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The effects of bay leaf (*Syzygium polyanthum*) infusion on the quality of soaked corn with high aflatoxin content during storage

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ABSTRACT

This study aimed to evaluate the effect of bay leaf (*Syzygium polyanthum*) infusion on preserving the quality of corn during storage. The research employed an experimental approach using a Completely Randomized Design (CRD) with five treatments and four replications. The treatments included A (control corn), B (25% (v/v) bay leaf infusion), C (50% (v/v) bay leaf infusion), D (75% (v/v) bay leaf infusion), and E (100% (v/v) bay leaf infusion). The observed variables included damaged kernels, moldy kernels, moisture content, and aflatoxin content. Corn with high aflatoxin content was immersed in bay leaf infusion according to the respective treatments and then stored for four weeks. The research results showed that soaking corn with high aflatoxin content in bay leaf infusion (had a significant effect ($P<0.01$) on moisture and aflatoxin content but did not have a considerable impact ($P>0.05$) on damaged kernels and moldy kernels. Based on the research findings, it was concluded that the application of 100% (v/v) bay leaf infusion improved the quality of corn during a 4-week storage period, with the percentage of damaged kernels at 4.75%, moldy grains at 0.75%, moisture content at 14.60%, and a decrease in aflatoxin content by 41.11% (150-88.33 ppb).

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1- Introduction

The storage of maize can lead to a decline in both its quality and quantity due to variables such as elevated moisture levels, which can result in contamination by microorganisms, especially fungi that produce aflatoxin. The presence of aflatoxin in corn and its derivatives has been identified as a hazardous substance that could endanger the well-being of both animals and humans [1].

Typically, farmers employ direct sunshine to desiccate corn. While this practice is frequently observed, it is essential to acknowledge that it heightens the potential for microbial contamination in the immediate vicinity. One of the most common contaminants found in corn is *Aspergillus flavus* and *Aspergillus parasiticus*. These fungi can grow well at 20-30°C temperatures with substrate moisture content above 14% and humidity 75-85%. They produce mycotoxins, including toxic compounds known as aflatoxins [2].

Aflatoxin is a toxic secondary metabolite compound. In poultry and ruminant livestock, aflatoxin can cause various negative impacts, including decreased weight gain, reduced egg production, decreased livestock immune response, high livestock mortality rates, and liver organ damage. Furthermore, aflatoxin can be residual in livestock products, harming human health. Aflatoxins can significantly affect human and animal health because they can be carcinogenic, are very heat-stable, and are thus challenging to destroy during processing [3]. Continuous exposure to small amounts of aflatoxin in humans can cause liver organ damage and other chronic health impacts, including decreased immune response, higher risk of infection, liver cirrhosis, and even the risk of liver cancer [4].

The main challenge in dealing with aflatoxin is its heat-stable nature and resistance to physical or chemical treatment. Therefore, effective and appropriate handling efforts are needed. Some chemical handling efforts have been made, such as using compounds that inhibit fungal growth and aflatoxin production. Some fungicides, such as difenoconazole and thiophanate, have inhibited the field's fungal growth and aflatoxin production [5, 6].

There is a need for safer and more efficient management options that utilize readily accessible plant materials that are cost-effective and capable of preventing mold growth and producing aflatoxin. Several plant materials, including turmeric, oranges, cloves, curcuma, neem leaves, and Mindi leaves, can impede mold growth and the production of aflatoxin. Bay leaves are a noteworthy botanical specimen that possesses antibacterial properties due to the presence of many components, including flavonoids, tannins, and essential oils [7].

Alternative methods, such as plant-based materials like bay leaves (*Syzygium polyanthum*), are being explored to overcome the challenge of aflatoxin contamination. Bay leaves contain active ingredients, including flavonoids, tannins, and essential oils, which have antimicrobial properties [8]. This research aims to evaluate the potential of bay leaves in improving corn quality during storage, reducing the risk of aflatoxins, and supporting food security and health.

