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Potential of Turmeric Extracts as an Antifungal for Extending Storage Periods and Maintain Corn Nutrition Quality

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Original Research Article

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Abstract: This study aims to determine the effect of turmeric extract with macerated a distilled water solvents in inhibiting the growth of Aspergillus parasiticus, and maintaining the nutrient content of corn in some storage time. This study consisted of 2 stages: stage I (the minimum concentration of extract test which still showed inhibitory ability and as an antifungal against Aspergillus parasiticus), stage II (the best potential concentration of turmeric extract from stage I as antifungal against Aspergillus parasiticus and the ability to maintaining nutrients and fungal /mold content and total aflatoxin in corn for a period of time in storage. Stage I experiments of turmeric extract compared to several levels of concentration (K1 = 25% K2 = 50%, K3 = 75%, K4 = 100%). Stage II the best concentration of turmeric extract from the first stage compared to the second factor: storage time (L1 = 0 days, L2 = 7 days, L3 = 14 day, L4 = 21 days, L5 = 28 days). Variables observed are inhibitory ability, antifungal activity, moisture, crude protein content, gross energy, % corn contaminated with fungal/mold, % contaminated aflatoxin, and totally contaminated with aflatoxin on a quantitatively. The results showed that the minimum concentration of turmeric extract has inhibitory ability is at a concentration of 25% and the best antifungal activity at the concentration of 100%. The extract turmeric gives significant effect (P <0.01) on the ability to maintain the nutritional content of corn and inhibit the growth of fungi/mold and aflatoxin both qualitatively and quantitatively for 28 days of corn storage. From the results of this study it can be concluded that the addition of turmeric extract, at a concentration of 100% can inhibit fungal/mold growth and aflatoxin in corn and also can maintain the nutritional content of corn for 28 days of storage.

Keywords: turmeric, corn, distilled water, storage time, Aspergillus flavus, aflatoxin.

INTRODUCTION

The condition of Indonesia with a tropical climate had a high temperature and humidity will accelerate the decline in the quality of feed raw materials and fungal growth during storage. Grains generally contain water, carbohydrates, proteins including enzymes, fats, minerals, and vitamins so that these feed ingredients are easily contaminated with fungi. Therefore, improper handling of feed ingredients (corn) will accelerate the growth of fungi which will further increase the level of aflatoxin in the feed.

Corn is one of the plants at risk of contamination with fungi Aspergillus sp. especially Aspergillus flavus and Aspergillus parasiticus which produce aflatoxins which are carcinogenic and harmful to humans and animals. Aspergillus sp. can pollute corn plants while still in the garden or during storage. According to [1]. Rahayu Aspergillus flavus is one type of fungal that often contaminates food; this type of fungus can cause Aspergillosis infection and is also a fungus that produces the most aflatoxin. Aflatoxin is a type of toxin that is carcinogenic and can cause poisoning with symptoms of nausea and vomiting, and if long-lasting illnesses that arise are liver cancer which results in death. If someone consumes food that is contaminated with low concentrations of aflatoxin continuously, it can damage the liver and reduce the immune system in the body.

Fungal growth Aspergillus sp. directly affected by several things during post-harvest handling of corn, including moisture, storage temperature, relative humidity, and storage duration [2]. Increased contamination of Aspergillus sp. in food such as corn usually occurs at the level of collectors. This happens because storage conditions are not suitable so that it is prone to the development of Aspergillus sp[3].

In Indonesia the maximum limit of aflatoxin levels, based on the Indonesian National Standard (SNI) for the starter layer and grower layer is 50 ppb, 60 ppb on the layer (lay egg period). In the broiler / starter broiler ration the

maximum limit was 50 ppb, and 60 ppb in broiler finisher, in the pork the maximum limit was 50 ppb, in quail feed the maximum limit was 40 ppb, in duck feed the maximum limit was 20 ppb and in dairy cattle concentrate and beef cattle maximum limit is 200 ppb. Corn contaminated with Aspergillus sp. the amount of aflatoxin content can increase rapidly due to improper post-harvest handling, so the procedure for storing corn until processing needs to be considered. Usually corn farmers store dried corn in a silo or sack stored in a storage warehouse before being distributed or processed into food after being given synthetic antifungals.

Synthetic antifungals are widely used because they are quite effective in killing fungi. Although the use of synthetic antifungals is quite effective, but in addition to expensive prices can also have a negative impact on the environment in the form of environmental pollution and human health that cause cancer when used less wisely. To avoid unwanted side effects from the use of synthetic antifungi, another alternative antifungal is needed to be developed which does not have a harmful effect on humans or the environment.

In the face of this challenge, the authors are looking for new sources of materials and / or additives, one of which is a possible source of agro-food waste. According to [4] Kordi the best method for pest and disease management is a method that does not have an impact on the environment, both short and long term. The use of natural ingredients in the management of pests and diseases, especially fungi is considered to be environmentally friendly. One alternative that can be done is the use of natural antifungi. The use of natural ingredients continues to be studied such as the use of turmeric extract.

According to [5] Mujim, natural antifungals have advantages over synthetic antifungi, because they are easy to decompose, easy to apply, easy to obtain, and safe for humans if they are used in the right dosage, and are environmentally friendly. Besides that the benefits of plants are widespread throughout the world and are like sources of secondary metabolism that are useful against pathogen attacks such as viruses, bacteria and fungi, herbivores (such as insecticides and mammals) and environmental stresses such as ultraviolet and ozone rays. Natural ingredients of turmeric extract have been widely used as traditional medicine, food and beverage flavorings and perfumes. Research to prove the potential of phytochemical compounds from plants as antifungals has also been carried out. Even so, information about the antifungal activity of turmeric extract is still limited.

Such antimicrobials are due to the presence of bioactive substances such as flavonoids, terpenes, coumarins and carotenes [6]. Bioactive components in seasonings, especially from the Zingiberaceae group, which are mostly from flavonoids which are the largest phenolic and terpenoids, known as fungistatic compounds and fungicides. Other forms of bioactive compounds are from terpenoids. This group is known as the main group in plants as a constituent of essential oils. Terpenoids have a basic formula (C5H8) n or with one isopren unit. The number n shows the classification on terpenoids known as monoterpenes, sesquiterpenes, diterpenes, triterpenes, tetraterpenes and politerpenes. The terpenoids are cyclic and some are not [7, 8] Rukmana states that the main content of turmeric is essential oils and curcuminoids and also the compounds contained in turmeric have biological activities as anti-bacterial, antioxidant and anti-hepatotoxic. The results of several studies show that secondary metabolites found in turmeric can inhibit the growth of fungal mycelium, so that turmeric can be used as a control for plant diseases caused by fungi.

Based on [9] Nurhayati's research *et al*. there were barriers from the crude extract of turmeric rhizoma to fungus *Alternaria Ellis porri* growth due to the presence of active compounds contained in the crude extract of rhizoma turmeric which has both anti-fungal and anti-microbial properties. The antifungal compounds contained in the turmeric extract are thought to originate from the components of rhizoma turmeric essential oil containing secondary metabolites which belong to the sesquiterpenes. The derivative compound from rhizoma essential oil of turmeric which belongs to the class of sesquiterpenes are: turmerone, turmerol, ar-turmeron, curlon, ar-curcumin and other essential oil derivatives are thought to have antifungal properties [10] Moghadamtousi et al states that curcuminoid derived compounds, curcumin, are a group of polyphenols in turmeric juice which has anti-fungal activity. Several studies of turmeric in vitro, showed that the active compounds of turmeric rhizome, namely flavonoids, saponins, polyphenols and alkaloids can inhibits the growth of fungi, viruses, and bacteria[11].

