ASSESSING THE EFFECT OF TEBUCUNAZOLE ON ASPERGILLUS FLAVUS AND FUSARIUM VERTICILLIOIDES GROWTH IN MAIZE SEEDS IN UGANDA

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ABSTRACT

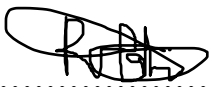
Tebuconazole, a triazole fungicide is used for effective control of the fungal pathogens in other crops. Little information regarding the use of tebuconazole fungicide is known. Therefore, this study aims at evaluating the effect of tebuconazole on growth of *A. flavus* and *F. verticillioides* in maize seeds. Completely randomized design (CRD) with three replications was used to determine fungal growth and changes in physical traits of maize seeds and the germination rate. Data was collected on fungal growth (colonies, capping, and abnormal seeds) and seedling growth. The results showed that was a significant difference ($p< 0.004$) among the tebuconazole concentrations on the fungal growth, physical traits and germination rate of maize seeds. The high dose had the highest normal seeds (72.65 ± 16.14^{a}) with the lowest number of abnormal seeds (23.47 ± 15.14^{c}) and capping (5.13 ± 8.45^{ab}) while Control had the lowest number normal seeds (50.57 ± 28.33^{c}) with highest number of abnormal seeds (44.40 ± 27.57^{a}) and capping (7.94 ± 8.04^{a}). White fungal colonies were present in 100% of the samples across all treatments, high dose was the most effective in reducing presence of yellow, green, grey and brown fungal colonies. The highest germination percentage (96.67 ± 4.880^{c}) was recorded in the control while the recommended dose had the lowest germination percentage (63.33 ± 9.759^{a}). Since, the recommended dose (0.5ml) of tebuconazole effectively controlled the fungus, it is the most effective dosage for controlling of *A. flavus* and *F. verticillioides* in maize seeds.

DEDICATION

I dedicate my research to my family, my parents Miss Korweto Leonida and Mr Muhwezi John Wilbroad, and my sisters who supported me financially and spiritually throughout my studies.

DECLARATION

I, Mutambi Ritah, declare that this research dissertation is done by me and it's my original work and has not been submitted by any other institute for a degree award.

Signature: 

Date: .....

Approval

This research dissertation has been submitted for examination with my approval as the University supervisor.

Signature: ..... Date: 16/April/2025

MADAM NAMUTOSI WINNIE

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LIST OF ABREVIATIONS.

F.verticilliodes	*Fusarium verticillioides.*
A.flavus	Aspergillus flavus.
FH	High dose of tebuconazole.
FL	Low dose of tebuconazole.
FR	Reference dose
PC	Recommended dose of tebuconazole.
NC	Control.

CHARPTER ONE

1.0 INTRODUCTION

1.1 Background.

Maize is one of the staple food crops for most of the population in African including Uganda. Maize is the one of the most important cereal crops cultivated throughout the year. It is used as feed (63.0%), food 23.0%) and in starch industries (12.0%), and also widely used for the production of biofuels and industrial products. It is used in manufacturing starch and glucose in industries dealing with poultry and animal feeds. (Tang et al., 2022). The demand for starch consumption in food is growing at 10−12% yearly. It has greater calorific value and rich in amino acids with less toxins compared to grains like millet and broken rice. (Buyinza & Wambede, 2008)

Despite the economic importance of Maize, more than 60 diseases attack maize during production and storage hence reducing the production and productivity of maize. (Xu et al., 2023) Fungi are one of the principal causes of deterioration and yield loss to maize farmers and seed companies during storage. Maize is susceptible to contamination by aflatoxin, which are produced by fungi called *Aspergillus flavus* (Sserumaga et al., 2020). During seed storage, the common fungal species include: *Aspergillus flavus* and *fusarium verticillioides*. These fungus mostly affect seed quality through production of mycotoxins that cause discoloration of seeds, reduction in seed viability and germination where the mycotoxins can directly damage the embryo leading to decreased germination rates and also increase susceptibility to diseases which makes them more vulnerable to fungal diseases thus reducing quality and yield in maize production.(Sserumaga et al., 2020) *Aspergillus flavus* thrives and

multiplies in storage areas with high moisture and temperature and it is a major producer of aflatoxin which is a toxic secondary metabolites that pose serious health risks to both humans and animals. (Mateo et al., 2017).*Aspergillus flavus* has L Morph type with large sclerotia that produces fewer conidia compared to the S morph type. The L morph type of *Aspergillus flavus* is the most prevalent type found in Uganda. *Aspergillus flavus* colonies have varying appearances with green, yellow and black mycelium. (El-Dawy et al., 2024) *Aspergillus flavus* destroys stored seeds causing loss of viability which impacts the ability of the seeds to germinate successfully, and a musty odor which indicates spoilage and potential health risks. The prevalence and severity of aflatoxin in maize depend on the composition of *Aspergillus* populations present. The toxigenicity of There are varying levels of aflatoxin in the Aspergillus flavus strains where some strains produce high levels of aflatoxin while others produce little or nothing hence the maize stored is affected by a specific strain depending on its ability to produce the aflatoxin.(Massomo, 2020). *Aspergillus ssp* produces different aflatoxin for example *Aspergillus flavus* produces B1and B2 aflatoxin while *A.parasiticus* produces G1 and G2 aflatoxin which is known as the most potent in potent aflatoxin production.