According to a prior study conducted by Trimasdika [9] (2016), it was found that an infusion of bay leaf can effectively hinder the growth of

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Streptococcus pyogenes when used at a concentration of 45%. Cita et al. [10] (2018) studied using bay leaf infusa. They found that soaking goat meat in a bay leaf infusion with a concentration of 15% can effectively reduce the amount of germs present. A study conducted by Sumono and Wulan [11] (2009) found that a 70% concentration of bay leaf infusion can effectively decrease the quantity of Streptococcus sp. colonies. Suciari et al. [12] (2017) found that bay leaf decoction, when used at doses of 20%, 40%, 60%, 80%, and 100%, demonstrated the ability to inhibit the development of Staphylococcus aureus in a laboratory setting.

While there has been some research on using bay leaves to control aflatoxin in microorganisms, further investigation is required to assess the potential of bay leaves to enhance the quality of stored maize [13]. This research is expected to provide valuable insights into using bay leaves as a natural material that can improve corn quality, reduce the risk of aflatoxins, and support food security and livestock health.

2-MATERIALS AND METHODS

2- 1- Materials

The tools used in this research are a blender, scale, woven plastic bags, filter paper, Erlenmeyer flask, stirrer, and pipette. A Kett PM410 Moisture Tester is used to test moisture levels. A set of aflatoxin testing equipment uses UV light with a wavelength of 360 nanometers. The materials used include corn sorted from PT. Japfa Comfeed Indonesia Ltd Padang branch, with an aflatoxin content of 150 ppb and moisture content of 15%, is under conditions where the corn has been damaged and contaminated, bay leaves, water, label paper, and writing tools.

2- 2- Methods

Using bay leaves, efforts can be made to reduce aflatoxin contamination in shelled corn. This study employed a Completely Randomized Design (CRD) with five treatments and four replications, namely:

treatment A = corn without bay leaf treatment
treatment B = corn + 25% bay leaf infused
treatment C = corn + 50% bay leaf infused
treatment D = corn + 75% bay leaf infused
treatment E = Corn + 100% bay leaf infused

2- 3- Parameters

2- 3- 1- Percentage of Damaged Seeds (%DS)

Damaged corn seeds (physically broken or with holes caused by insects) were separated from intact corn seeds after four weeks of storage. The percentage of damaged seeds was calculated as follows [14]:

$$\%DS = \frac{\text{Weight of damaged seeds}}{\text{Weight of corn after four weeks}} x100\%$$

2- 3- 2- Percentage of Fungal-Infected Seeds (%FS)

After four weeks of storage, fungal-infected seeds were visually assessed, and their percentage was calculated as follows [14]:

$$\%FS = \frac{\text{Weight of Corn after four weeks} - \text{Weight of intact}}{\text{Weight of corn after four weeks}}$$

2- 3- 3- Moisture Content Determination (AOAC)

The moisture content of corn was determined using a Moisture Tester Kett PM410, a device commonly used by PT.

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Japfa Comfeed Tbk in Padang. Corn stored for four weeks (800 grams per treatment) was placed in a stainless steel cup with 100 grams of corn. The cup was evenly positioned in the device, which automatically measured the moisture content of the corn [15].

1- 3- 4- Qualitative Aflatoxin Contamination Assessment (UV Light)

Corn observed in the previous stages, whose weight was known, was further assessed visually using a device equipped with UV light with a wavelength of 360 nanometers. The corn was finely ground with a grinder, spread evenly on a tray, and placed under the UV light. Aflatoxin content was determined using the PT method. Japfa Comfeed Tbk in Padang [16].

2- 4- Procurement of Bay Leaf Powder

Fresh bay leaves were washed and then dried in a location not directly exposed to sunlight at room temperature. The dried leaves were ground into bay leaf powder.

2- 5- Preparation of Bay Leaf Infusum

Fifty grams of bay leaf powder were weighed, and then it was mixed with 500 mL of sterile distilled water at a 1:10 ratio in an Erlenmeyer flask. The mixture was heated for 15 minutes at 100°C. Afterward, the solution was filtered using filter paper [17].

2- 6- Corn Storage

Corn obtained from PT. Japfa Comfeed, with an aflatoxin content of 150 ppb and a moisture content of 15%, was treated with bay leaves according to the respective treatment. It was then placed in woven plastic bags and stored for four weeks. After four weeks, the corn was ready for analysis (damaged seeds, fungal-infected seeds, moisture content, and the percentage of corn contaminated with aflatoxin).

