



Effect of Selected Fungicides in the Control of Aflatoxin-producing Fungi in Pre-harvest Maize (*Zea mays* L) in Anambra State

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Contamination of maize by toxigenic fungi is the process that occur yet in the field. Aflatoxin contamination in several foodstuffs has been a recurrent problem. This study is aimed to evaluate the effect of selected fungicides in the control of aflatoxin-producing fungi affecting growth in pre-harvest maize (*Zea mays* L). Maize seeds treated with organic and synthetic fungicides were sown immediately after land preparation comprising of 13 plots. Five plants each were randomly selected from 13 plots for collecting data for 12 weeks at 2 week interval. Effect of organic and synthetic fungicide in the control of aflatoxin production on the growth parameters of maize showed that maize treated with *Azadirachta indica* recorded significant growth in the leaf area for all treatments for weeks 8, 10 and 12 while at week 6, maize treated with *Cymbopogon citratus* showed significant growth in the leaf area at 0.05% for TLg25%, TLg75% and TLg100%. Maize treated with synthetic fungicide (mancozeb) showed significant growth in the leaf area for weeks 8, 10, 12 in all treatments. Maize treated with *Azadirachta indica* recorded a significant difference in the plant height for week 4, 10 and 12 in all treatments. For maize treated with *Cymbopogon citratus*, weeks 2 and 8 recorded no significant difference in the plant height but showed a significant growth in the plant height at weeks 4 and 12 (for all treatments) while weeks 6 and 10 recorded significant growth in the plant height for some of the treatments. Maize treated with synthetic fungicide showed significant growth in the plant height for weeks 4, 10 and 12. Fungal isolates identified in this study

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were: *Aspergillus* spp. *Fusarium* spp. and *Penicillium* spp., with *Aspergillus* spp. having the highest frequency of occurrence of 4 in treatments T_{Ai}100%, T_{Lg}75%. The result showed that both organic and synthetic fungicide were effective in the control of aflatoxin-producing fungi on the maize yield but was more effective with the organic fungicide. The use of *Azadirachta indica* and *Cymbopogon citratus* is recommended as a means of biofungicides to control aflatoxin-producing fungi in maize.

Keywords: Maize; *Azadirachta indica*; *Cymbopogon citrates*; aflatoxin.

1. INTRODUCTION

Zea mays L. is the world's most noteworthy provider of calories with the caloric supply of almost 19.5% giving more calorie than rice (16.5%) and wheat (15.0%). Maize has grown to be a nearby 'cash crop' and the foremost imperative staple nourishment in Nigeria [1]. Globally, demand for maize is believed to increase by 50%, from 558 million metric tonnes in 2020, this is due to its importance in food processing, animal feed and ethanol production. It could be an essential staple nourishment grain in numerous parts of the world counting Africa, Latin America, and Asia [2]. In Nigeria, maize was presented within the 16th Century and has gotten to be the third most vital cereal after sorghum, millet and rice. It has been recognized to be one of the longest ever developed nourishment crops. It has been alluded to as the world's best adjusted trim since it is developed in a few regions of the world [3]. The request for maize in Nigeria is expanding at a faster rate day by day and this may be due to the reality that the grain is being utilized for bolstering poultry and conjointly serve as the most nourishment for numerous family units [4,5].

Agreeing to Ramirez-Camejo et al. [6], *Aspergillus*, *Fusarium* and *Penicillium* genera are prevailing toxigenic organisms' species, they are known contaminants of maize and produce mycotoxins. *Aspergillus flavus*, which is broadly dispersed, could be a saprophytic/pathogenic organism and cereal grains, tree nuts, and vegetables are known best for their colonization. The defilement of maize by toxigenic organisms and their mycotoxins in the method that it can happen however within the field during collect and afterward amid the capacity until the utilization [7]. Aflatoxin defilement in a few foodstuffs in Africa has been a repetitive issue [8]. Pre-harvest biocontrol organisms speak to a great and hence distant broadly connected strategy to diminish aflatoxin dangers in nourishment by ensuring plants from pathogens. They both diminish financial misfortune caused by contagious diseases and lower poison levels

in products [9]. Biocontrol operators compete for supplements and space, may secrete antifungals or indeed parasitize molds, and can too stimulate and have plant resistance [10] and, subsequently, they diminish the hazard of plant contaminations and their undesirable consequences [11,12]. Amid the pre-harvest stage, the fitting agronomic practices may depend on (i) the utilize of crop assortments or cross breeds, which are safe to contagious diseases, (ii) the application of pesticides and fungicides, (iii) satisfactory administration of weeds and crop residues, (iv) the utilization of crop rotation, tillage, fertilization and irrigation and (v) the application of biocontrol specialists, e.g., bacteria, yeasts, or atoxigenic strains of *A. flavus* or *A. parasiticus* [13,9, 14, Peng et al., 2018].