The color colonies for *fusarium verticillioides* are pink, grey and white and it is the most prevalent disease in the Ugandan maize exhibited with a genetic differentiation from the strains that are in other countries. (Wokorach et al., 2021 The infection caused by F.*verticilliodes* during the maize seed storage is severe and leads to high levels of damage where it damages embryo and endosperm which causes low levels of germination when the seeds are planted. (Moharana et al., 2020) It also causes other

symptoms like visible rotting on the seeds which affects their quality and produces Fumonisin that are toxic to human health and the animals. The Fumonisin contamination known as a group of mycotoxins. *F. verticillioides* causes visible rot symptoms on stored seeds hence affecting their quality and produces fumonisins known as a group of mycotoxins that pose serious health risks to humans and animals. In addition, Uganda fusarium strains exhibit a unique haplotype diversity which leads to a localized evolutionary patterns and adaptation. (Xu et al., 2023)

There are different methods of seed treatments to control fungal diseases which include biological methods like use of *trichoderma harzianum* which can parasitize and kill other fungi including *Aspergillus* and *Fusarium*. Physical methods like hot water treatment where seeds are soaked in hot water to kill fungal spores on the seed surface for a specific time and temperature and also seed sorting and selection where damaged, discolored, or moldy seeds are removed visibly before storage to reduce overall fungal load. Chemical seed treatments include: Carboxyl a systemic fungicide that controls seed-borne diseases like smuts, Thiram a contact fungicide that protects the seed surface from fungal infections and prodione a broad-spectrum fungicide effective against a wide range of fungi, including Aspergillus and Fusarium. Chemical seed protectants have been recommended for the control of seed borne diseases, but have not been completely effective and can also cause phytotoxicity and negative growth. The storage resistance of seeds that have been treated with saromyl fungicide was used as a reference for maize storage seeds (Rahmawati and Aqil, 2021).

Tebuconazole is a triazole systemic fungicide that is used worldwide in the agriculture sector to protect the crops from a variety of fungal pathogens. (Rajasekar et al., 2015) Its efficacy it affected by the regional variation for example in India which is subtropical, tebuconazole has shown significant efficacy against fungal pathogens while in warmer climates , studies have shown resistance rates of up to 95% in treated corn seeds under high dosages. (Smith et al., 2023). This research evaluated the impact of different tebuconazole dosages on the growth of *A. flavus* and *F. verticillioides* in stored maize seeds, as well as its effects on seed germination rates and physical traits, to determine optimal application strategies in Uganda.

1.2 Statement of the problem.

Maize is one of the most important crops in Uganda that highly contributes to food security and economic stability though its production and storage is greatly damaged by fungal pathogens especially *Aspergillus flavus* and *fusarium verticillioides*. These two fungi affect the quality of the seeds and also reduce its germination rates, they also produce aflatoxin that are harmful to both humans and animals. Despite the economic importance of maize to our country, the contamination caused by these two fungi during maize seed storage still remains a significant challenge leading to post harvest losses and reduced market value (Sserumaga et al., 2020; Xu et al., 2023). *Aspergillus flavus* and *Fusarium verticillioides* are the predominant fungal species affecting maize during storage in Uganda. These fungi thrive in warm and humid conditions, which are common in the region, and their presence leads to seed discoloration, reduced germination rates, and mycotoxin contamination (Massomo, 2020; Wokorach et al., 2021).

The control methods used include: Biological, physical and chemical methods that are used to manage fungal growth. Tebuconazole, a triazole fungicide that is a chemical method has shown efficacy in the control of the fungal pathogens in other crops and countries. (Rajasekar et al., 2015; Gxasheka et al., 2020). However the effectiveness of tebuconazole in controlling *A.flavus* and *F. verticillioides* in maize under the Ugandan storage conditions is still not known though it is widely used in control of fungal pathogens, there is limited information on its effectiveness in managing of *Aspergillus flavus* and *fusarium verticillioides*. The optimal dosage of tebuconazole for controlling these two fungi is not well established. There is lack of the standardized dosage level with no negative effect on the germination and physical traits of maize seeds (Tang et al., 2022).

There is a need to determine the efficacy of tebuconazole in controlling *Aspergillus flavus* and *Fusarium verticillioides* growth in stored maize under Ugandan conditions. Specifically, the optimal dosage of tebuconazole that effectively inhibits fungal growth while maintaining or improving seed germination rates and physical characteristics must be established though the impact of tebuconazole on seed quality during storage needs to be evaluated to provide practical recommendations for maize farmers and storage facility managers. (Sserumaga et al., 2020) This study aims at evaluating the effect of tebuconazole on the growth of *Aspergillus flavus* and *fusarium verticillioides* in the stored maize seeds and finding out the impact it has on the germination rate and physical traits. This experiment will consist of varying doses of tebuconazole in order to identify the optimal concentration that will effectively control these two fungi growth without affecting the seed quality. The findings of this

study will provide a clear view on how to develop am effective seed treatment strategy to mitigate fugal contamination in the maize seed storage in order to improve food security.

