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Comparative Studies on The Efficacy of Some Fungicides Activities on Fungal Seed Borne Pathogens of Commercial Maize Seeds

Egbo Nwakaku Jane[1], Ugoh Sylvester Chukwudi[1], Olisa Babafemi Sunday[1]*
1) Department of Microbiology, University of Abuja, FCT, Nigeria.
**) Corresponding Author: janenwakaku@gmail.com*

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Abstract*— This study investigated the efficacy of some fungicides activities on fungal seed borne pathogens of two commercial maize seeds (Sammaz 15 and Sammaz 52). A total of 2500 seeds were randomly picked for each variety to represent a working sample from the seed reference collections. Out of the 2500 seeds, 400 seeds were surface–sterilized with 10% sodium hypochlorite solution, cultured and examined microscopically for the presence of fungal pathogens. Molecular analysis confirmed that all isolated pathogens matched with the reference accession from NCBI molecular database. Seeds were treated with different dosages of fungicides viz Thiram and Metalaxy + Tebuconazole and incubated for 5 days. The effect of the various fungicides on the incidence of seed- borne fungi, effect on seed germination and interaction of the fungicide were evaluated and analyzed. Percentage occurrence of the fungal pathogens revealed that Sammaz 15 have F. solani (29.0%), A. flavus (20.3%), A. niger (19.0%) and Rhizpus stolanifera (15.0%). In Sammaz 52, A.niger (40.0%), A. flavus (19.7%) and F. solani (27.0%). It was observed that F. solani was predominant in sammaz 15 while A. niger was predominant in Sammaz 52. Thiram (45.88 mm) at all doses showed greater inhibitory effects on mycelia growth than Metalaxyl + Tebucanozole (24.25 mm). The correlation analysis performed on the data set reveals that there is a negative significant correlation between zone of inhibition and percent disease incidence (r = -0.99), but the relationship was positive with number of non-infected seeds (r = 0.99***). This shows that increasing the zone of inhibition will increase the number of non-infected seeds and the normal seedling (germination percentage). Thiram fungicides observed to be more effective in reducing the disease incidence than Metalaxyl fungicides.***

Keywords*— Commercial maize seeds, Fungal seed borne pathogen, Fungicides

I. INTRODUCTION

Seed is one of the main inputs in agriculture, and its quality is one of the primary factors for establishing any crop. Seed as the only living input has the potential of transmitting the seed borne pathogens, therefore, sowing contaminated or infected seeds can reduce seed quality such as germination potential, seedling vigour and potential yield by transmitting pathogens from seed to plant. This underscores the importance of seed treatment in controlling seed borne pathogens and maintaining seed quality [1].

Seed treatment has been used as a tool for protecting seeds in the field and in storage for the purpose of maintaining physiological quality [2], and it is a valuable method for controlling and/or preventing pest and pathogen attacks. Lack of this initial protection can have a direct impact on yield. Chemical treatment consists of incorporating artificially developed chemical products on the seeds. This modality has been increasingly adopted by farmers since it is easy to perform and can be practiced in a controlled environment through the ease of uniformly distributing small amounts of products in growing areas, through reduced need for complementary applications of pesticides on developing crops, and through its low relative cost, which, even so, provides significant increases in final yield. Some factors affect the performance of the seed chemical treatment, such as type of seed, physical and physiological condition of the seed lot to be treated, seed size, product formulation, active ingredient, and application rate of the product [2].

Inadequate application of chemical products on seeds can increase risks of deterioration of their physiological quality due to possible phytotoxic effects and disease infestation [3] evaluated the effect of treatment of maize seeds with insecticides in different storage periods and concluded that seed treatment with insecticide and storage affect seed germination. According to these authors, the interaction of seed treatment and storage affect the number of germinated seeds. In contrast, the advantages of using seed with protection against external biological agents, such as fungi, insects, nematodes, etc. are well-known [4]. The fact that chemical treatment of maize seeds is a widely used practice before storage and near the time of sowing highlights the importance of conducting studies on the chemical products used for treatment, as well as their effect on the quality of seeds during storage and after storage.