As of late, broad use of manufactured fungicides (chemical fungicides) in farming is the major development and has taken put amid the last 60 years. This has been the major way of fungal malady control within the world amid the past decades and these days it plays a major part in crop protection. Chemical build ups in any case tend to stay on the plant or inside its tissues taken after fungicidal treatment. Fungicide buildups in plants and their natural products cause an incredible wellbeing risk to the consumer, thus the ought to explore for secure options to engineered fungicides. Already, agriculturists frequently overlooked or did not recognize the impact that parasitic pathogens had on the abdicate and quality of their crops. In addition, the development of safe strains of pathogens is as a result of the abuse of these engineered fungicides and this has ended up a major worldwide issue since the frequency of mutant phenotypes within the populaces is high [15,16]. A few of these fungicides are suspended due to their high harmfulness, and there's expanded weight on the nourishment esteem chain to either expel these agents or grasp characteristics options for the upkeep or expansion of a product's shelf life [17]. These impediments give new opportunities to explore common options for unused preservatives to be

connected to crops. The proceeding advancement of fungicide resistance in plants and human pathogens requires the revelation and advancement of modern fungicides. Hence, a wide run of chemicals has been evaluated for their potential to utilize as an alternative to the current fungicides e.g plants extracts and a few compounds gotten from plants [18]. Plant fungicides have been detailed to be secure to beneficial living beings such as pollinating creepy crawlis, night crawlers and to humans.

Khalid and Shad [19], detailed that the harmful impact is brief and vanishes within 14-21 days, hence phyto-fungicides are environment-friendly. Phytochemicals, a term is given to happening, non-nutritive naturally dynamic chemical compounds of plant beginning, have a few defensive or disease-preventive properties. A few phytochemicals are destructive to organisms and may well be utilized to secure crops, creatures, humans' nourishment and feed against toxigenic organisms and mycotoxin. Phyto-fungicides may be arranged or formulated from the leaves, seeds, stem bark or roots of plants of pesticide noteworthiness and can be applied in form of extract, powders and cakes or as exudates (Owino and Wando, 1992; Anjorin and Salako, 2009). Phytochemical examination on the leaves of *Azadirachta indica* and *Cymbopogon citratus* showed the presence of alkaloids, saponins, tannins, steroid, terpenoid, glycoside, flavonoid, phenol, oxalic acid, cardiac glycosides and coumarins [20, 21].

Due to exceptionally tall and unbalanced money related trade rate, engineered fungicides are now more-costly for most of the resource-poor agriculturists. A few manufactured fungicides such as methyl bromide are phytotoxic and frequently take off undesirable buildups when connected to the developing crops [22]. Thus, the hunt for an elective or complement to manufactured fungicide. This study is aimed to assess the impact of chosen-fungicides within the control of aflatoxin-producing fungi influencing development in pre-harvest maize (*Zea mays*.L).

2. MATERIALS AND METHODS

The experiment was carried out at the trial field located behind the Bioscience Lecture Hall, Science Village, Nnamdi Azikiwe University, Awka.

Maize seeds (Plate 1) were obtained from local farmers situated in the three zones in Anambra

State and stored in sterile paper bags. Leaves of Neem (*Azadirachta indica*) and Lemon grass (*Cymbopogon citratus*) -organic fungicides were gotten from Nnamdi Azikiwe University, Main Campus Awka ((Plate 2 and 3) while mancozeeb (synthetic fungicide) was procured from Eke-Awka market, Awka.

The experimental design was a randomized complete block with three replicates. Treatments consisted of evaluating maize seeds treated with *Azadirachta indica*, *Cymbopogon citratus* and a synthetic fungicide (Mancozeeb).