1.3 Main objective.

- To evaluate the effect of tebuconazole on fungal growth and seed quality of maize for effective management of fungal diseases and reduce the yield losses caused during storage.

1.3.1 Specific objectives.

1. To assess the effect of tebuconazole dosage on *Aspergillus flavus* and *Fusarium verticillioides* growth and physical traits in maize seed.
2. To assess the effect of tebuconazole dosage on the germination percentage of maize seeds.

1.3.2 Hypothesis.

1. Increased dosages of tebuconazole reduce the growth of *A. flavus* and *F. verticillioides* in maize seeds.
2. Low to moderate dosages of tebuconazole affect the germination percentage of maize seeds negatively.

1.4 Significance.

The results of this study will provide scientific evidence for the efficacy of tebuconazole in controlling local fungal growth and its impact on maize seed quality during storage. The results will provide valuable information for policymakers and regulatory agencies involved in setting standards for the use of fungicides in

agriculture. The study will also benefit maize farmers, food and feed industries, researchers and extension workers by providing them with practical recommendations for the safe and effective use of tebuconazole to prevent fungal contamination of

1.5 Justification.

Tebuconazole, a triazole fungicide has shown promising results in controlling a wide range of fungal pathogens including *Fusarium* species in various crops. (Gxasheka et al., 2020). Previous studies have demonstrated the efficacy of tebuconazole in inhibiting mycelia growth of *F. verticillioides* and reducing fumonisin production in vitro(Falca & Mycotoxin, 2011).However, there is a lack of information regarding the use of tebuconazole as a seed dressing agent for maize in Uganda due to the varying climatic conditions. This research will address this knowledge gap by investigating the effects of different tebuconazole concentrations on fungal growth, specifically targeting the dominant *A. flavus* and *fusarium verticillioides*. The study will also examine the impact of tebuconazole on seed germination and other crucial physical characteristics of maize seeds. The findings will provide a practical solution for fungal control and the optimal dosage will reduce wastage and develop effective fungicide application guidelines that will have no negative effect on maize seed physical characteristics and germination rate valuable information for developing effective seed treatment strategies.

1.6 Scope of the study.

This study will be conducted to evaluate the effect of tebuconazole on fungal growth and maize seed characteristics and germination. The focus will be on maize seeds treated with tebuconazole during storage. The study will be conducted at Makerere

agricultural research institute at kabanyolo. The fungi used in the study will be isolated from maize samples collected from different storage facilities in Uganda.

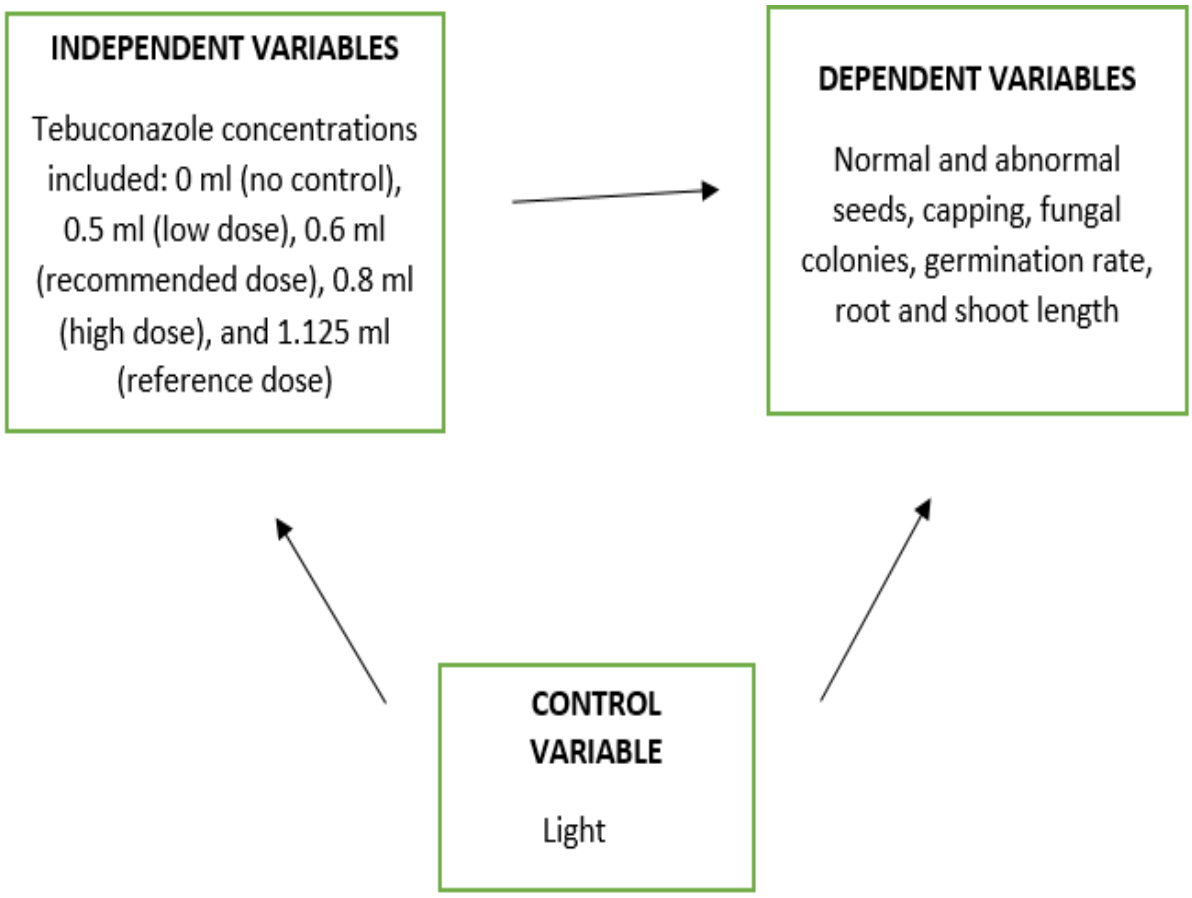
1.7 Theoretical framework.

