize for the first four weeks, the moisture content decreased from the water content before storage. The type of packaging in the form of vacuum plastic (VP) has decreased drastically due to its low oxygen permeability and no permeability to carbon dioxide gas and low water vapor transmission rate.

In accordance with the opinion of Imdad and Nawangsih, (1999) packaging is a container or media used to wrap materials or commodities, as well as to provide protection for materials or commodities. Different packaging can affect the moisture content can be seen in Table 1. It shows that the moisture content value of corn in vacuum plastic packaging has the lowest value compared to other types of packaging up to 4 weeks of storage, this is because vacuum plastic packaging does not have pores. pores compared to other packaging types.

Susanto (2008) added that the ecological factors that influence the growth of fungi are water activity (wa), moisture content, temperature, O2 substrate, CO2,

microbial interactions, mechanical damage, insect infection, number of spores, and storage time. Syarief, et al (2003) added that the growth and metabolic activity of microorganisms require water to transport nutrients or waste materials into and out of cells. All of these activities require water in liquid form. According to (Syarief and Halid, 1993) Water content is the amount of bound water and free water contained in the material expressed in percent.

3.2. Percentage of Visually Contaminated Corn with Fungus / Mold

Table 2. Effect of giving mimba leaf extract in the form of packaging on moldy seeds in maize with a storage time of 4 weeks.

Treatment	Percentage of Moldy Seeds (%)
Plastic Bag (PB)	0,0[a]
Paperboard (P)	0,0[a]
Open Container (OC)	3,7[b]
Vacuum Plastic (VP)	0,0[a]
SE	0,15

Note: Different superscripts in the same column show a very significant difference (P <0.01)

The analysis of variance in Table 2 shows that the effect of the type of packaging given mimba leaf extract at 4 weeks of storage has a significantly different effect (P <0.05) on the percentage of moldy seeds. The DMRT further test results showed that the mean moldy seeds in the treatment of packaging types in the form of PB, P, and VP were not significantly different (P> 0.05), but significantly different (P <0.05) higher than OC.

The type of packaging in the form of an open container (OC) was significantly different (P <0.05) with a higher value than the type of packaging for PB, P, and VP. The high type of OC packaging (open container) due to temperature and relative humidity are the main factors in mold growth. This is in accordance with the opinion of Titik et al. (2001) stated that *Aspergillus flavus* was detected in corn storage at 2 weeks and the fungi *Aspergillus flavus* and *Aspergillus parasiticus* were able to grow at low water content. In contrast to the types of packaging PB, P, and VP, the absence of mold/mold fungi are stored for 4 weeks, this is because there is no or little oxygen circulation in the package, at a level where there is insufficient oxygen content, as well as other microorganisms very much determined by the oxygen level in a certain condition (Moreno-Martines et al. 2000) this proves that the three packages have almost the same ability to maintain the quality of shelled corn.

3.3. Percentage Qualitative Aflatoxin Contaminated Corn (UV Light)

The aflatoxin content of maize used in this study was 200 ppb, this corn is corn that is not used in feed factories because of its high aflatoxins. By giving mimba leaf extract and stored for 4 weeks in various packages, it is expected that a decrease in aflatoxin will occur. The average aflatoxin content and reduction of maize stored in various packages, storage for 4 weeks can be seen in Table 3.

Table 3. Effect of giving mimba leaf extract on the type of packaging on aflatoxins in maize with a storage time of 4 weeks.

Treatment	Aflatoxin content (ppb)	Decreased Aflatoxin (%)
Plastic Bag (PB)	91,50[a]	54,25%
Paperboard (P)	124,75[b]	37,62%
Open Container (OC)	173,00[c]	13,50%
Vacuum Plastic (VP)	129,00[b]	35,50%
SE	10,55	

Note: Different superscripts in the same column show a very significant difference (P <0.01)

The results of the analysis of diversity are in Table 3. It shows that giving mimba leaf extract to the type of packaging at 4 weeks of storage has a very significant effect (P <0.01) on aflatoxins. The DMRT further test results showed that the average aflatoxin in the 4 week storage period for the types of packaging in the form of cardboard (P) and plastic sacks (PB) was not significantly different (p> 0.05) higher in the type of cardboard packaging (P), and different. significantly (P <0.05) higher with open containers (OC) and significantly different (p <0.05) lower with plastic bags (PB).

The high reduction in aflatoxins occurred in the type of packaging in the form of plastic sacks (PB). This is presumably because plastic bags have a better ability to protect corn from moisture and oxygen so that the administration of mimba leaf extract (Azadirachta Indica A. Juss) is able to work well. During storage, there was a decrease in aflatoxins in corn kernels in all types of packaging PB, P, VP, and OC. This was because the mimba leaf extract was thought to contain very strong

phenolic compounds, triterpenoids, and coumarin, which are antioxidant compounds that will give up one or more electrons to radicals. free radicals so they can stop the damage caused by free radicals, as free radical scavengers and prevent chain reactions (Dyatmiko et al., 2000). This is thought to be the cause of the decrease in aflatoxins. Therefore, one of the purposes of giving mimba leaf extract in this study is to interfere with the metabolic process of Aspergillus sp in producing aflatoxins, wherein this extract there are phytochemical compounds that have an antifungal function.

This is supported by research (Lillehoj, 1989). The decrease in aflatoxin content is thought to be due to the absence of fungal growth in shelled corn so that there is also no aflatoxin production. Aflatoxins are toxins produced by fungi. In accordance with the opinion of Goldbalt (1969), which states that the formation of aflatoxins will continue to increase if the fungi increase in number and in a long storage time.

Of the 12 types of aflatoxins that have been identified according to Goto (1990), aflatoxins B1, B2, G1, G2 are commonly found in food and feed ingredients and aflatoxin M1 in milk, and it is known that aflatoxin B1 is produced mostly by Aspergillus flavus mushrooms in Indonesia (Dharmaputra 2002). Among these, aflatoxins B1 and M1 are the toxins that receive major attention because of their toxicity to animals and humans (Bhatnagar et al, 2006) and because they are the most dangerous, AFB1 is often used as the maximum threshold for aflatoxin in food and feed (Goto 1990).

According to Miskiyah, et al (2009) there are currently six types of aflatoxins, namely B1, B2, G1, G2, M1, and M2. Aflatoxins M1 and M2 are hydroxylated metabolites of aflatoxin B1 and B2 and can be found in milk and milk products obtained from animals consuming aflatoxin-contaminated feed. The order of toxicity levels based on the study of the effect of aflatoxins on liver cells in vitro is B1> G1> G2> B2. Aflatoxin B1 is the dominant type of aflatoxin in maize (cobs and shelled 23-367.4 ppb) and corn products (cornstarch, popcorn, and crackers 10-40 ppb) obtained from farmers, collectors, collectors, wholesalers, and markets. self-service (Dharmaputra et al. 1995; Dharmaputra and Putri 1997).

IV. CONCLUSION

The addition of 2.5% mimba leaf extract by using a type of packaging in the form of a plastic bag gave the best effect during 4 weeks of storage and could reduce the aflatoxin content by 54.25%.

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