Seed-borne diseases are plant diseases that are transmitted by seed. Planting seed that is free of seed-borne pathogens is the primary means of limiting the introduction of pathogens, especially new pathogens into a field. The seed-borne pathogens may result in loss of germination, discoloration, shriveling and

development to plant diseases and toxin production in infected seeds. Control of seedling diseases is a major priority in many cropping systems. In practice seed borne pathogens could be managed/controlled through seed testing, quarantine and treatment [5]

Seed treatments are defined as chemical or biological substances that are applied to seeds or vegetative propagation materials to control disease microorganisms, insects, or other pests. Seed treatment pesticides include bactericides, fungicides, and insecticides. One of the main reasons for seed treatment with fungicides is the control of microorganisms. Most seed treatments are applied to true seeds, such as maize, wheat, or soybean, which have a seed coat surrounding an embryo. However, some seed treatments can be applied to vegetative propagation materials, such as bulbs, corms, or tubers (such as potato seed pieces). Seed treatments ensure uniform plant/crop stand establishment by protecting against insects, soil-borne and seed borne pathogens. Seed treatments have had phenomenal success in eradicating seed-borne pathogens, such as smut or bunt, from wheat, barley, and oats. Seed treatments can be used to suppress root rots in certain crops. Seed-borne pathogens are vulnerable; the seed borne phase is often the weak link in the lifecycle for many plants pathogen thus using fungicide seed treatments to control seed-borne pathogens are often very effective for disease control and precision targeting. The maize seed treatment has traditionally been accomplished with some fungicides, captan and thiabendazole being the most used products. Recently other products have been commercialized, such as fludioxonil, metalaxyl and tolylfluanid, with a wider spectrum of active ingredients. The use of mixtures of products with complementary mode of action for treating seeds has been a strategy, which in addition to expanding range of pathogens to be controlled prevents development of resistance by population of target-microorganisms of these products [2].

Nigeria and indeed most African countries uses fungicides seed treatment products more compared to other pesticides chemicals. The treatment of maize seeds with fungicide is a practice that is becoming more important and common among agricultural producers by the direct reflexes on health and physiological quality of seeds that are intended for sowing. However, most of the problems of farmers lie in the product choice and dosage applied [2]. Methods of control vary considerably from one disease to another depending on the kind of pathogens, the host and the interaction of the two. The various control methods could be generally classified as regulatory, cultural, biological, physical and chemical depending on the nature of the agent employed to control the disease [1].

The use of mixtures of products with complementary mode of action for treating seeds has been a strategy, which in addition to expanding range of pathogens to be controlled prevents development of resistance by population of target-microorganisms of these products [2]. However, inadequate application of fungicide products on seeds can increase risks of deterioration of their physiological quality, due to possible phytotoxic effects and poor performances of some of the chemical products. The aim of this study is to determine the efficacy of some fungicides activities on seed borne pathogens of commercial maize seed.

The objectives of the research are to: isolate and identify seed borne fungi pathogens associated with two commercial maize seed varieties produced in Nigeria, characterize the isolates by molecular method, determine the activities of the fungicides on the pathogen species, and determine the interactive effect of fungicide on the performance of maize seed lots.

II. Materials And Methods

A. Study Area

This research was carried-out at the Seed Health Unit, Central Seed Testing Laboratory of National Agricultural Seeds Council (NASC). NASC is located on Km 29, Abuja-Lokoja Highway, Sheda, Kwali Area Council Abuja, Federal Capital Territory (FCT), Nigeria.

National Agricultural Seeds Council is a Government Agency charged with the overall development and regulation of the National Seed Industry. Central Seed Testing Laboratory is a standard laboratory established by the agency for the purpose of quality assurance, seed testing and agricultural research. The Laboratory was designed and equipped to meet the International Standard of Seed Testing.

Kwali is a local Government area in the Federal Capital Territories in Nigeria. It has an area of 1,206 km2 and a population of 85,837 at the 2006 census. Its geographical coordinates are 8° 52’ 2” North, 7° 0’ 18” East. It is notable for Agriculture and pottery making which also elevate an indigene of the area, Ladi Kwali to global recognition and honour.

Fig. 1. Image of *Guibourtia coleosperma* seeds collected from Shakawe, Botswana

B. Source of Seed

The first seed samples were randomly collected by gathering Two (2) Varieties of Commercial maize seeds (Sammaz 15 and Sammaz 52) were collected from Seed Reference unit submitted by seed companies actively involved in seed production..

C. Sample Collection and Disinfection

Two thousand five hundred (2500) maize seeds each of the two varieties, Sammaz 15 and Sammaz 52 of open pollinated commercial maize seeds were obtained from commercial seed companies that are actively involved in seed production. The Samples were collected in polyethylene bags according to International Seed Testing Associations [9] methods and stored at 4ºC until needed for further analysis [6].