Dependent variables: Seed weight was assessed by using a weighing scale in grams. The color of the fungal colonies growing on the seeds were assessed visually. Seeds were visually assessed for any abnormalities including discoloration, shriveling and rotting. Normal seeds are classified as healthy and had no signs of fungal growth or any other physical damage. Capping were assessed visually by counting the number of capped seeds. Germination percentage is determined by counting the number of seeds that have germinated expressing this as a percentage of the total number of seeds (10). (Moharana et al., 2020)

Independent variables : A range of tebuconazole concentration were used 0ml for no control, 0.5ml as high dose, 0.6ml as recommended dose, 0.8ml as lower dose and 1.125 dose as reference dose. Distilled water was used to dilute the tebuconazole fungicide.

Control variable: Light was controlled during the experiment in the laboratory. (Moharana et al., 2020)

1.7.1 Conceptual framework.



CHARPTER TWO

2.0 Literature review.

2.1.1 Importance of maize

Maize (Zea mays) is a critical staple food for many populations worldwide, particularly in Sub-Saharan Africa, including Uganda. It serves as the most significant cereal crop and a staple food for the majority of households, contributing over 40% to total calorie intake through versatile consumption as fresh cobs, "posho," and porridge, and as a key food source for institutions. Economically, it stands as the primary income source for numerous farmers, especially smallholders, supporting the livelihoods of over two million households, traders, and millers, and has evolved into a major non-traditional export crop boosting foreign exchange and underpinning industries like brewing and livestock feed. Agriculturally, maize is widely cultivated across Uganda, predominantly by smallholder farmers who account for the majority of production and marketable surplus, making it a government priority for enhanced output. (Tang et al., 2022)

Aflatoxin contamination in maize in Uganda.

However, maize crops are highly susceptible to fungal infections, which can lead to significant yield losses and pose severe health risks due to aflatoxin contamination (Sserumaga et al., 2020). Aflatoxins, a group of carcinogenic and immunosuppressive mycotoxins produced primarily by *Aspergillus flavus*, contaminate maize during storage, especially in warm and humid environments. Several reports indicate alarming levels of aflatoxin contamination in maize from various regions in Uganda,

leading to economic losses from trade restrictions and reduced market value (Sserumaga et al., 2020).

2.1.2 Storage conditions for maize quality preservation.

Maintaining maize seed quality and preventing spoilage require adherence to specific storage conditions. Proper post-harvest management is essential, with a target moisture content of 13% recommended to inhibit fungal growth (Campos et al., 2015). Well-ventilated storage structures, such as raised platforms, are crucial for promoting air circulation and preventing moisture accumulation. Effective drying methods, like sun drying, should be employed to remove excess moisture and prevent mold growth. These practices reduce the risk of fungal growth and aflatoxin contamination during storage (Campos et al., 2015).

2.1.3 Fungal community dynamics influencing aflatoxin contamination.

Aspergillus section *Flavus* communities are composed of diverse individuals exhibiting varying phenotypic and genotypic characteristics (Sserumaga et al., 2020). The composition of these communities significantly influences the incidence and severity of aflatoxin contamination. Understanding the interactions and dynamics within these fungal communities is crucial for developing effective control strategies. Factors such as fungal strain, environmental conditions and agricultural practices can all impact the prevalence and toxigenicity of *Aspergillus* species in stored maize (Sserumaga et al., 2020).

2.1.4 Morphological and toxigenic variability in A. flavus populations.

Aspergillus flavus strains are categorized into different morph types, including the L and S morph types. In Uganda, the L morph type is more prevalent, accounting for approximately 6.66% of the *A. flavus* population (Massomo, 2020). The S morph type is known to produce higher levels of aflatoxin compared to the L morph type, posing a greater risk of contamination (Massomo, 2020).