2.1 Preparation of Plant Extract (Organic Fungicide)

One kilogram (1kg), each of the two plant materials (*A. indica* and *C. citratus*) was washed thoroughly with tap water, cut into small pieces and finely grounded using a ceramic pestle and mortar. The grounded plant materials were allowed to stand in two separate beakers for 24 hours with 500 ml of sterile distilled water added to each beaker. The solutions were sieved separately and filtered using Whatman No1 filter paper. The two filtrates obtained served as 100% w/v aqueous extract of *A. indica* (Ai100) and 100% of aqueous extract of *C. citratus* (Cc 100). Serial dilutions were prepared from each of the stock solutions of 75 ml, 50 ml and 25ml by making up the volume to 100ml with sterile distilled water. This gave a total of eight solutions namely; aqueous extract of *A. indica* and *C. citratus* at 100, 75, 50 and 25%, (Ai100, Ai75, Ai50 and Ai25) and (Cc100, Cc 75, Cc 50 and Cc 25) respectively.

2.2 Maize Seed Treatment

The method used by Wondimeneh et al. [23], was employed.

1. Treating seeds with synthetic fungicide
Synthetic fungicide viz: 5g, 2.5g, 1.25g and 0.625g powder was added each in a transparent plastic bag containing 1kg of maize seeds separately and thoroughly mixed by shaking the plastic bag until a uniform mixture is obtained. 5ml of sterile distilled water was added and thoroughly mixed to facilitate proper coating. The seeds were dried at room temperature before packing into sterile bags for future use.
2. Treating seeds with organic fungicide (plant extract)

Five (5) milliliters of each plant extract (*A. indica* and *C. citratus*) viz 100%, 75%, 50% and 25% concentrations were added in a transparent plastic bag containing 1kg of maize seeds. The procedure employed in treating synthetic fungicide was followed.

2.3 Land Preparation and Planting

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replicates. The experimental site was cleared, packed and tilled manually into rows consisting of 13 plots. Each plot was 3.6m long and 3m wide consisting of 3 rows with 30cm intra-row and 75cm inter-row spacing. Each plot was spaced 1m apart and 1.5m spacing between blocks.

Treated maize seeds were sown immediately after land preparation. Two seeds were planted per hole and were later thinned to one plant two weeks after emergence.

2.4 Effect of Organic and Synthetic Fungicide in the Control of Aflatoxin Production on the Growth Parameters and Cob Yield of Maize

Five (5) plants were randomly selected from each plot for collecting data on leaf area, plant height, plant girth and cob yield for 12 weeks at 2 weeks' intervals. The selected plants were tagged with polyethylene ropes to distinguish them from the rest of the plants. At harvest, maize cobs were properly labeled and dried before storage. The maize plants were labeled as follows:

TAi25%:	Maize seeds treated with <i>Azadirachta indica</i> extract of 25% concentration
TAi50%:	Maize seeds treated with <i>Azadirachta indica</i> extract of 50% concentration
TAi75%:	Maize seeds treated with <i>Azadirachta indica</i> extract of 75% concentration
TAi100%:	Maize seeds treated with <i>Azadirachta indica</i> extract of 100% concentration
TLg25%:	Maize seeds treated with <i>Cymbopogon citratus</i> extract of 25% concentration

TLg50%:	Maize seeds treated with <i>Cymbopogon citratus</i> extract of 50% concentration
TLg75%:	Maize seeds treated with <i>Cymbopogon citratus</i> extract of 75% concentration
TLg100%:	Maize seeds treated with <i>Cymbopogon citratus</i> extract of 100% concentration
TSf5g:	Maize seeds treated with 5g of synthetic fungicide
TSf2.5g:	Maize seeds treated with 2.5g of synthetic fungicide
TSf1.25g:	Maize seeds treated with 1.25g of synthetic fungicide
TSf0.625g:	Maize seeds treated with 0.625g of synthetic fungicide
CONTROL:	Maize plants without treatment

2.5 Frequency of Aflatoxin-Producing Fungi in Maize Seeds

Sabouraud Dextrose Agar (SDA) was used for the isolation, growth and maintenance of fungi associated with the maize seeds. This medium was prepared routinely according to the manufacturer's prescription and autoclaved at 121°C for 15 minutes [24,25].