In several studies it was stated that phytochemical compounds have various health benefits and are used in aromatherapy, the pharmaceutical industry and for the aroma and taste of food. And also as an antifungal alternative to fungi with better effectiveness, fewer side effects, lower prices, safe and natural, based on this, the authors are interested in conducting research on "The Potential of Turmeric Extracts as Antifungi in Extending Storage and Maintaining Corn Quality".

MATERIALS AND METHODS

This research will be conducted at the Laboratory of Animal Feed Industry Technology of the Faculty of Animal Husbandry, Andalas University and the Feed Center for Quality Assay and Feed Certification Bekasi, Ministry of

Agriculture, Indonesian of Republic. This research is 2 (two) stages of research: Stage I Research (Making Material Extracts with Aquadest, Secondary metabolic identification of turmeric extract, Minimum Concentration Test of Inhibitory Power of Extracts and Antifungal Activity Tests). Second Stage Research (Selected Extract Concentration Which Has the Best Antifungal Activity of the First Stage, Test Antifungal Ability Based on Corn Storage Time, Moisture Test, Crude Protein Content, Gross Energy and Aflatoxin Anti Nutrition Test Both Qualitatively (Visual and Ultraviolet Light) and Quantitative (UPLC).

Stage 1 Research

This phase I study was to determine the effect of extracts turmeric, which were macerated with aquadest in several concentrations so that the lowest concentration of extracts which still showed inhibitory ability and ability as antifungal to fungi Aspergillus sp.

Research Material

- The ingredients used include extracts turmeric, sterile distilled water, aluminum foil, cotton, tissue paper, label paper, PDA (Potato Dextrosa Agar) and stationery.
- Equipment needed include: oven, autoclave, petri dish, test tube, stirring rod, spatula, beaker, needle inoculation, analytic scales, gauze, cutting board, knife, basin, decantation separating funnel, centrifuge motor, centrifuge tube, blender dry, mortal and pistil, dark bottle, drop pipette, test tube, analytic balance / balance, microscope.

Method

Experimental design

Phase I research was carried out using an experimental method using Completely Randomized Design (CRD) with 4 Treatment 3 replications (based on Steel and Torrie (1991).
K1: Extract Tumeric Concentration 25%
K2: Extract Tumeric Concentration 50%
K3: Extract Tumeric Concentration 75%
K4: Extract Tumeric Concentration 100%

Measured parameters

Parameters measured in stage I are:

- Secondary metabolic identification of turmeric extract
- Test of Minimum Concentration Inhibitory of turmeric extracts
- Antifungal Activity Test Extracts
- Implementation

- **Preparation of Material Extract**

In making extracts from turmeric, the method used was a mechanical maceration method as follows: First, choose fresh turmeric, cleaned with clean water, then cut into small pieces, blended and soaked in aquadest in a ratio of 1: 1 means that in 100 grams of material dissolved in 100 ml of distilled water for 24 hours so that the active substances contained in the ingredients dissolve in the solvent. Then the turmeric soaking are drained and wrapped in thick cloth. After that it is pressed using a manually threaded or squeezed hydraulic tool. The resulting filtrate is considered to be 100% concentrated and then taken and diluted according to the treatment.

- Secondary metabolic identification of turmeric extract
- Test of Minimum Concentration Inhibitory of turmeric extracts

This test aims to determine the lowest concentration of extract which still shows the inhibitory ability of fungi aspergillus sp. The concentration of extract tested was 25%, 50%, 75% and 100% with the well diffusion method. PDA media (10 ml) which is still dilute (temperature ± 45^0C) is poured into the petri which has been filled with 1 ml of fungi spore suspension (the number of colonies is 107 CFU / mL) and allowed to solidify. The media is perforated with cork temporary / pipette then filled with extract. This test was repeated three times and the diameter of the obstacle zone formed on the third day was recorded.

- **Antifungal Activity Test Extracts**

The stages of antifungal testing of turmeric extract are as follows: PDA media that have been mixed with turmeric extract according to the desired concentration into sterile petridishes. The concentrations of turmeric extract were tested, 25%, 50%, 75% and 100%.

Furthermore, 7-day old Aspergillus parasiticus isolates were taken with a diameter of 5 mm and placed in the middle of the petridish. Then the media is stored in an incubator for 7 days at room temperature. Observations were made on fungal growth by measuring the diameter of the colony on the seventh day after being inoculated [12]. Measurement of antifungal activity by using the formula Mori *et al*. [13] as follows:

$$AFA = \frac{GC - GT}{GC - A} \times 100\ \%$$

Information

AFA = Antifungal activity
GC = Growth of mycelium control (mm)
GT = Growth of mycelium in material extracting medium (mm)
A = initial mycelium size incubation (mm)
d. Data analysis
The data obtained were analyzed using Analysis of variance (ANOVA). With the mathematical model:

$$Yij = \mu + \tau_i + \varepsilon_{ij}$$

Yijk = the value of observations the treatment level i and the j repetition
μ = general middle value
τ_i = effect treatment i dan repetition j
I = treatment (1, 2, 3, …)
J = repetition (1, 2, 3,..)
ε_{ij} =the effect of etror treatment i dan repetition j

If the treatment shows significantly different results (F count> F table 0.05), it will be continued with further testing using the Duncans Multiple Range Test (DMRT) according to Stell and Torrie [14].

Place and time

This research was conducted at the Laboratory of Animal Feed Industry Technology of the Faculty of Animal Husbandry, Andalas University for 1 month.

Stage II Research

The stage II study aims to determine the potential concentration ability of the best turmeric extract from the first stage as an antifungal against Aspergillus sp and the ability to maintain the content of moisture, crude protein content, and gross energy in some storage time.

Material

The material used in this stage II is the result of selection from the concentration of extract material from the first stage which has been tested for antifungal activity and has the best ability, PDA, H2SO4, H2O2, H3BO3, NaCl, HCl, 70% Methanol, whatman paper no. 41, standard aflatoxin, indicator (red methyl sindur methyl or methyl purple), oxygen gas with 99.5% purity, sodium carbonate solution (3.76 g / l or 0.0709 N), standard benzoic acid (C6H5COOH)

Equipment

The equipment used at this stage include: UPLC (Ultra Performance Liquid Chormatography), kjedhal, oven, autoclave, petri dish, digestion stove, UV light, test tube, stirring rod, spatula, beaker, analytic scales, centrifuge motors, tubes centrifuge, mortal and pistil, dark bottle, drop pipette, test tube, petridist, label paper, microscope, corn storage container, Bomb Calorimeter Parr 6100.

Method

Experimental design

Stage II research was carried out using an experimental method using a Factorial Completely Randomized Design (CRD) 2 x 5 x 3 (based on Steel and Torrie [14].