2.1.5 Climatic drivers of fungal proliferation and aflatoxin production.

Climatic conditions play a significant role in the growth and aflatoxin production of *A. flavus*. A relatively hot and dry weather with an optimal growth rate of around 31°C favors fungal proliferation. High temperatures (exceeding 25°C) and high humidity (above 70%) create conducive environments for the spread of infection and the growth of aflatoxin (Marín et al., 2013).

2.1.6 Fusarium pathogens: Prevalence and genetic diversity in maize.

Fusarium verticillioides is another common fungal pathogen in maize, often found in multiple haplotype groups (Marín et al., 2013). This pathogen is prevalent in Ugandan maize, exhibiting genetic differentiation from strains found in other countries (Wokorach et al., 2021). *F. verticillioides* can cause severe damage to maize seeds during storage, leading to the production of fumonisins, which are toxic to human and animal health (Moharana et al., 2020). The unique haplotype diversity of Ugandan *Fusarium* strains indicates localized evolutionary patterns and adaptation, requiring region-specific management strategies (Xu et al., 2023).

2.1.7 Deterioration of seed quality due to fungal colonization.

The invasion and growth of *A. flavus* in maize seeds result in a loss of seed viability (Moharana et al., 2020). The fungus uses the seed's stored reserves as a food source, leading to a decline in the seed's ability to germinate. Fungal activity degrades the seed's physical and biochemical composition, resulting in an overall decline in seed quality (Moharana et al., 2020).

2.1.8 Musty odor development as an indicator of fungal activity.

The metabolic activity of *A. flavus* during its growth and colonization processes produces volatile compounds, resulting in a musty odor (Moharana et al., 2020). These compounds are byproducts of the fungus breaking down the seed's components. The presence of a musty odor serves as an indicator of fungal activity and potential spoilage of stored maize. (Moharana et al., 2020).

2.1.9 Current control methods of *A. flavus* and *F. verticillioides* in maize seeds

Various methods exist for controlling fungal diseases in stored maize, including biological, physical, and chemical approaches. Biological methods involve using beneficial microorganisms like *Trichoderma harzianum* to parasitize and kill other fungi (Xu et al., 2023). Physical methods include hot water treatments and seed sorting to reduce the fungal load. Chemical seed treatments, such as carboxyl, thiram, and prodione, have been used to control seed-borne diseases (Rahmawati and Aqil, 2021). Tebuconazole, a triazole systemic fungicide, has shown promise in protecting crops from fungal pathogens. It has curative and protective properties. It is a large class of compounds that were developed in 1960s to solve the problem of fungal growth which causes diseases in plants, it is widely used in agriculture sector

with the aim of protecting crops from a variety of fungal pathogens (Rajasekar et al., 2015).

2.1.10 Tebuconazole: Efficacy, regional variations, and resistance.

Tebuconazole's efficacy is influenced by regional variations and climatic conditions. In subtropical regions like India, it has demonstrated significant efficacy against fungal pathogens (Rajasekar et al., 2015). However, in warmer climates, studies have reported resistance rates of up to 95% in treated corn seeds under high dosages (Smith et al., 2023) *Fusarium species*, particularly *F. verticillioides*, are highly prevalent in Ugandan maize and exhibit significant genetic differentiation from strains in other countries, evidenced by variations in the TEF-α1 gene sequence. (Wokorach et al., 2021).

2.2 Identification of the fungal growth in maize seeds.

Capping: High number of maize seeds that were capped indicated high levels of fungal colonization while lower number of capped seeds indicated low levels of fungal growth. (Moharana et al., 2020). Fungal colonies: Presence of different fungal color colonies indicated the presence or incidence of the fungal species. (Adolph, 2016)

Normal seeds: High number of normal seeds indicated tebuconazole effectiveness to control fungal growth and maintain seed quality and lower number of normal seeds indicated the resistance of fungal pathogens and changes in the seed quality.

Abnormal seeds: High number of abnormal seeds indicated the resistance of fungal pathogens and changes in the seed quality while lower number of abnormal seeds indicated tebuconazole effectiveness to control fungal growth and maintain seed quality. (Moharana et al., 2020).

3.0 CHARPTER THREE

3.1 Specific objective 1. To assess the effect of tebuconazole dosage on *A.flavus* and *F.verticillioides* growth and physical traits in maize seed.

3.1.2 Study site.

The study was conducted at Makerere University Agricultural Research Institute Kabanyolo. MUARIK is located on spatial coordinates 0˚27'60"N, 32˚36'24" E at an altitudinal range of 1250 m to 1320 m above mean sea level. The study site is within the administrative boundaries of Nangabo Sub County, Wakiso district and about 14 km north of Kampala, Uganda's capital city. Kabanyolo is part of the Lake Victoria basin that receives an average annual precipitation of 1218 mm and slightly drier periods in June to July and December to February. The average annual temperature is 21.5˚C. (Makerere University et.at. 2025)

3.1.3 Materials.

Healthy maize seeds, tebuconazole fungicide, reference chemical (metalaxyl), 75 plastic tins, syringe, weighing scale and distilled water.