A direct isolation method was employed. Twenty relatively healthy maize seeds were surface-sterilized with 10% sodium hypochlorite (NaOCl) solution for 10 minutes and rinsed in three changes of sterile distilled water and dried between sterile filter papers. Five maize seeds were inoculated in each Petri dish with three replicates. The Petri dishes were sealed with paraffin to prevent contamination and incubated at 28±2°C for 5 days.

The Petri dishes were sealed with paraffin to prevent contamination. A completely randomized design was used for the determination of the frequency of fungi. Isolates were identified based on colony characterization, strain morphology and macroscopic features.

2.6 Data Analysis

Data obtained from the experiments in this study was subjected to two-way analysis of variance using sigma plot. Treatment means were separated and compared using the Duncan's multiple range test (DMRT) at $p \leq 0.05$ [26].



Plate 1. Maize seeds



Plate 2. Neem Leaves



Plate 3. Lemon Grass Leaves

3. RESULTS

3.1 Effect of Organic and Synthetic Fungicides in the Control of Aflatoxin Production on the Leaf Area of Maize

Table 1 recorded that maize treated with *Azadirachta indica* showed no significant growth in the leaf area at week 2 for all treatments, but progressed at week 4. For week 6, only TAI50% and TAI100% showed significant growth at 20.000 ± 0.495^a and 26.000 ± 0.141^a respectively. Week 8, 10 and 12 showed significant growth in the leaf area for all treatments.

For maize treated with lemon grass, week 2 recorded no significant growth in the leaf area but showed a significant growth at week 4, 8, 10 and 12 (for all treatments). Week 6 showed significant growth in the leaf area at 0.05% for TLg 25%, TLg 75% and TLg 100% at 15.100 ± 0.141^a , 18.550 ± 0.778^a and 20.550 ± 0.778^a respectively.

The result in this study showed that maize treated with synthetic fungicide showed significant growth in the leaf area for weeks 8, 10, 12 while week 2 recorded no significant growth in the leaf area. Week 4 showed significant growth in the leaf area only for TSf5g and TSf0.625g while week 6 recorded significant growth in the leaf area for TSf2.5g (15.100 ± 0.14^a) and TSf1.25g (38.150 ± 0.212^a).

3.2 Effect of Organic and Synthetic Fungicides in the Control of Aflatoxin Production on the Plant Height of Maize

In Table 2 maize treated with *Azadirachta indica* showed no significant growth in the plant height at week 2 in all treatments while all treatments were significant for plant height at week 4. For week 6, only TAI50% showed significant growth in the plant height at 20.500 ± 0.000^a . Week 8 showed no significant difference in the plant height for all treatments while there was a significant difference in the plant height recorded at week 10 and 12.

For maize treated with lemon grass, week 2 and 8 recorded no significant difference in the plant height but showed significant growth in the plant height at weeks 4 and 12 (for all treatments) while weeks 6 and 10 recorded significant growth in the plant height for some of the treatments.

The result in this study showed that maize treated with synthetic fungicide showed significant growth in the plant height for weeks 4, 10 and 12. Week 2 showed significant for only TSf5g (8.000 ± 0.000^a) while week 6 showed significant growth in the plant height for TSf5g and TSf2.5g at 10.900 ± 0.000^a and 16.300 ± 0.000^a respectively. Week 8 showed significant growth in the plant height for most of the treatments except for TSf2.5g (16.300 ± 0.495^b).

3.3 Effect of Organic and Synthetic Fungicides in the Control of Aflatoxin Production on the Plant Girth of Maize

Table 3 showed that maize treated with *Azadirachta indica* recorded significant growth in the plant girth at week 4, 6, 8 and 12 for all treatments. Week 2 and 10 showed significant growth in the plant girth except for TAI50% at 9 ± 0.00^b and 19 ± 0.00^b respectively.

For maize treated with lemon grass, week 2 recorded a significant difference in the plant girth for TLg25% (11 ± 0.00^a) and TLg75% (10 ± 0.00^a). Week 4, 6, 8 and 12 showed significant difference in the plant girth for all treatments while at week 10, there was significant growth in the plant girth except for TLg25% (26 ± 0.00^b).

In this study, the result showed that maize treated with synthetic fungicide showed significant growth in the plant girth at week 6, 10 and 12. Week 2, 4 and 8 recorded significant growth in the plant girth except for TSf5g (9 ± 0.00^b), TSf5g (9 ± 0.00^b) and TSf2.5g (14 ± 0.00^b) respectively.