The first factor is the concentration of the best extract from the first stage:
E1: Concentration of n% Turmeric Extract
E2: Control/Distilled Water

The second factor is

L1: 0 Days of Corn Storage Time
L2: 7 Days of Corn Storage Time
L3: 14 Days of Corn Storage Time

L4: 21 Days of Corn Storage Time
L5: 28 Days of Corn Storage Time

Measured parameters
Parameters measured in Stage II are

- Measurement of Moisture Content
- Measurement of Crude Protein Content
- Measurement of Gross Energy Content
- Measurement of % Corn of Contaminated Fungal in a Qualitative Visual manner
- Measurement of % Corn of Aflatoxin Contaminated Qualitatively Using Ultraviolet Light
- Quantitative Measurement Corn of Total Contaminated Aflatoxin Using Ultra Performance Liquid Chromatography (UPLC)

Implementation of Stage II Research
Preparation

The way it works at this stage is first we harvest the fresh corn and dry it to reduce the moisture to a maximum of 14%, the best extract concentration from the material from the first stage is put into a sprayer as much as 10 ml, the corn is put into a weighing container each of 400 grams according to the number of concentrates from the selection of stage I research, sprayed into the corn which has been piped, then the corn is allowed to dry air around 30-60 minutes is expected so that the extract that has been sprayed is absorbed by corn. In this second stage use control. For control only use distilled water.

Research Implementation

Before the corn is put in a storage container, take the treated corn according to the desired concentration for testing moisture, crude protein content, qualitative and quantitative aflatoxin analysis by purposive sampling, after which the corn is stored in a closed container for 7 days, 14 days, 21 days and 28 days at room temperature. Repeat the moisture test, crude protein content, test aflatoxin qualitatively and quantitatively at any given storage time.

Determination of Moisture [15]

The aluminum cup used is weighed appropriately and the value is recorded (c). The sample weight was also weighed with the analytic balance and recorded as the sample wet weight (a). Samples and dishes were dried in an oven with a temperature of 130 ± 3 ˚C for 1 hour. Calculation of 1 hour starts when the temperature has reached 130 ˚C. After 1 hour the aluminum cup containing the sample is then cooled in a desiccator and weighed. The weight obtained is then called the dry weight of the sample + cup (b). The data obtained is then calculated using the following equation.

$$\textbf{MOISTURE (\%)} = \frac{a-(b-c)}{a}\frac{a-(b-c)}{a} x100 \;\; x100$$

Determination of Crude Protein Contents

Digesti stove

Turn on the stove digested until it reaches a temperature of 420ºC. Weigh each example (w). For example with 3 25% protein, weigh about 1 g of the sample. With 25-50% protein, about 0.5 g for example: and> 50% protein. Approximately 0.3 g of example. Weigh 1 g sample stirring and weigh with low-weight paper Nitrogen. Fold the weigh paper containing the sample and put it in the kjeldahl tube.

Standard

Conduct a quality control analysis and standard analysis on each type of ammonium and glycine salt p. The tolerance of sulfonate is the first time as a check in distillation efficiency and titration accuracy because it is a fast digestion. Lysin and nicotinic acid p lovene sulfonate as a check in digestibility efficiency because it is difficult to digest.

Digestion

Add 2 catalyst tables to each tube. Add 12 mL H2SO4 to each tube using a dispenser pipette; for example with high fat (> 10%), add 15 mL, mix. If mixing takes out the foam, slowly add 3 mL of H2O2 30-35%. The reaction is carried out in an acid chamber or exhaust system. Store the tube rack in a heater, place the tube cover and turn on the drain. After 10 minutes, reduce the drainage. After the acid smoke enters the exhaust. The condensation area should be in the tube. After sulfuroxide smoke is produced at the beginning of digestion, reduce the vacuum source to prevent H2SO4 loss. Digesti added 50 minutes. The total digestion is approximately 60 minutes.

Turn off the digestion stove. Store the tube rack in the exhaust chamber, let it cool until 10-20 minutes. Cooling can be done using an air blower or by being placed in a water channel. When it's not smoky, move the tube and turn off the blower. Place a cold tube.

Use gloves and eye protection. Before a ing distilled water. Carefully add a few mL of distilled water to each tube. If there is a spark, Baung is still hot. Chill a few more minutes. Add water to each tube to a total volume of approximately 80 mL.

Distillation

Place 40% NaOH in the alkaline tank in the distillation unit. Add a volume of 50 mL. Place the digestion tube in unit distillation or use it automatically if possible. Place 500 mL of erlenmeyer titration containing 30mL of H3BO3 solution with the receiving indicator, and dip the condenser into the source of the H3BO3 solution. (when using an automatic titration system, titration immediately after distillation starts 1% H3BO3 can be replaced). Distilled steam becomes> 150 mL of distillation collected (> 180 mL total volume) remove the receiving solution. Titration of H3BO3 receiving solution with a standard of 0.1000 M HCl to purple. Record mL HCl. Until it's approaching 0.05 mL.

If done using steam distillation with automatic titration. Follow the instructions to operate distillation / titration.

$$\textbf{\% KJELDHAL NIT} = \frac{V_S - V_B \times N \times 14{,}01}{W \times 10}$$

$$\textbf{\% PK = \% KJELDHAL N X F}$$

Where:
Nit : Nitrogen
VS : Volume titration example
VB : Volume of blank titration
N : Normality standard HCl
W : Weight of sample / standard
10 : Conversion factor mg / g to percent (%)
14.01 : Atomic weight of element N
F : Correction factor N Protein
5.70 for soybeans
6.25 for fishery, animal husbandry and forage products

Gross Energy Determination

Preheat the tool for + 20 minutes, Weigh 0.5 - 1 g of sample, wire (7 - 10 cm long) is attached to the end of the bomb tube until the wire touches the sample, insert 1 ml of distilled water into the bomb, cover the bomb tightly, then fill with oxygen with a pressure of 20-30 atmospheres, put the bomb that has been closed into a calorimeter vessel, fill the vessel with distilled water as much as 2000 g + 0.5 g with a volumetric flask, press the button on the Bomb Calorimeter, root the sample in the bomb with sw on the transformer, rinse the remaining combustion with distilled water into Erlenmeyer, titration with sodium carbonate solution (Na2CO3) with an indicator of methyl red or methyl purple. The results of the recording are recorded, turn off the switch on, remove the bomb from the calorimeter vessel, remove the remaining oxygen and open the lid, measure the remaining wire to burn.

$$\text{EE} = \frac{(HxB\ ) + e1 + e2 + e3}{T}$$

Where:
EE = equivalent energy (cal / 0C)
H = heat burning of benzoate acid (C6H5COOH) which is usually mentioned in the certificate.
B = weight of benzoate acid (g)
e1 = correction of combustion heat of HNO3, heat of formation of nitric acid (HNO3) which can be measured from the number of ml of Na2CO3 solution used.
e2 = sulfur correction (usually worth 0)
e3 = correction of the burning heat of the burning wire

The combustion heat of the combustion wire is 1.484 kcal / g for the type of iron Fe, usually mentioned in the certificate of the wire concerned.

T = temperature increase (T1 - T0)
T0 = the initial water temperature
T1 = water temperature after burning

Gross Energy Determination Example

Weigh 0.5 - 1 g of charcoal sample into the burning cup, attach the cup to the end of the combustion valve in the available place, attach the combustion wire 7 or 10 cm long and pinch it with the sample in the cup. Then it is done according to the method of determining the calorimeter value in the procedure.