D. Asparatus, Reagent, and Media

Moisture content

The apparatus used were seed mixer and divider, autoclave, spectrophotometer, microscope, plastic pipette, wire loop, petri dishes, beaker, conical flask, laminar flow chamber, Erlenmeyer flasks, incubator, aluminum foil, forceps, planting tray, sterilized river sand, cork-borer, test tubes etc. While the media used were Potatoes Dextrose Agar (PDA), Muller Hinton Agar and potatoes dextrose broth.

E. Media Preparation and Sterilization

All the glass wares used were sterilized in the autoclave at 121ºC for 15 minutes. Ethanol (70%) was used to swab the workbench and to sterilize other apparatus. Forty grams (40gm) of PDA was weighed and distilled water was added. The media was than autoclaved at 121ºC for 15 minutes, allowed to cool to about 45ºC before the addition of 50mg of Chloramphenicol.

F. Isolation and Identification of Seed Borne pathogen

Agar method was used for the isolation and detection of seed borne fungi following ISTA rules for seed testing [7]. In this method, four hundred (400) seeds were counted from the two maize seed varieties respectively after thorough mixing. The Seeds were surface - sterilized by dipping them in 10% aqueous Sodium hypo-chlorite solution for 2 minutes. Thereafter, the Seeds were rinsed with distilled water thrice and dried on paper towel. Potatoes Dextrose Agar (40 g) was prepared according to manufacturer's instructions, allowed to cool to about 45ºC before the addition of 50 mg of Chloramphenicol. It was then poured into 9cm plastic petri dishes aseptically and allowed to solidify. Ten (10) seeds per plate in four (4) replications were placed on medium for each of the varieties as recommended by International Rules for Seed Testing Association [7]. The plates were sealed with parafilm wax and incubated for 5 days at 25ºC under 12 hrs alternating light and darkness. After incubation, each individual incubated seeds were examined under Stereo-binocular Microscope at 16x and 25x magnifications according to [8] for growth habit of associated fungi pathogens. The identification of the isolates was based on morphological and sporulation characteristics. Morphological identification was done examining under compound microscope. The colony appearance, texture and pigmentation on the plates were observed [9]. Data were recorded as number of germinated seed (%), number of non-germinated seeds, number of infected seeds (%) and name of pathogens isolated (%).

Obtaining pure culture

The fungal pathogens identified during the examinations were sub-cultured onto a fresh plate of Potato Dextrose Agar (PDA). Distinct fungal colonies from the primary cultures were cut using a sterile blade and was transferred aseptically into the freshly prepared PDA plates. The plates were incubated for 5 days at 25ºC under 12 hours alternating light and darkness to obtain the pure cultures [10]. The plates were observed daily for contaminations. Pure isolates of single species were obtained from the two maize varieties and preserved for further studies. The isolates were identified by microscopy and morphological structures. The PDA used were prepared according to the manufacturer's instructions and the pure cultures were preserved in refrigerator at 5ºC until used.

Molecular analysis

DNA extraction procedure

The fungal cells (approximately 100 mg, mycelia) were harvested from the culture vessel into sterile mortar and ground with pestle in 1000µL/ 1ml of DNA Extraction Buffer (DEB) containing proteinase K (5µl, 0.05mg/ml). The ground sap was transferred into 1.5 ml Eppendorf tube, 50 µl of 20% Sodium Dodecyl Sulphate (SDS) was added and briefly vortex mixed. The mixture was then incubated in a water bath at 65°C for 30 minutes to complete cell lyses and the tubes were allowed to cool to room temperature.

Equal volume (600ul) of a mixture of Chloroform and Isoamyl alcohol (24:1) were then added to each sample, thoroughly vortex mixed and centrifuged at 13000 rpm for 10 minutes. From different phases, 450ul supernatant of the aqueous layer was pipetted into a new 1.5ml Eppendorf tube and 300ul isopropanol was added, briefly mixed by gentle inversion 5-10 times before incubated in the freezer at -20 °C for 1hour to allow DNA precipitation. This was later centrifuged at 13000 rpm for 10 minutes and the supernatant discarded. DNA pellet was observed and 500ul of 70% ethanol added to wash and centrifuged again at 13000 rpm for 5 minutes and the supernatant was finally discarded. The DNA pellet was kept to dry in 37ºC incubator and finally dissolved in 50ul sterile distilled water and store at -20°C for further lab analysis.

Quantity and quality check

The DNA extracted was checked on a Nano drop spectrophotometer, model 2000 from Thermo-Scientific to quantify the concentration of the extracted DNA and also determine purity by measuring at 260/280 nm amount of protein contaminants left. Then, the DNA was used as template for PCR amplification assay.