3.4 Effect of Organic and Synthetic Fungicides in the Control of Aflatoxin Production on the Cob Yield of Maize

In Table 4, the cob yield components of maize showed that TAI25% recorded the highest fresh weight (411g) while TLg25% gave a fresh weight of 120g. For the dry weight, TAI25% recorded the highest weight (310g) while the lowest was TLg25% at 73g. The number of cobs per plant recorded 1 for the treatments except in TLg25% and TSf2.5g which gave a record of 2 maize cob per plant. The cob diameter recorded TLg75%, TLg100%, and TSf1.25g as the highest at 8cm while TAI25%, TAI50%, TAI100%, TLg50%, TSf5g, TSf1.25g and TSf0.625g recorded the lowest at 4cm. The cob length gave the highest reading at 13cm (TLg100%) and the lowest at 8cm for TAI75% and TAI100%.

Table 1. Effect of Organic and Synthetic Fungicides in the Control of Aflatoxin Production on the Leaf Area of Maize

S/N	Samples	WK 2 (cm ²)	WK 4 (cm ²)	WK6 (cm ²)	WK8 (cm ²)	WK10 (cm ²)	WK12 (cm ²)
1	TAi25%	5.500±0.707 ^b	24.000±0.00 ^a	24.050±0.354 ^b	26.000±0.00 ^a	26.150±0.212 ^a	28.050±0.0707 ^a
2	TAi50%	10.000±2.828 ^b	15.000±0.00 ^a	20.000±0.495 ^a	21.000±0.00 ^a	23.100±0.141 ^a	24.150±0.212 ^a
3	TAi75%	8.500±0.707 ^b	16.000±0.00 ^a	19.050±0.354 ^b	20.000±0.00 ^a	20.050±0.070 ^a	28.500±0.707 ^a
4	TAi100%	19.000±7.071 ^b	26.000±0.00 ^a	26.000±0.141 ^a	27.000±0.00 ^a	28.500±0.707 ^a	29.200±0.283 ^a
5	TLg25%	5.000±1.414 ^b	12.000±0.00 ^a	15.100±0.141 ^a	18.000±0.00 ^a	19.200±0.283 ^a	26.100±0.141 ^a
6	TLg50%	8.500±0.707 ^b	16.000±0.00 ^a	16.050±0.070 ^b	17.000±0.00 ^a	18.100±0.141 ^a	18.000±0.000 ^a
7	TLg75%	6.500±0.707 ^b	18.000±0.00 ^a	18.550±0.778 ^a	18.000±0.00 ^a	19.100±0.141 ^a	19.000±0.000 ^a
8	TLg100%	8.500±0.707 ^b	18.000±0.00 ^a	20.550±0.778 ^a	21.000±0.00 ^a	21.000±0.000 ^a	22.000±0.000 ^a
9	TSf5g	9.500±0.707 ^b	15.000±0.00 ^a	16.050±0.070 ^b	17.000±0.00 ^a	17.420±0.000 ^a	17.300±0.000 ^a
10	TSf2.5g	13.000±2.828 ^b	14.000±0.00 ^b	15.100±0.141 ^a	16.000±0.00 ^a	16.000±0.000 ^a	16.000±0.000 ^a
11	TSf1.25g	23.500±0.707 ^b	37.000±0.00 ^a	38.150±0.212 ^a	27.000±0.00 ^a	37.000±0.000 ^a	40.000±0.000 ^a
12	TSf0.625g	4.500±0.707 ^b	5.000±0.00 ^a	17.050±0.0707 ^a	18.000±0.00 ^a	18.000±0.000 ^a	18.500±0.000 ^a
13	CONTROL	7.500±0.707 ^b	15.000±0.00 ^b	17.050±0.070 ^a	18.000±0.00 ^a	25.500±0.707 ^a	28.050±0.0707 ^a

Results are in Mean± Standard deviation. Values with a superscript 'a' are significant at 0.05% while values with superscript 'b' are not significant at 0.05%

Table 2. Effect of Organic and Synthetic Fungicides in the Control of Aflatoxin Production on the Plant Height of Maize