Calculation

$$GE = \frac{(EE\ \Delta t) - k - T}{B}$$

Information

GE = Gross Calorific Value (kcal / g)
EE = Equivalent Energy
B = Sample Weight (g)
Δt = Initial and Late Temperature Difference
K = Length of Burned Wire (cm)
T = Volume Titration (mL)

Determination % Contaminated Corn of Fungal/Mold

This test is carried out visually with the sense of sight, the treated corn which has been known to be initially separated and then weighed and calculated.

$$\%\ \text{Contaminated Corn of Fungal/Mold} = \frac{\text{the weight of contaminated corn}}{\text{weight of sample corn}} \times 100\ \%$$

Determination % Contaminated Corn of Aflatoxin Qualitatively (UV Light)

The corn that we have observed in the previous stage and it has been known that the weight is followed by visual observation under UV light (360 nm) , after observing it under UV light, then we calculate the percentage of corn contaminated by Aspergillus sp, corn which means the luminescence is contaminated with aflatoxin.

$$\%\ \text{Contaminated Corn} = \frac{\text{Weight of Corn contaminated with Aflatoxin}}{\text{Weight of corn contaminated with mold}} \times 100\ \%$$

Quantitative Determination of Aflatoxin Contaminated Corn Using Ultra Performa Liquid Chromatography (UPLC)

Each sample was weighed 25 g then added 5 g NaCl and 125 mL 70% methanol then smoothed with a blender for 2 minutes at high speed. The liquid results are then filtered using Whatman 41 filter paper and the filtrate results are taken as much as 15 mL. The filtrate is then added with 30 mL of distilled water and filtered using a glass microfibre filter. 15 mL filtration filtrate was taken then added to the AflaTest column containing monoclonal antibodies that are specific to aflatoxins B1, B2, G1 and G2 for purification. Then the column was washed using 20 mL of distilled water and aflatoxin eluted from the column with 1 mL of methanol. The eluent filtrate with 1 mL of methanol was then collected in a vial and then 1 mL of distilled water was added. The filtrate is then exported and then injected into the UPLC. The UPLC conditions used are as follows,

- Column: Column Poroshell 120 SB C18, 4.6x150 mm, 2.7um
- Post Column: Photochemical Reactor Derivatization (PHRED) Brand AUR
- Detector: Kratos 950 Fluorescence Detector
- Wavelength: Excitation = 365 nm. Emission = 465 nm
- Eluen: H2O: ACN: MeOH = 60:20:20
- Flow rate: 1.0 mL / min
- Elution system: Isocratic
- Injection volume: 20 uL
- Column temperature: 40 0C
- Post column temperature: 40 0C
- Running time: 15 minutes
- Pressure: 400-450 bars

The analysis phase of aflatoxin concentration begins with the manufacture of standard solutions of aflatoxin B1, B2, G1 and G2 which are made with concentrations of 10, 20, 30, 40, 50 and 60 ng / mL respectively. The standard solution is injected into the HPLC to produce peak areas. Furthermore, the standard curve is made by plotting the peak area to the concentrations of aflatoxins B1, B2, G1 and G2. Aflatoxin concentration is calculated using the formula:

$$\text{AFLATOXIN CONCENTRATION: } \frac{\frac{area - a}{b} x VSolvent \times VFinal}{WSample \times VAliquot}$$

Information
Area: Measured area of aflatoxin (ng / mL)
a: Intersep (LU.s)
b: Slope (LU.s) / (ng / mL)
Final V: Final volume of sample solution (mL)
V aliquot: Volume of pipette solution for IAC (mL)
V solvent: Volume of methanol: water for extraction (mL)
W sample: sample weight (g)
3. Data Analyzed

The data obtained were analyzed using Analysis of variance (ANOVA). With the mathematical model:

$$Yijk = \mu + £i + ßj + (£ß)ij + \sum ijk$$

Yijk = the value of observations in one experiment that received the treatment level i of factor A, the j level of factor B and the k is repetition
μ = general middle value
£ I = influence of the level of i from factor A
ßj = effect of the j level of factor B
(£ ß) ij = the effect of the interaction of the level of the factor A and the level of the factor B
∑ijk = the effect of error on the unit that gets the third treatment from factor A, the j level of factor B and the k repetition

If the treatment shows significantly different results (F count> F table 0.05), it will be continued with further testing using the Duncans Multiple Range Test (DMRT) according to Stell and Torrie [14].

Place and Time
The second phase of the research was conducted at the Feed Quality Assay and Certification Center Bekasi, Ministry of Agriculture, Indonesian of Republic for 2 Months.

RESULTS AND DISCUSSION
Stage I Research

Making Extracts
The results of the analysis of secondary metabolic identification extracts of the ingredients were soaked with distilled water for 24 hours showed in table 1

Table-1: Secondary Metabolic Identification Turmuric Extract*

Secondary Metabolic Types	Level
Flavonoids	-
Phenolic	-
Saponin	-
Triterpenoid	++
Steroids	-
Alkaloids	-
Cumarin	-

*) Test Results of Chemical Laboratory the Mathematics and Natural Sciences Faculty of Andalas University.
Information: - : No ++ : Strong
+ : Weak +++ : Very Strong

Phenolic compounds interact with cell membrane proteins which cause precipitation and denaturation of cell membrane proteins [16]. Damage to the cell membrane causes changes in permeability in the membrane, resulting in lysis of the fungal cell membrane [17]. Chismirina *et al*. [18] added that phenol works by denaturing bacterial cell proteins so that cell activity is disrupted and causes cell death. So this compound is widely used as an oral antiseptic. In

accordance with the study of Lisangan *et al*. [19] that the extract of HEM of kebar grass leaves containing phenolic compounds is one of the causes of inhibition of AFB1 production. Kumar *et al*. [20] suggested that the presence of phenolic components in essential oils Ocimum sanctum can reduce mold growth and AFB1 production. Inhibition of α-atoxin production by phenolic components was also stated by Kim *et al*. [21] which state that mitochondria play a role in the supply of acetyl-CoA which is a major precursor in biosynthesis of a atoxin. Damage to the mitochondrial respiration chain caused by phenolic components is part of the inhibition of the production of atoxin. This indicates that the HEM extract of kebar grass leaves is not only able to inhibit the growth of both A. flavus isolates but also is able to inhibit the production of AFB1.

Terpenoids, including triterpenoids and steroids are bioactive compounds that function as antifungals. These compounds can inhibit fungal growth, either through the cytoplasmic membrane or interfere with the growth and development of fungal spores [22]. Razzaghi-Abyaneh *et al*. [23] stated that bioactive metabolism of plants can be divided into several major parts, namely terpenes (terpenoids, isoterpenoids), phenylpropanoids (flavonoids, tannins, glycosides, and lignins), phenolic and nitrogen components (alkaloids and heterocyclic aromatics). Harboune [24] added, terpenoids are fat soluble, one of the terpenoids which has the potential as an antimicrobial is triterpenoid. While steroids are fat groups and are part of the triterpenoid. Yuharmen *et al*. [25] suggested that the antimicrobial effects of terpenoids are their ability to damage bacterial cell membranes, while essential oils can inhibit growth or kill bacteria by disrupting the formation of membranes or cell walls; the membrane or cell wall is not formed or formed imperfectly, so that the cell's osmotic pressure is disrupted and the microbes die.