2- 7- Statistical Analysis

Data obtained were analyzed using analysis of variance (ANOVA) to determine treatment effects using SPSS version 26. The subsequent step involves conducting the Duncan Multiple Range Test when there is a statistically significant difference in the impact ($p< 0.05$).

3- RESULTS AND DISCUSSION

Based on the research results, the average percentages of damaged seeds during the study period are presented in Table 1.

Table 1: Percentage of damage to corn seeds during storage after treatment

Treatment	Damaged Seeds (%)	Fungal-Infected Seeds (%)	Moisture Content (%)	Aflatoxin Content (ppb)	Aflatoxin Reduction Percentage (%)
A	4.40	0.92	14.37^{e}	165.67^{a}	10.45
B	4.77	1.34	14.93^{ab}	112.33^{c}	25.11
C	4.70	1.17	14.97^{ab}	99.00^{cd}	34.00
D	4.49	1.16	14.83^{b}	96.33^{cd}	35.76
E	4.75	0.75	14.60^{d}	88.33^{d}	41.11

Note: A (control), B (25% v/v bay leaf infusum), C (50% v/v bay leaf infusum), D (75% v/v bay leaf infusum), E (100% v/v bay leaf infusum).

Damaged Seeds During Storage

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Table 1 displays the percentage of damaged seeds with bay leaf treatment for each treatment during storage, ranging from 4.40% to 4.77%. The analysis of variance results showed no significant differences (P>0.05) in the treatment's effect on the percentage of damaged seeds. This could be attributed to the fact that the corn used by Japfa Comfeed Indonesia Tbk Padang branch is already sorted. Additionally, the storage conditions, including temperature and air humidity, were suitable for corn storage, reducing the risk of damage.

The storage temperature during the study ranged from 24°C to 30°C, with a relative humidity of 80%. These conditions were unfavorable for the growth of Sitophilus zeamays beetles and other potential contaminants. This aligns with the findings of those who stated that temperatures of 30°C and relative humidity of 80% prevent insect damage to corn [18]. However, it suggested that the suitable temperature for *Sitophilus Zea mays* beetle development ranges from 18°C to 35°C, with an optimum temperature of 30°C and relative humidity ranging from 25% to 100%, with an optimum of 80%-89% [19]. While sorting for beetles had been conducted before the study, it was not entirely practical, as some beetle eggs remained, which could have developed further, as bay leaves alone were insufficient to inhibit their growth. The absence of damaged seeds during storage is also due to the corn being pre-screened by the feed company.

Fungal-Infected Seeds During Storage

The average percentage of seeds attacked by fungus during storage with bay leaf infusion treatment is presented in Table 2. The incidence of seeds being attacked by fungus with bay leaf infusion treatment for each treatment during the study ranged from 0.75% to 1.44%. The analysis of variance showed no significant difference (P>0.05) in the effect of treatment on the percentage of seeds attacked by fungi with the bay leaf treatment. However, if you look closely, there was a decrease in the number of corn seeds attacked by fungus with the addition of bay leaf infusion. This shows that different concentrations of bay leaf infusion provide almost the same results inhibiting fungal growth on shelled corn during storage. So, increasing the percentage dose of bay leaf infusion when storing corn seeds is necessary.

Several factors, such as the storage environment and corn moisture content, influenced fungal growth on shelled corn during the research. Similar storage conditions, including humidity, ventilation, and temperature, can cause mold growth on corn. This aligns with observations that state that the storage environment significantly affects fungal growth, especially regarding the warehouse's ventilation, temperature, and humidity [20]. In addition, the water content of corn before storage is 15%, making it susceptible to fungal growth. This is based on findings that state that safe corn storage requires a humidity level below 14%. At this water content, fungal growth is inhibited, and the spread of fungal spores can be prevented.

Corn Moisture Content During Storage

The percentage of corn moisture content with bay leaf treatment for each treatment during storage ranged from 14.37% to 15.00%. The analysis of variance results showed a highly significant difference (P<0.01) in the treatment's effect on corn moisture content with bay leaf treatment during storage. Duncan's

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Multiple Range Test revealed that treatment A (without bay leaf treatment) significantly reduced corn moisture content during storage compared to other treatments. Treatment A had the lowest moisture content at 14.37%. The reduction in moisture content occurred due to respiration processes and water utilization by microorganisms for metabolic processes. The statement is that corn's moisture content decreases during storage due to microbial growth, which requires water to transport nutrients or waste materials in and out of cells [22]. Fungal metabolism ultimately produces CO_2, H_2O, and energy. According to respiration processes, grains generate heat, which could lead to the natural accumulation of moisture in the storage area, resulting in evaporation and decreased corn moisture content during storage [23].