S/N	Samples	WK 2 (cm ²)	WK 4 (cm ²)	WK6 (cm ²)	WK8 (cm ²)	WK10 (cm ²)	WK12(cm ²)
1	TAi25%	10.100±0.141 ^b	13.000±0.000 ^a	14.500±0.283 ^b	15.050±0.070 ^b	17.000±0.000 ^a	18.000±0.00 ^a
2	TAi50%	15.050±0.0707 ^b	19.200±0.000 ^a	20.500±0.000 ^a	20.100±0.141 ^b	21.000±0.000 ^a	22.500±0.00 ^a
3	TAi75%	13.050±0.0707 ^b	15.000±0.000 ^a	15.850±0.0707 ^b	15.100±0.141 ^b	16.000±0.000 ^a	17.100±0.00 ^a
4	TAi100%	10.100±0.141 ^b	13.000±0.000 ^a	13.850±0.0707 ^b	13.050±0.070 ^b	17.000±0.000 ^a	20.000±0.00 ^a
5	TLg25%	16.150±0.212 ^b	16.900±0.000 ^a	17.000±0.000 ^b	17.00±0.141 ^b	29.100±0.000 ^a	33.200±0.00 ^a
6	TLg50%	14.100±0.141 ^b	17.800±0.000 ^a	19.900±0.000 ^a	19.000±0.000 ^b	19.500±0.000 ^b	23.500±0.00 ^a
7	TLg75%	12.150±0.212 ^b	18.900±0.000 ^a	18.900±0.000 ^b	18.900±0.141 ^b	24.000±0.000 ^a	28.900±0.00 ^a
8	TLg100%	10.050±0.0707 ^b	12.000±0.000 ^a	16.600±0.000 ^a	16.600±0.141 ^b	19.000±0.000 ^a	28.200±0.00 ^a
9	TSf5g	8.000±0.000 ^a	10.000±0.000 ^a	10.900±0.000 ^a	12.150±0.212 ^a	16.000±0.000 ^a	18.200±0.00 ^a
10	TSf2.5g	10.200±0.283 ^b	14.200±0.000 ^a	16.300±0.000 ^a	16.300±0.495 ^b	17.000±0.000 ^a	27.000±0.00 ^a
11	TSf1.25g	15.050±0.0707 ^b	15.900±0.000 ^a	16.100±0.141 ^b	18.150±0.212 ^a	19.000±0.000 ^a	23.000±0.00 ^a
12	TSf0.625g	15.350±0.495 ^b	16.500±0.000 ^a	16.500±0.000 ^b	18.150±0.212 ^a	26.000±0.000 ^a	29.100±0.00 ^a
13	CONTROL	15.050±0.070 ^b	17.600±0.000 ^a	17.550±0.495 ^b	18.050±0.070 ^b	18.100±0.000 ^b	18.000±0.00 ^b

Results are in Mean± Standard deviation. Values with a superscript 'a' are significant at 0.05% while values with superscript 'b' are not significant at 0.05%

Table 3. Effect of Organic and Synthetic Fungicides in the Control of Aflatoxin Production on the Plant Girth of Maize

S/N	Samples	WK 2	WK 4	WK6	WK8	WK10	WK12
1	TAi25%	12±0.00 ^a	13±0.00 ^a	15±0.00 ^a	16±0.00 ^a	16±0.00 ^a	16.5±0.00 ^a
2	TAi50%	9±0.00 ^b	10±0.00 ^a	13±0.00 ^a	13±0.00 ^a	19±0.00 ^b	22±0.00 ^a
3	TAi75%	14±0.00 ^a	14±0.00 ^a	15±0.00 ^a	15±0.00 ^a	17±0.00 ^a	18±0.00 ^a
4	TAi100%	12±0.00 ^a	13±0.00 ^a	16±0.00 ^a	17±0.00 ^a	17±0.00 ^a	16±0.00 ^a
5	TLg25%	11±0.00 ^a	14±0.00 ^a	18±0.00 ^a	18±0.00 ^a	26±0.00 ^b	28±0.00 ^a
6	TLg50%	9±0.00 ^b	13±0.00 ^a	16±0.00 ^a	18±0.00 ^a	19±0.00 ^a	20±0.00 ^a
7	TLg75%	10±0.00 ^a	11±0.00 ^a	13±0.00 ^a	16±0.00 ^a	18±0.00 ^a	20±0.00 ^a
8	TLg100%	8±0.00 ^b	10±0.00 ^a	13±0.00 ^a	15±0.00 ^a	17±0.00 ^a	18±0.00 ^a
9	TSf5g	9±0.00 ^b	9±0.00 ^b	13±0.00 ^a	15±0.00 ^a	16±0.00 ^a	18±0.00 ^a
10	TSf2.5g	10±0.00 ^a	11±0.00 ^a	12±0.00 ^a	14±0.00 ^b	15±0.00 ^a	17±0.00 ^a
11	TSf1.25g	11±0.00	13±0.00 ^a	16±0.00 ^a	16±0.00 ^a	18±0.00 ^a	20±0.00 ^a
12	TSf0.625g	12±0.00 ^a	15±0.00 ^a	15±0.00 ^a	17±0.00 ^a	19±0.00 ^a	20±0.00 ^a
13	CONTROL	10±0.00 ^a	11±0.00 ^a	12.3±0.00 ^a	13±0.00 ^b	13±0.00 ^b	13.5±0.00 ^b