Whereas secondary metabolites produced by Zingiberaceae plants generally can inhibit the growth of pathogens that harm human life, including Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Neurospora sp, Rhizopus sp. and Penicillium sp. [26]. In several studies it was stated that essential oils have various health benefits and are used in aromatherapy, the pharmaceutical industry and for the aroma and taste of food. In fact, it is also reported that essential oils from Vietnam's sweet orange peel have high antioxidant and antimicrobial activity [27, 28] Choi [29] Hung [30]. Furthermore, essential oils from citrus fruit peel of C. aurantium from Iran also have antidermatophytic activity, cytotoxicity, anxiolytic, sedative and gastro-protective effects [31, 32] Sanguinetti *et al*. [33] Carvalho-Freitas and Costa [34] Pultrini *et al*. [35] Moraes.

Test the Minimum Concentration of Inhibitory Extracts

The average inhibitory power formed in each treatment can be seen in Table 2, and the variance in Appendix 1 shows that the treatment is not significantly different from inhibitory power in some level concentration of extract.

Table-2 Minimum Concentration Test of Turmuric Extract

Extract	Concentration (K)				Average
	K1 (25%)	K2 (50%)	K3 (75%)	K4 (100%)	
Turmeric	0,72	0,75	0,83	0,85	0,79

In Table 2 show that the diameter smallest inhibitory formed at a concentration of 25% of 0.72 mm and the largest at a concentration of 100% of 0.85 mm. And an extracts showed inhibitory zones at a concentration of 25% against Aspergillus parasiticus, but the amount of inhibitory power (the light zone formed) was different. This means that the minimum inhibitory concentration (MIC = Minimum Inhibition Concentration) of an turmeric extracts is 25% of Aspergillus parasiticus. The effect of extract concentration on the treatment is showed that the higher the extract concentration make of clear zone diameter formed larger. According to [37]. Martoredjo that the concentration of a substance that functions as an antimicrobial is one of the determinants of the size of the ability to inhibit the growth of microbes tested. [38]Sari states that at different concentrations of red ginger rhizome decocta shows different inhibitory power. The higher the concentration, the greater the diameter of the inhibitory zone formed. Fresh extracts of ginger rhizome can inhibit the growth of microbial assays with the average variation of the diameter of microbial-free regions formed this is due to the fresh extract of ginger-ginger rhizomes containing anti-microbial compounds [39]. Mulyani added that the fresh extract of ginger root ginger contains several components of essential oils which are composed of a-pinene, kamfena, caryophyllene, ß-pinene, a-farnesena, cineol, dl-kamfor, isocariophylene, caryophylleneoxide, and germacron which can produce antimicrobials to inhibit microbial growth.

Based on the results obtained, antifungal activity needs to be done by entering the appropriate components. Phytochemical compounds that is able to have the ability as antifungals such as alkaloids, flavonoids, saponins, tannins, triterpenoids and steroids [40]. And according to [41] Selvyana *et al*. the formation of inhibition zones around the rhizome sample wells contain fungicides against Aspergillus fungi flavus. Damage caused by antimicrobial components can be fungicidal (kill fungi) and fungistatic (temporary growth of fungi). A component will become fungicidal or fungistatic depending on the properties of the active compound, concentration, and eye media. Damage caused by

antimicrobial components can be fungicidal (kill fungi) and fungistatic (temporary growth of fungi). A component will become fungicidal or fungistatic depending on the properties of the active compound, concentration, and eye media.

Antifungal Activity Test Extracts

The average antifungal activity of extracts on the growth of Aspergillus parasiticus in each treatment can be seen in Table 3.

Table-3: Antifungal Activity Tests on the Growth of *Aspergillus parasiticus* (%)

Type of Extract (E)	Concentration (K)				Average
	K1 (25%)	K2 (50%)	K3 (75%)	K4 (100%)	
Turmeric	70,08^{B}	75,41^{B}	86,48^{A}	95,08^{A}	81,76

Description: Superscripts with different uppercase letters in different lines a significantly different effect (P <0.01)

From Table 3 shows that the higher the antifungal activity of the growth of fungal colonies is due to the antifungal activity of the extract at the higher the extract concentration. In accordance with Sitepu's [42] that each addition of extract concentration showed an increase in inhibitory ability, because the greater the concentration of extract contained in the medium, the increases number of extracts that diffuse into the fungal cell, causing disruption of fungal growth and even death.

The antifungal activity of the plant extract was thought to be related to the content of phytochemical compounds in the extract. Phytochemical compounds that are thought to have the ability as antifungals such as alkaloids, flavonoids, saponins, tannins, triterpenoids and steroids [40]. Terpenoids, including triterpenoids and steroids are bioactive compounds that function as antifungals. These compounds can inhibit fungal growth, either through the cytoplasmic membrane or interfere with the growth and development of fungal spores Yuharmen *et al*. [22, 25] stated that the antimicrobial effect of terpenoid compounds is their ability to damage bacterial cell membranes, while essential oils can inhibit growth or kill bacteria by disrupting the formation of membranes or cell walls; the membrane or cell wall is not formed, so that the cell's osmotic pressure is disrupted and the microbes die [26]. Added that ginger-ginger rhizomes contain antimicrobial compounds of phenols, flavonoids, terpenoids and essential oils found in ginger extract are a class of bioactive compounds that can inhibit microbial growth. The inhibition of microbial growth by the fresh extract of ginger rhizome is due to the presence of bioactive compounds contained in the extract [39]. Mulyani stated that fresh extracts of ginger root ginger contain several components of essential oils which are composed of a-pinene, kamfena, cariophyllene, ß-pinene, a-farnesena, cineol, dl-kamfor, isocariophylene, cariophylleneoxide, and germacron which can produce antimicrobials to inhibit microbial growth.

According to [41] Selvyana each addition of extract concentration showed an increase in inhibitory power. this is due to the greater concentration of extracts contained in the medium, the number of extracts that diffuse into the fungal cells increases, causing disruption of fungal growth and even death. The mechanism of inhibition of microorganisms by antimicrobial compounds can be caused by several factors, including: (1) interference with the constituent cells of the cell, (2) increased permeability of cell membranes which can cause loss of cell fluid, (3) inactivate enzymes, and (4) destruction or the function of genetic material [43].

The occurrence of microbial inhibition on the growth of bacterial colonies is also caused by damage to the structural components of the bacterial cell membrane. Cell membranes composed of proteins and lipids are very susceptible to chemicals that can reduce surface tension. Damage to cell membranes causes disruption of nutrient transport (compounds and ions) so that bacterial cells experience a lack of nutrients needed for their growth [44, 45]Velázquez-Nuñez states that higher concentrations of essential oils are needed to delay fungal growth; and reported synergistic inhibitory effects of the surrounding gas composition of volatile volatile oil compounds. [46]Carson, *et al*. and [47] Tyagi and Malik state that essential oils cause different changes in the nature and function of microbial cell membranes by increasing membrane fluidity and changing membrane permeability; while low concentrations change their permeability, high concentrations cause severe damage, loss of homeostasis, and death [48]. Nychas reports that several components of essential oils can change the size of enzymes responsible for germination spores, energy production and synthesis of structural compounds or interfere with the amino acids involved in germination.

Stage II Research

Based on the results of the first stage (I), the best concentration used to go to the next stage of research or the second stage (II) is the treatment of extract turmuric at a concentration level of 100%.

Measurement of Moisture

The average Moisture of corn in each treatment and storage time can be seen in Table 4.