3.1.4 Experimental design.

Completely Randomized Design (CRD) with three replications for statistical validity including treatment groups. (Moharana et al., 2020) Healthy maize seeds were obtained from a maize storage facility.300g of maize seed was weighed for each plastic tin using a weighing scale. Treatments were inoculated with the infected maize seed except the control treatment group.

The tebuconazole treatments groups included; 0ml (control), 0.5ml (recommended dose), 0.6ml (low dose), 0.8ml (high dose) and 1.125ml (reference dose) which were diluted with distilled water (3ml). (Moharana et al., 2020)

The solution was mixed with the seeds, shaking manually in plastic bags for the uniform distribution of the fungicide. The maize seeds were then placed in the plastic tins to observe the fungal growth. Maize seeds in the tins were monitored for fungal growth and changes in physical traits of the seeds every after 7days for a month. (Moharana et al., 2020).

Experimental layout.

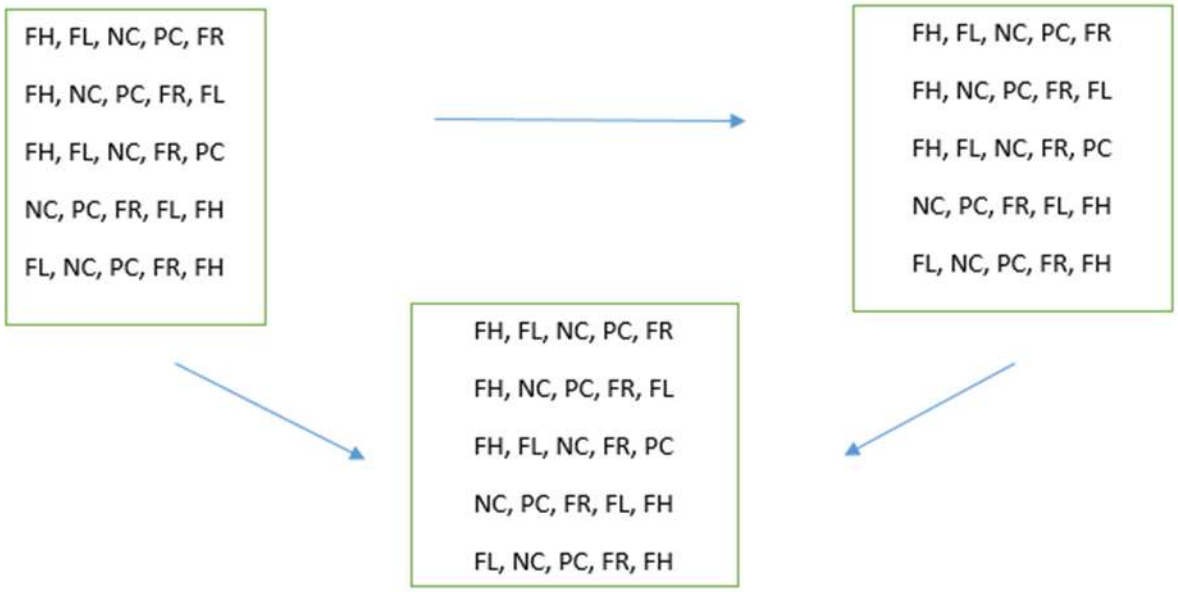


Figure 1: Experimental layout

3.1.5 Data collection.

Data collected: Quantitative and qualitative data was collected.

Variables: Independent variable (tebuconazole concentration), dependent variables (fungal colonies, capping, abnormal seeds and normal seeds), control variable (light)

A sample of 100 maize seeds were chosen to assess the number of abnormal seeds, normal seeds and capping visually. The color of the fungal colonies growing on the seeds were assessed visually. (Adolph, 2016). Seeds were visually assessed for any abnormalities including discoloration, shriveling and rotting. (Moharana et al., 2020). Normal seeds were classified as healthy and had no signs of fungal growth or any other physical damage. (Moharana et al., 2020)

3.1.6 Data analysis.

Data was entered in excel then imported to Genstat 12edition for analysis. Descriptive statistics was done to calculate mean and standard deviation for numerical variables (normal, abnormal seed and capping), and for categorical variables (high dose, low dose, reference dose, Control, and recommended dose. (Moharana et al., 2020). One way ANOVA was done to compare means of dependent variables across different treatment groups. This was followed by Duncan's multiple range test was used to determine which treatments are significantly different from each other.

3.2 Specific objective 2. To assess the effect of tebuconazole dosage on the germination of maize seeds.

3.2.1 Study site

This study was conducted from Uganda Christian University, Mukono main campus with approximately 8,000 students, is in the town of Mukono, approximately 25 kilometers (16 mi), by road, east of Uganda's capital city, Kampala, on the Kampala-

Jinja Highway. The coordinates of the main campus are 0°21'27.0"N, 32°44'29.0"E (Latitude: 0.357500; Longitude: 32.741389) with a tropical rainforest climate. (Uganda Christian Unversity.et al., 2025)

3.2.2 Materials.

Healthy maize seeds, tebuconazole fungicide, reference chemical (metalaxyl), 75 sterilized petri dishes, pipettes, gloves, cotton and distilled water

3.2.3 Experimental design.

Completely Randomized Design (CRD) with three replications for statistical validity including treatment groups.