Results are in Mean± Standard deviation. Values with a superscript 'a' are significant at 0.05% while values with superscript 'b' are not significant at 0.05%

Table 4. Effect of Organic and Synthetic Fungicides in the Control of Aflatoxin Production on the Cob Yield of Maize

S/N	Sample No:	Fresh Weight(g)	Dry Weight(g)	No of cob/plant	Cob Diameter(cm)	Cob Length(cm)
1	TAi25%	411	310	1	4	9
2	TAi50%	251	210	1	4	10
3	TAi75%	301	213	1	6	8
4	TAi100%	410	300	1	4	8
5	TLg25%	120	73	2	6	10
6	TLg50%	312	273	1	4	9
7	TLg75%	412	218	1	8	10
8	TLg100%	321	216	1	8	13
9	TSf5g	201	150	1	4	10
10	TSf2.5g	204	98	2	8	10
11	TSf1.25g	311	290	1	4	12
12	TSf0.625g	240	170	1	4	10
13	Control	330	210	1	5	10

Table 5. Frequency of Aflatoxin-Producing Fungi in Maize Seeds

S/N	Sample No:	<i>Aspergillus</i> spp.	<i>Fusarium</i> spp.	<i>Penicillium</i> spp.	Total Frequency
1	TAi25%	2	0	2	2
2	TAi50%	3	0	2	5
3	TAi75%	3	0	0	3
4	TAi100%	4	0	1	5
5	TLg25%	3	2	1	6
6	TLg50%	3	0	2	5
7	TLg75%	4	2	0	6
8	TLg100%	3	0	2	5
9	TSf5g	2	1	2	5
10	TSf2.5g	0	1	1	2
11	TSf1.25g	2	2	1	5
12	TSf0.625g	2	1	1	4
13	Control	3	2	1	6

3.5 Frequency of Aflatoxin-Producing Fungi in Maize Seeds

Fungal isolates associated with maize observed in this study include: *Aspergillus* spp. *Fusarium* spp. and *Penicillium* spp. (Table 5). *Aspergillus* spp. recorded the highest incidence of occurrence as 4 for TAI100% and TLg75% and the lowest occurrence as 0 for TSf2.5g. For *Fusarium* spp. the highest incidence of occurrence recorded 2 (TLg25%, TLg75%, TSf1.25g and CONTROL) while the lowest was 0 for TAI25%, TAI50%, TAI75%, TAI100%, TLg50% and TLg100%. *Penicillium* spp. recorded TAI25%, TAI50%, TLg50%, TLg100% and Tsf5g as the highest incidence of occurrence as 2 while TAI75% and TLg75% recorded 0 as the lowest. TLg25% had a total frequency of fungal isolates as 6 while TAI25% and Tsf2.5g recorded the lowest at 2.