Table-4: Effect of Extracts for Moisture Content Corn at Various Storage Time (%)

Type Of Extract	Storage Time (Day)					Average
	0 (L1)	7 (L2)	14 (L3)	21 (L4)	28 (L5)	
Turmuric (E1)	$13{,}53^{Ba}$	$12{,}82^{Bb}$	$12{,}21^{Bc}$	$11{,}85^{Bc}$	$11{,}74^{Bd}$	12,43
Control/ Distilled Water (E2)	$14{,}70^{Aa}$	$13{,}69^{Ab}$	$13{,}32^{Ac}$	$13{,}25^{Ac}$	$12{,}85^{Ad}$	13,56
Average	14,12	13,25	12,77	12,55	12,30	

Description: Superscripts with different uppercase letters in different coloumn and lowercase letters in the same lines show a significantly different effect (P <0.01)

In Table 4 it can also be seen that the longer the storage of corn, the moisture in corn will be reduced, this is in accordance with the study of [49] Widianingrum *et al.* of corn storage the first four weeks of moisture has decreased from the initial moisture content [50]. Susanto states that ecological factors that influence the growth of fungi are water activity (aw), moisture content, temperature, O2 substrate, CO2, microbial interactions, mechanical damage, insect infection, number of spores, and storage time [51]. Syarief *et al.* added that the growth and metabolic activities of microorganisms need water to transport nutrients or waste materials into and out of cells. All of these activities require water in liquid form.

Susanto [50] also argued that moisture is positively correlated with water activity (aw) in seeds and in milling on all three levels of management because water activity is free water that can be used by microorganisms, the greater the moisture in the material will also have the tendency of water availability free which can be used for metabolism of microorganisms, then both variables have a positive correlation.

Measurement of Crude Protein Content

The average content of crude protein content of corn in each treatment and storage time can be seen in Table 5.

Table-5: Effect of Extracts on Protein Corn Levels at Various Storage Periods (%)

Type Of Extract	Storage Time (Day)					Average
	0 (L1)	7 (L2)	14 (L3)	21 (L4)	28 (L5)	
Turmuric (E1)	8,34	8,69	8,71	8,72	8,75	8,64
Control/ Distilled Water (E2)	8,41	8,52	8,60	8,66	8,83	8,60
Average	$8{,}38^{B}$	$8{,}61^{A}$	$8{,}65^{A}$	$8{,}69^{A}$	$8{,}79^{A}$	

Description: Superscripts with different uppercase letters in the same line show a significantly different effect (P <0.01)

The results of variance in show that between the treatment of factor A (type of extract), and the interaction of factor A (type of extract) with factor B (storage time) there is no very significant difference, while factor B (length of storage) there is a very significant difference (P <0.01) to the level of corn water.

From Table 5 we can see that the longer the storage of corn, the crude protein content in corn will increase even though it is not significant. This is consistent with the research of Widianingrum *et al.* [49] that the protein content in corn kernels stored with CO2 fluctuated in the 2nd week to the 8th week but the value was relatively stable [52] Syaifurrisal added that the addition of water volume in commercial feed can result in a decrease in crude protein content in unsaved samples, while the stored samples show increased crude protein values. This shows that the presence of fungi in feed can increase the protein content in feed. This is in accordance with FAO in Susi [53], fungi have a high protein content reaching 13.8%. So that the protein content of fungi can increase the protein content of feed.

In addition, according to Purwadaria in Haryati [54], Aspergillus mushroom can produce hydrolytic enzymes of mananase and cellulase which can reduce crude fiber and increase crude protein. Lim *et al.* added. in Simon [55] stated that fermentation of feed ingredients using Aspergillus flavus can increase crude protein content. Lina, *et al.* [56] said R. oryzae was able to increase protein levels and reduce HCN levels from mocaf flour. And Sugiyono in Ria [57], A. niger fermentation in sago pulp can increase protein content by 1.9% within 12 days. A. niger is a single culture often used in feed processing because of its ability in degradation of cellulose and starch and increasing protein levels.

According to Takahashi and Kiyosha [58], in millet commodities, by using chemical methods it can be seen that a large number of chemical changes occur in barley protein during storage. Protein breaking occurs due to the hydrolysis of the proteolytic enzyme into a polypeptide, which then produces amino acids. Fennema [59] caused this process to run slowly during the fruit or grain ripening process, at the beginning of storage, the protein content of corn seeds was 5.90% and increased in the second week to 6.08%; 6.76%; 7% and 6.28% in storage with open control treatment, 0% CO2, 40% CO2, and 70% CO 2 and although there were some fluctuations, the levels were relatively stable up to 8 weeks of storage. According to Winarno [60], during storage of starch, total nitrogen was largely unchanged, but nitrogen from

protein decreased slightly. Storage treatment of corn seeds with CO2 treatment can maintain the protein content so that it remains stable and even tends to increase in storage until the 8th week.

Measurement of Gross Energy Content

The average gross energy content of corn in each treatment and storage time can be seen in Table 6.

Table-6: Effect of Extracts on Gross Energy Levels at Various Storage Periods (%)

Type Of Extract	Storage Time (Day)					Average
	0 (L1)	7 (L2)	14 (L3)	21 (L4)	28 (L5)	
Turmuric (E1)	3797,52	3885,22	3899,03	3915,92	3927,73	3885,08
Control/ Distilled Water (E2)	3786,29	3839,34	3852,45	3859,82	3872,00	3841,98
Average	3791,91^{A}	3862,28^{A}	3875,74^{A}	3887,87^{A}	3899,86^{A}	

Description: Superscripts with different uppercase letters in the same line show a significantly different effect (P <0.01)

The results of variance in show that between the treatment of factor A (type of extract), and the interaction of factor A (type of extract) with factor B (storage time) there is no very significant difference, while factor B (length of storage) there is a very significant difference (P <0.01) to the level of corn gross energy. From Table 6 it can be seen that the longer the storage of corn the gross energy content will increase even though it is not significantly different, this is in accordance with Susanto [50] opinion that organic matter will increase if the moisture decreases or organic material will increase if the good value is in the form of milled and seeds go down, and vice versa. Decrease in moisture at a safe limit, in addition to protecting against the attack of aflatoxin-producing fungi also increases the concentration of nutrients in corn. And added by Nafiah [61] the addition of additives can protect the material so as not to degrade too much. As in his research the addition of propionic acid can prevent the proliferation of mold by damaging membrane cells, disrupting the activity of enzymes in cells and inhibiting microbial transport nutrient because the structure of 3 carbon atoms cannot be broken down by microbes that can cause material damage so that quality can be maintained.

Measurement % Contaminant Corn of Fungal/Mold on a Qualitative Visual

Table-7: The average% of corn contaminated with mold / mold can be seen in

Type Of Extract	Storage Time (Day)					Average
	0 (L1)	7 (L2)	14 (L3)	21 (L4)	28 (L5)	
Turmuric (E1)	0,00^{Ea}	3,33^{Db}	3,98^{Cb}	15,69^{Bb}	19,52^{Ab}	10,63
Control/ Distilled Water (E2)	0,00^{Da}	8,18^{Ca}	8,35^{Ca}	24,30^{Ba}	69,95^{Aa}	27,69
Average	0,00	5,75	6,16	19,99	44,74	

Description: Superscripts with different uppercase letters in same lines and lowercase letters in the different coloumns show a significantly different effect (P <0.01).