Treatment B (1% ethanol extract) had a significant effect compared to the 25% and, 100% water extract and 100% infused treatments but showed no significant difference compared to other treatments. This can be attributed to the smaller fungal population growing on shelled corn during storage. Additionally, changes in moisture content were influenced by temperature and relative air humidity. Therefore, storage temperature and humidity need to be carefully managed. This aligns with the assertion that low temperature and high humidity can increase moisture content [19]. In contrast, rising temperatures lead to moisture release from stored materials due to the interconnected relationship between moisture content, temperature, and humidity.

Table 1 shows that the 25% water extract had a highly significant effect on aflatoxin content compared to other treatments. This is due to the low concentration and fewer active compounds in reducing aflatoxin. Conversely, using a 100% concentration resulted in a higher reduction in corn moisture content. This is a consistent statement that higher concentrations lead to more significant reductions in moisture content [24].

Aflatoxin Content in Corn

The aflatoxin content in corn, with the application of bay leaves for each treatment during storage, ranged from 88.33 ppb to 165.67 ppb. The research results demonstrated a significant impact of bay leaf application on aflatoxin content ($P<0.01$). Subsequently, Duncan's Multiple Range Test (DMRT) showed that treatment A (without bay leaf application) had a highly significant effect compared to the other treatments. In this treatment, there was a 10.45% increase in aflatoxin content. This increase occurred due to contributions from fungi capable of producing aflatoxins. Additionally, the rise in aflatoxin content was attributed to the absence of active compounds that could inhibit the growth of aflatoxin-producing fungi in stored corn that the increase in aflatoxin content is caused by the absence of active compounds that inhibit enzymes responsible for aflatoxin biosynthesis, leading to aflatoxin formation [25].

Regarding the use of bay leaf infusum, it was observed that the application of 25% concentration had a significant but not highly significant effect compared to 50% and 75% infusum concentrations. Still, it had a highly effective impact compared to 100% infusum concentration. Apart from differences in concentration, the high aflatoxin content at 25%, 50%, and 75% could also be attributed to corn moisture

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content during storage. This is consistent with Monica's [26] assertion that raw materials with high moisture content are highly susceptible to aflatoxin-producing fungi. Aflatoxin production can be caused by the growth of fungi, including Aspergillus species. Identified *Aspergillus flavus, Aspergillus parasiticus*, and *Penicillium citrinum* in corn sourced from PT—Japfa Comfeed Tbk's Padang branch [27].

Bay leaves contain alkaloids, flavonoids, essential oils, and tannins. The antifungal properties of bay leaves may be attributed to alkaloids, flavonoids, and essential oils [28]**.** Alkaloids are active compounds found in plants that serve as medicine and potent activators of the immune system, capable of destroying viruses, bacteria, and aflatoxin-producing fungi [29]**.** Alkaloids can strongly bind to ergosterol, creating holes that lead to membrane leakage, causing irreversible damage and death to fungi. Furthermore, GC-MS analysis of essential oil resulted in identifying 25 compounds. 1.8-Cineol (44.72%), a-Terpinyl acetate (12.95%), and Sabinene (12.82%) were the main components [30].

Penelitian sebelumnya terkait dengan penggunaan daun

Flavonoids and essential oils act as antifungal agents [31]**.** Flavonoids, as antifungals, contain genistein compounds that inhibit cell division or proliferation. Meanwhile, essential oils, rich in sesquiterpenoid compounds, can damage microbial cell membranes, disrupting membrane formation [32]**.** Tannin compounds are suspected to be effective in inhibiting fungal growth [33]**.** Tannins have a shrinking effect and can precipitate proteins from solutions with insoluble compounds.

4- CONCLUSION

Based on the research findings, using bay leaf infused (*Syzygium polyanthum*) at a concentration of 100% (v/v) can help maintain the quality of shelled corn during storage, especially in reducing aflatoxin.

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