4. DISCUSSION

In this study, organic fungicides and synthetic fungicides were used as a pre-harvest approach in the control of aflatoxin-producing fungi in maize. The effect of organic and synthetic fungicides in controlling aflatoxin production on the growth parameters of the maize samples were considered. Comparing the effect of organic and synthetic fungicides on the leaf area, it was observed that there was significant growth in the leaf area (for organic fungicide) in all the treatments at week 8, 10 and 12 while it varied for week 2, 4 and 6. The result showed that both plant extracts used in this study were effective in controlling aflatoxin production and hence had a positive effect on the leaf area of the maize samples. This can be seen in the works of Owino and Wando, [27] and Anjorin and Salako, [28]

that phyto-fungicides could be prepared or formulated from the leaves, seeds, stem bark or roots of plants of pesticidal significance and could be applied in form of extract, powders and cakes or as plant exudates. Phytochemical analysis on the leaves of *Azadirachta indica* and *Cymbopogon citratus* showed the presence of alkaloids, saponins, tannins, steroid, terpenoid, glycoside, flavonoid, phenol, oxalic acid, cardiac glycosides and coumarins [20,21]. These findings support the results in this study that plant extracts were more effective in controlling aflatoxin production in the growth of the maize plant than the synthetic fungicide.

In comparing the effect of organic and synthetic fungicide on the plant height, it was observed that there was a significant difference in the plant height (at 0.05%) for week 4, 10 and 12 in all treatments while weeks 2, 6 and 8 showed significant growth in the plant height for some of the treatments. The result showed that both types of fungicides were effective and hence a significant growth in the plant height.

It was also observed that there was significant growth in the plant girth at weeks 4,6 and 12 for all the treatments According to some researchers, plants possess a range of tools for combating fungal infections. Biocontrol agents compete for nutrients and space, may secrete antifungals or even parasitize molds, and can also stimulate host plant resistance [10] and, thereby, they mitigate the risk of plant infections and their undesirable consequences [11,12]. These findings may have led to effective growth in the maize plants undertaken in this research.

A comparison on the effect of organic and synthetic fungicide in controlling aflatoxin

production on the cob yield components of maize, showed that maize treated with *Cymbopogon citratus* recorded the highest weight at harvest while *Azadirachta indica* at 25% concentration had the highest dry weight. The number of cobs per plant recorded 1 for the treatments except for TLg25% and TSf2.5g which had 2. The cob diameter and length varied in the treatments with maize treated with *Cymbopogon citratus* showing the highest cob yield.

Fungal isolates identified in this study were: *Aspergillus* spp. *Fusarium* spp. and *Penicillium* spp. With *Aspergillus* spp. having the highest frequency of occurrence of 4 in treatments TAI100%, TLg75%. This result supports Zorzete et al. [7], that there are many potential toxigenic fungi species that contaminate grain, from which species from *Aspergillus*, *Fusarium* and *Penicillium* genera are dominant species, as contaminants of maize and produces mycotoxins. The contamination of maize by toxigenic fungi and their mycotoxins is the process that can occur yet in the field during harvest and later during the storage until consumption. These fungi pose serious phytopathological and mycotoxicological risks at pre-harvest and post-harvest stages, as well as in processed food products. The result in this study confirms the report given by Ramirez-Camejo et al. [6], that *Aspergillus flavus*, which is widely distributed, is a saprotrophic/pathogenic fungus and cereal grains, tree nuts, and legumes are known best for their colonization.

5. CONCLUSION AND RECOMMENDATION

The effect of the organic fungicide on the growth parameters showed that maize treated with *Azadirachta indica* was the most effective in the control of aflatoxin production in maize. From the study, the result showed that both the organic and synthetic fungicides were effective in the control of aflatoxin-producing fungi on the maize yield but was more effective with the organic fungicide. The use of *Azadirachta indica* and *Cymbopogon citratus* is recommended as a means of biofungicides to control aflatoxin-producing fungi. Much attention should be given to medicinal plants to explore their effectiveness in the control of organisms that contaminate food materials and help to reduce food shortages in the world over. This study also recommends that maize seeds should be treated with *Azadirachta indica* and *Cymbopogon citratus* before planting

as they are excellent bio-fungicides and bio-pesticides. In adapting the methods used in this study in the control of aflatoxin-producing fungi, considerations should be given to the type of fungicide, fungicide application, different concentration, *Azadirachta indica* and *Cymbopogon citratus* leaves extract as this had variations in controlling the test organism. This study recommends that extensive research should be carried out on other plant crops which are prone to aflatoxin contaminations and provide solutions. Also, there should be a continual awareness of the insidious activity of aflatoxin-producing fungi and its implication in human health [29].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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