The results of variance in show that between the treatment of factor A (type of extract), factor B (length of storage) and the interaction of factor A (type of extract) with factor B (storage time) there is a very significant difference (P <0.01) to the level of % Contaminant Corn of Fungal/Mold. In Table 7 it can be seen that the longer the corn storage increases the percentage of corn contaminated with fungal/mold, but the corn treated with the percentage of corn contaminated with fungal/mold is lower than the control treatment which is not given extract. This is consistent with the opinion of Cushnie and Lamb [62] which states that the addition of anti-fungal substances can improve the quality of active substances. Nafiah [61] added that preservation is an effort to inhibit or prevent damage, maintain quality, avoid damage, facilitate handling and storage and workability of preservatives generally by: disrupting nutrient liquids in microbial cells or by damaging membrane cells, disrupting the activity of enzymes in microbial cells, disrupting the genetic system of microbes. The mechanism of action of preservatives consisting of organic acids, based on the permeability of microbial cell membranes to undissosiated acid molecules. The addition of additives in the treatment can protect the material from mold and yeast attack which utilizes the nutrient content of the material so as to reduce the content of dry matter and organic matter, thus storing up to the 6th week does not damage organic matter.

Nafiah [61] stated that corn storage for 6 weeks experienced a marked increase in the total change in corn kernels that were damaged compared to the previous week. Where factors that influence the cracking of seeds are: changes in moisture due to weather changes, improper shelling and warehouse pest attacks. Damage to feed ingredients due to changes in moisture is the most common case, thus facilitating the growth of microorganisms, especially mold. Microorganisms take and eat food from seeds or other raw materials that cause damage to the protective material. In addition to causing physical damage due to its migratory nature, microoranism can remove fungal spores which destroy feed ingredients and pave the way for other microoranism contamination such as mold that produces mycotoxins which can increase damage to feed ingredients such as holes, break and break. Imdad and Nahwangsih [63] add that the

temperature and humidity fructuations in the storage environment naturally will cause the movement (transfer) of water vapor from the material so that it will encourage qualitative damage (physically) to the stored material. The thing that is not profitable in storage is the loss of nutrients or certain substances needed by both animals and humans during the storage process.

Qualitative Measurement of% of Corn Aflatoxin Contamined Using Ultraviolet Light

Table-8: The average% of corn contaminated with aflatoxin which is seen using ultraviolet light can be seen in

Type Of Extract	Storage Time (Day)					Average
	0 (L1)	7 (L2)	14 (L3)	21 (L4)	28 (L5)	
Turmuric (E1)	$0,00^{Ca}$	$0,00^{Cb}$	$0,00^{Cb}$	$8,86^{Bb}$	$19,09^{Ab}$	5,59
Control/ Distilled Water (E2)	$0,00^{Da}$	$2,32^{Ca}$	$4,87^{Ca}$	$36,55^{Ba}$	$46,25^{Aa}$	18,00
Average	0,00	1,16	2,44	22,70	32,67	

Description: Superscripts with different uppercase letters in same lines and lowercase letters in the different coloumns show a significantly different effect ($P < 0.01$)

The results of variance in show that between the treatment of factor A (type of extract), factor B (length of storage) and the interaction of factor A (type of extract) with factor B (storage time) there is a very significant difference ($P < 0.01$) to the level of Measurement of% of Corn Aflatoxin Contamined Using Ultraviolet Light. From Table 8 corn on trains began to be contaminated with aflatoxin at 7 days storage by 2.32% and the most fungi contaminated with aflatoxin also in trains at 28 days storage period was 46.25%. This is in accordance with the opinion of Titik *et al*. [64] stated that Aspergillus flavus was detected at corn storage for 2 weeks and fungi Aspergillus flavus and aspergillus parasiticus were able to grow at low moisture [65]. Garcia and Park (1999) stated that damaged corn seeds will provide and facilitate the route of infection and growth of Aspergillus flavus and aflatoxin production compared to other fungi so that it can damage feed ingredients. Where the optimum temperature and the time to produce aflatoxin by Aspergillus flavus is 25 ° C within 7–9 days, the temperature is 30 ° C within 5-7 days and at 20 ° C it takes 11–13 days. Aspergillus parasiticus produces aflatoxin, most of the total aflatoxin is produced at 25 ° C during the incubation period of 7-15 days [63], Syarief and Halid [51] stated that mold growth occurs at temperatures of 26-35 ° C and relative humidity 70-90%.

Toxicity properties of Aspergillus flavus in nature can be broadly classified into aflatoxigenic (also known as toxic strains) and non-toxic. Toxic strains are strains capable of producing aflatoxin secondary metabolites, while non-toxic strains are strains that do not produce toxins [66] Rahmianna *et al*. [66] stated that the toxin compounds were named according to the fluorescent color characteristics at the time of detection using ultraviolet 1 wave (λ = 365 nm) after the separation of compounds using thin layer chromatography. AfB1 and AfB2 produce blue fluorescent colors, while AfG1 and AfG2 produce green fluorescent colors [67]. Of the 12 types of aflatoxins that have been identified [68] aflatoxins B1, B2, G1, G2 are commonly found in food and feed as well as aflatoxin M1 in milk, and it is known that aflatoxin B1 is produced by Aspergillus flavus in Indonesia [69]. Among them, aflatoxin B1 and M1 are the toxins that receive major attention because of their toxicity to animals and humans [70], and because they are most dangerous, AfB1 is often used as the maximum threshold for aflatoxin in food and feed [68].

According to Miskiyah *et al*. [72] there are currently six types of aflatoxins, namely B1, B2, G1, G2, M1, and M2. Aflatoxin M1 and M2 are metabolized aflatoxin B1 and B2 which are hydroxylated and can be found in milk and dairy products obtained from animals that consume feed contaminated with aflatoxin. The order of toxicity level based on the study of the effect of aflatoxin on liver cells in vitro is B1> G1> G2> B2. Aflatoxin B1 is the dominant type of aflatoxin in corn (tuna 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 [69] Dharmaputra Dharmaputra and Putri [72]. According to [66] Rahmianna *et al*. toxic strains are able to produce blue fluorescent velocity colors on observations under UV light (λ = 365 nm) which are not found in non-toxic strains. That's why corn contaminated by fungi/mold is not necessarily contaminated with aflatoxin.

Quantitative Measurement Corn of Total Aflatoxin Contaminated Using Ultra Performance Liquid Chromatography (UPLC)

Table-9: The average number of maize contaminated with aflatoxin which was seen using UPLC can be seen in

Type Of Extract	Storage Time (Day)					Average
	0 (L1)	7 (L2)	14 (L3)	21 (L4)	28 (L5)	
Turmuric (E1)	0,00	0,00	0,11	0,16	0,33	$0,12^{A}$
Control/ Distilled Water (E2)	0,02	0,16	0,40	0,46	1,20	$0,45^{A}$
Average	$0,01^{b}$	$0,08^{b}$	$0,26^{ab}$	$0,31^{ab}$	$0,77^{a}$	

The results of variance in show that between the treatment of factor A (type of extract), factor B (length of storage) there is a very significant difference (P <0.01) but the interaction of factor A (type of extract) with factor B (storage time) no very significant difference to the level of Corn Total Aflatoxin Contaminated Using Ultra Performance Liquid Chromatography (UPLC). In Table 9 shows that the longer the storage of corn, the total contaminated aflatoxin is highert. Although in extract treatments there was an increase in total aflatoxin in corn during the storage period, but based on the Indonesian National Standard (SNI) it was still far below the maximum limit of aflatoxin levels where on concentrate chicken feeds were 50 ppb for starter / child layer and grower layer / adults and 60 ppb at the laying / laying period, the concentrate ration of broiler / starter broiler is 50 ppb and 60 ppb in the broiler finisher, the pork ration is 50 ppb, the quail feed is 40 ppb, the duck is 20 ppb and dairy cattle concentrate concentrate and 200 ppb beef cattle.