Healthy maize seeds were obtained from storage maize facilities. Maize seeds were soaked in distilled water for 12 hours to improve germination.

Tebuconazole concentrations were prepared, low dose(0.5ml),high dose(0.8ml), recommended dose (0.6ml) and reference dose (1.125ml) that were diluted with 0.3ml of distilled water. (Moharana et al., 2020). The maize seeds were treated, apart from control group where the mixture and the seeds were put in polyethene bag and shaken manually for uniform distribution of the fungicide.

Treated 10 maize seeds, apart from control group were placed in each petri dish. Moist cotton was placed in the petri dishes where the treated seeds were placed. The seeds were then covered with a black polyethene back to trap moisture and prevent direct light from inhibiting germination. (Moharana et al., 2020)

Moisture of the cotton was monitored daily and watered to prevent inhibition of seeds causing by drying of the seeds. The germination process took 7days. (Moharana et al., 2020) (figure 1).

3.2.4 Data collection.

Data collected: Quantitative data was collected.

Variables: Independent variable (tebuconazole concentrations, distilled water), dependent variable (germination percentage, root length and shoot length), control variable (light)

The number of maize seeds that germinated were recorded after 7days using the excel sheet.

Germination percentage was calculated by dividing the number of germinated seeds by the total number of seeds in each petri dish, which expressed the result as a percentage. The root and shoot length data was collected using a ruler in cm by measuring the root and length of the seedlings. (Moharana et al., 2020)

3.2.5 Data analysis.

Data was entered in excel then imported to Genstat 12edition for analysis. Descriptive statistics was done to calculate mean and standard deviation for numerical variables (germination percentage, shoot length and root length), and for categorical variables (high dose, low dose, reference dose, Control, and recommended dose. One way ANOVA to compare means of dependent variables across different treatment groups and correlation analysis to determine the relationship between germination percentages, root and shoot lengths. Duncan's multiple range test used to determine which treatments are significantly different from each other. (Moharana et al., 2020)

CHARPTER FOUR

4.0 RESULTS

4.1 For objective One: To assess the effect of tebuconazole dosage on *A.flavus* and *F.verticillioides* growth and physical traits in maize seed.

4.1.1 Effect of tebuconazole on fungal growth.

The results showed that there was a significant difference at p- 0.004 among the dosages of tebuconazole on fungal growth on maize seeds (Table 1). White fungal colonies were present in 100% of the samples across all treatments, suggesting potential resistance to tebuconazole. The high dose of tebuconazole appeared to be most effective in reducing the presence of yellow, green, grey, and brown fungal colonies.

Table 1: Effect of tebuconazole on fungal growth

Treatment	White (%)	Yellow (%)	Green (%)	Grey (%)	Brown (%)
Recommended Dose	100.0	24.4	6.7	0.0	17.8
Reference dose	93.3	2.2	0.0	0.0	0.0
Low dose	95.6	13.3	20.0	31.1	22.2
High dose	100.0	13.3	6.7	8.9	11.1
Control	100.0	11.1	28.9	48.9	17.8
P-value	< 0.05	< 0.01	< 0.001	< 0.001	< 0.01

4.1.2 Effect of tebuconazole dosage fungal growth and appearance of physical traits.

The results showed that there was a significant difference at p- 0.001 among the dosages of tebuconazole on physical traits of maize seeds (normal seeds, abnormal and capping) table 2. High dose of tebuconazole resulted in the highest number of normal seeds (72.65 ± 16.14^{a}) and the lowest number of abnormal seeds (23.47 ± 15.14^{c}), hence there was effective control of fungal growth. The control group had the lowest number of normal seeds (50.57 ± 28.33^{c}) and the highest number of abnormal seeds (44.40 ± 27.57^{a}), hence there was significant fungal impact in the absence of treatment.

Table 2: Effect of tebuconazole dosage fungal growth and appearance of physical traits

Treatment	Normal Seeds	Abnormal Seeds	Capping
Recommended dose	65.73 ± 21.39^{b}	29.97 ± 20.76^{b}	6.86 ± 7.45^{ab}
Reference dose	62.42 ± 25.84^{b}	34.83 ± 24.87^{ab}	3.84 ± 4.31^{b}
Low dose	53.14 ± 26.77^{c}	42.77 ± 20.62^{a}	7.80 ± 7.59^{a}
High dose	**72.65 ± 16.14^{a}**	**23.47 ± 15.14^{c}**	**5.13 ± 8.45^{ab}**
Control	**50.57 ± 28.33^{c}**	**44.40 ± 27.57^{a}**	**7.94 ± 8.04^{a}**
P-value	<0.001	<0.001	<0.01
LSD	8.24	7.95	2.47

P=0.004 shows significant difference, a, b, c ab separates means of the different treatments

4.2 For Objective 2: To assess the effect of tebuconazole dosage on the germination of maize seeds.

4.2.1 Effect of tebuconazole dosage on germination percentage.

The results showed that there was a significant difference at p- 0.004 among the dosages of tebuconazole on germination of maize seeds and seedling traits. Table 3 indicates that low dose of tebuconazole resulted in the highest germination percentage (96.67 ± 4.880^{c}), shoot length (5.327 ± 3.619^{b}) and root length (7.247 ± 4.391^{c}) while the recommended dose of tebuconazole resulted in the lowest germination percentage (63.33 ± 9.759^{a}), shoot length (3.420 ± 2.723^{a}) and root length (5.200 ± 1.715^{b}).