Polymerase Chain Reaction (PCR) amplification

Amplification was carried out using PCR system thermal cycler (Applied Biosystem Inc., USA) with PCR profile of an initial denaturation, 94°C for 5 min; 35 cycles of 94°C for 30s, 55°C for 30s and 72 °C for 1 minute 30 seconds; and a final extension at 72 °C for 10mins.

Purification of amplified PCR product

The amplified DNA fragments of target sequences in PCR were ethanol purified in order to remove the PCR reagents. Briefly 7.6µl of Na acetate 3 M and 125 µl of 95% ethanol were added to each about 50µl PCR amplified products in a new sterile 1.5µl tube Eppendorf, mixed by simple inversion and stored at -20°C for 30 min. The tubes were centrifuged for 10 min at 13000 g followed by removal of supernatant after which the pellet was washed by adding 500µl of 70% ethanol and mix then centrifuge for 15 min at 7500g and 4°C.

Pellet were washed repeatedly with 70% ethanol and centrifuged. Tubes were inverted on paper tissue to tap dry and left further to dry in the incubator set at 37°C for 25 min. then re-suspend with 25µl of sterile distilled water and kept in -20°C. The presence or absence of expected band size of amplified target ITS gene sequence was checked when purified fragment was run on a 2% agarose gel electrophoresis at a voltage of 100 V for 1 hr. It was viewed under UV light and picture was taken.

Sanger sequencing and analysis

The purified PCR products were subjected to sanger sequencing using the following reaction mixture: Amplified PCR Product 10 µl, ExoSAP Mix 2.5µl, mixed well and incubated at 37°C for 30 min and the reaction was stopped by heating the mixture at 95°C for 5 min. Fragments were sequenced using the Nimagen, Brilliant Dye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000 according to manufacturer's instructions. The labelled products were then cleaned with the ZR-96 DNA Sequencing Clean-up Kit (Catalogue No. D4053). The cleaned products were injected on the Applied Biosystems ABI 3500XL Genetic Analyzer with a 50 cm array, using POP7 and Sequence chromatogram analysis is performed using BioEdit analysis software.

DNA quantity and quality assessment

This assessment confirmed good quality DNA were extracted from fungal isolates. The amount of DNA extracted was read in concentration of ng/µl and at 260/280 nm permissible amount of protein impurities that will not hinder success of polymerase chain reaction was measured which is between a range of 1.7-2.1 while 260/230 nm measured the permissible level of alcohol solvent used in the washing of the DNA also at a range of 1.8-2.1. BLAST (Basic Local Alignment Search Tool) analysis was used to match the % identity of sequences obtained from this study to those already submitted to the database. The true identity of isolated organisms as identified by conventional method was confirmed by molecular method.

Experimental layout

A completely randomized experimental design were used for all experiments, with treatments arranged into a factorial scheme of 64 blocks replication of (2 varieties x 2 fungicide type x 4 rep x 4 treatment dosages: 50%, 100%, 150% and 200%).

TABLE 1. LIST OF SEED TREATMENT CHEMICAL COMBINATION IN THIS STUDY

S/N	Trade Name	Chemical Combination	Chemical Group	Mode of Action
1	Dress force	20% Metalaxy 20% Imidacloprid 2% Tebuconazale (WS)	Acylalanines-Metalaxy	Fungicide: Systemic Action Pesticide: Systemic Action Systemic, preventive, curative, eradicative action
2	Seed care	10 % Thiram-10% WS 20% Imidacloprid	Acylalanines-Thyram	Fungicide: Systemic Action Pesticide: Systemic Action

Efficacy of fungicides seed treatment for the control of seed borne pathogens of maize seed.

According to International Seed Testing Association [9] methods, Four hundred (400) seeds were counted from the two maize seed varieties (Sammaz 15 and Sammaz 52) respectively and placed inside 125 ml sterile Erlenmeyer flask each containing 50%, 100% 150% and 200% doses of fungicides. Table (2) shows the preparation of fungicides at different doses. Seeds from each varieties were treated with the two different fungicides at different dosages (50%, 100%, 150% and 200%) respectively. The Erlenmeyer flasks containing different doses of fungicide were sealed with aluminum foil and placed onto a rotary shaker for 24hours. Each variety of the treated seeds at different doses were plated at 10 seeds per plate in four replications using standard potatoes dextrose agar (PDA) (Difco, 39 g/l) medium supplemented with 50 microgram of Chloramphenicol in 9 cm plastic Petri dishes as recommended by [9] and plates were incubated for 5 days at 25 ºC under 12 hours alternating light and darkness. After incubation, fungal growths on the seed samples were examined microscopically and the effects of the fungicides for the control of seed borne pathogens were observed. The incidence of a particular pathogen on the tested seed lots originating from a given treatment and dose were calculated by dividing the number of infected seeds by the total number of the tested seeds and multiplying the obtained ratio by 100.