In accordance with [50] Susanto's research, a long storage time will increase the aflatoxin content, because with increasing time it will provide opportunities for aflatoxin-producing fungi to produce aflatoxin secondary metabolites [73]. Rachmawati *et al*. suggested that observations of aflatoxin content in feed showed an increase in aflatoxin levels during the 10-day incubation period Goldbaltt [74] added that the formation of aflatoxin will continue to increase if the fungus increases and the storage time is long, as long as the optimum growth limit is 7-15 days. Diener And Davis [75] stated that the optimum temperature and time to produce aflatoxin by Aspergillus flavus was 25 °C within 7 - 9 days, a temperature of 30 °C within 5-7 days and at a temperature of 20 °C it took 11-13 days. Aspergillus parasiticus produces aflatoxin B1 at a temperature of 30 °C to 35 °C, and produces G1 at a temperature of 25 °C to 30 °C. most of the total aflatoxin is produced at a temperature of 25 °C to 30 °C during incubation 7-15 days.

Titik *et al*. stated that Aspergillus sp. Aspergillus and Pinicillium, the fungi were able to grow at low water levels. Dharmaputra *et al*. [69] stated that total aflatoxin will increase with increasing storage time because aflatoxin is not easily decomposed, so it will accumulate with time. The amount of aflatoxin produced by mold is influenced by certain molds, overgrown substrate, moisture, CO2 and oxygen content and the interaction of other microbes that grow on the material. Like what Tian *et al*. [76] there is a direct correlation between mold growth and AFB1 production. Mycelium growth correlates with the synthesis of enzymes that play a role in the production of AF, so that the growth of dense mycelium in Aspergillus flus also causes high fl atoxin production. It is stated that the environmental conditions needed for the formation of conidium, sclerosium, and secondary metabolism are the same [77].

Nonetheless, inhibition of AFB1 production is not always caused by reduced mold enzymes which ultimately reduce the ability of A. avus to produce AFB1 [78]. Pitt [79] suggested that inhibition of atoxin production might be caused by enzymes released during lysis of the mold mycelium. Added by Namazi *et al*. [80] damage to mycelium and conidium fungi is one of the characteristics of the process of deactivation of atoxin. Inhibition of α-atoxin production by phenolic components was also stated by Kim *et al*. [81] which states that mitochondria play a role in the supply of acetyl which is the main precursor in biosynthesis of a atoxin. Damage to the mitochondrial respiration chain caused by phenolic components is part of the inhibition of the production of atoxin.

CONCLUSION

Based on the results of the study several conclusions can be drawn, the first : The minimum concentration of turmeric extract, which still has the ability to form clear zones is at a concentration of 25%., second turmeric extract, have the best ability of activating Aspergillus parasiticus antifungal at 100% concentration. Third Addition of turmeric extract, can maintain the nutritional content of corn (moisture content, crude protein content, gross energy content) and inhibit the percentage (%) of corn contaminated with fungi/mold and aflatoxin qualitatively and totally aflatoxin quantitatively for 28 days of storage.

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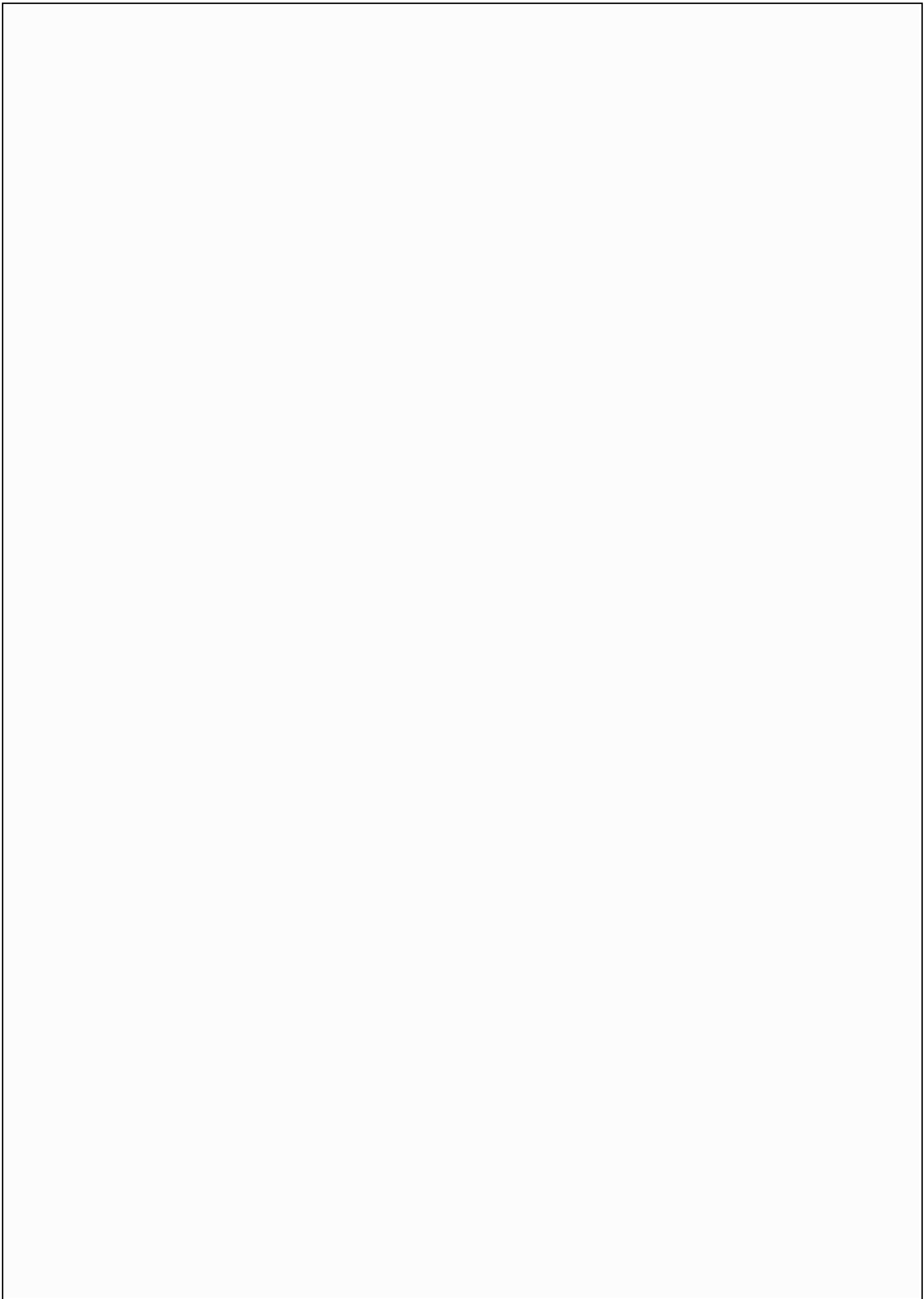

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