Table 3: Effect of tebuconazole dosage on germination percentage and seedling growth

Treatment	Germination %	Shoot length	Root length
High dose	70.00 ± 8.452^{b}	4.387 ± 1.230^{ab}	2.987 ± 1.183^{a}
Low dose	**96.67 ± 4.880^{c}**	**5.327 ± 3.619^{b}**	**7.247 ± 4.391^{c}**
Reference Dose	80.00 ± 22.361^{b}	4.493 ± 1.702^{ab}	4.193 ± 1.688^{b}
Control	83.33 ± 4.880^{b}	4.213 ± 1.409^{a}	3.800 ± 1.440^{b}
Recommended Dose	**63.33 ± 9.759^{a}**	**3.420 ± 2.723^{a}**	**5.200 ± 1.715^{b}**
P value	0.001	0.001	0.01
LSD	5.737	1.3727	1.662

P=0.004 shows significant difference, a, b, c, ab separates means of different treatments.

CHAPTER FIVE

5.0 DISCUSSION.

5.1 For objective one: To assess the effect of tebuconazole dosage on *A.flavus* and *F.verticillioides* growth and physical traits in maize seed.

The findings of this study confirm tebuconazole's effectiveness in reducing fungal growth and maintaining the physical quality of maize seeds during storage. Specifically, the high dose of tebuconazole led to a higher number of normal seeds and a lower number of abnormal seeds, indicating effective control of fungal growth. This aligns with previous research demonstrating tebuconazole's ability to protect crops from various fungal pathogens. (Rajasekar et al. (2015) which shows that tebuconazole's efficacy in protecting crops from fungal pathogens. However, the consistent presence of white fungal colonies across all treatments, as observed in this study, suggests potential resistance or the presence of non-targeted fungal species which is the same observation that was reported in warmer climates with resistance rates of up to 95% in treated corn seeds under high dosages. (Smith et al. 2023)

Implication.

Tebuconazole significantly affects the growth of *A. flavus* and *F. verticillioides*, as well as the physical traits of maize seeds.

For objective two: To assess the effect of tebuconazole dosage on the germination of maize seeds.

The results indicated that low to moderate dosages of tebuconazole (0.5–0.6 ml) had minimal negative effects on germination percentage hence safe for seed viability.

This finding is consistent with the broader understanding that while fungicides can protect seeds from fungal pathogens, they must do so without compromising the seed's ability to germinate. However, the reference dosage (1.125 ml) negatively impacted germination due to phytotoxicity which can damage the seed embryo and inhibit germination. The same observation was reported regarding the importance of establishing optimal tebuconazole dosages to avoid negative effects on germination and physical traits of maize seeds where high doses of tebuconazole affected germination with an incidence of 10-20% (Tang et al. 2022).

Implication.

Low to moderate dosages of tebuconazole have minimal negative effects on seed germination and they are safe for seed viability.

Limitations.

The study observed white fungal colonies in 100% of the samples across all treatments, suggesting potential resistance to tebuconazole or the presence of non-targeted fungal species.

The research did not include field trials in different environmental conditions and with different maize varieties, which could limit the generalizability of the results.

CHAPRTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions.

The results from objective one for assessing the effect of tebuconazole dosage on *A.flavus* and *F.verticillioides* growth and physical traits in maize seed showed that there was a significant difference (<0.004) among the tebuconazole concentrations on the fungal growth and physical traits of maize seeds, therefore I fail to reject my hypothesis which states that increased dosages of tebuconazole reduce the growth of *A. flavus* and *F. verticillioides* in maize seeds.

The results from objective two for assessing the effect of tebuconazole dosage on the germination percentage of maize seeds showed that was a significant difference (< 0.004) among the tebuconazole concentrations on germination percentage, therefore I fail to reject my hypothesis which states that low to moderate dosages of tebuconazole affect the germination percentage of maize seeds.

6.2 Recommendations.

A recommended dose of tebuconazole (0.5ml) is recommended for usage due to its effectiveness to control fungal growth and minimal effect on the germination percentage.

Investigate long-term effects of tebuconazole on maize crop performance and explore integrated approaches combining chemical and biological treatments.

Filed trials should be conducted in different environmental conditions and with different maize varieties to ensure generalizability. The presence of white fungal

colonies across all treatments suggests potential resistance to tebuconazole hence it is recommended to conduct further investigations to identify the specific fungal species present and their susceptibility to tebuconazole.

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Appendices

Appendix 1: Pictures showing setting up of the experiment.

	

Appendix 2: Pictures showing effect of tebuconazole on fungal growth on maize.

Appendix 3: Pictures showing monitoring and data collection.