$$Level\ of\ incidence = \frac{number\ of\ infected\ seeds}{total\ number\ of\ seed\ plated} x\ 100$$

TABLE 2. PREPARATION OF FUNGICIDES AT DIFFERENT DOSES

S/N	Doses (%)	Number of seed	WT. of fungicides (g)	Conc. Of water (Ml)
1	50	400	0.24	2
2	100	400	0.48	2
3	150	400	0.72	2
4	200	400	0.96	2

G. Interactive Effect of Fungicide on The Performance of Maize Seed Lots

One hundred treated seeds in four replications each at different dosages (50%,. 100%, 150% and 200%) of the experimental maize seed samples of the two varieties (Sammaz 15 and Sammaz 52) were planted in a clean plastic germination tray containing sterilized river sand and placed in the germination bench for 7 - 14 days according to [9]. Untreated seeds of the different varieties were used as the control. The interactive effect of fungicide seed treatment on seed-to-seedling disease transmission was assessed on maize seedling on 7 and 14 days after planting (dap). Growth of each replications were monitored daily. At the end of the assessment date, seedlings of each treatment were carefully removed from the container, washed with water to remove soil from the radicle and blotted dry in sterile paper towels. After washing, primary radicle, length of coleoptile and shoot lengths (mm) were measured with a ruler and results recorded.

H. Determination of Zone Of Inhibition for Fungicidal Activity

To determine the fungicidal effect on the seed borne pathogen of maize seed lots, Agar well method was used. A

suspension of the pure culture of each isolated fungi pathogens were spread evenly over a potatoes dextrose agar contained in a 9 cm plastic Petri dishes and allowed to dry. After drying, a hole was punched aseptically with a 10.5 mm sterile cork borer at the centre of the agar. Different concentrations (50%, 100%, 150%, and 200%) of the fungicides were poured inside the wells on different Petri dishes. The agar plates were incubated for 5 days at 25 ºC under 12hrs alternating light and darkness. The clear zone (zone of inhibition) that appeared around the test product was measured and results recorded. Samples with strong fungicidal activities formed a larger zone of inhibition and those with lesser zone of inhibition indicates weaker fungicidal activities.

I. Statistical Analysis

Analysis of variance (ANOVA) was conducted on data generated from variables measured in each growth chamber experiment. Least significant different (LSD) was used to separate the means from each treatment and within each sampling date. Contrasts was used to compare the treatment effect of different fungicide groups on measured variables. Data analysis was conducted using the General Linear Model procedure (PROC GLM) of SAS version 9.1 (SAS Institute, 200).

III. Results And Discussion

A. Identification of Pathogen Species and Molecular Analysis

Pathogen isolated from Sammaz 15 includes Fusarium solani with percentage occurrence of 29.0%, followed by Aspergillus flavus (20.3%), Apergillus niger (19.0%) and Rhizopus stolonifer (15%). Among others, F. solani was more predominant when compared with the remaining pathogens. Pathogen isolated from Sammaz 52 include A. niger, F. solani, and A. flavus while A. niger was more predominant with percentage occurrence of 40.0%, followed by F. solani with percentage occurence of 27.00% (Table 3).

TABLE 3. FREQUENCIES OF OCCURRENCE OF THE ISOLATED PATHOGEN SPECIES AND TOTAL PERCENT DISEASE INCIDENCE

	Species				Total Disease
Variety	*Fusarium solani*	*Aspergillus niger*	*Aspergillus flavus*	*Rhizopus stolonifer*	
Sam-maz 15	29	19	20.3	15	83.3
Sam-maz 52	27	40	19.7	-	86.7
Mean ±s.e	28±1	29.5± 10.5	20±0.3	15	85.0± 1.7

TABLE 4. PERCENT IDENTITY OF ISOLATED SPECIES AFTER BLAST MOLECULAR ANALYSIS

S/n	Organism (Species)	Percent Identity	Accession number
1	*Aspergillus flavus* isolate LUOHE	99.83%	MT645322.1
2	*Aspergillus niger* voucher MSR3	98.75%	KJ881376.1
3	*Fusarium solani* isolate SY1	99.43%	MT605584.1
4	*Rhizopus stolonifer* strain Jus7	99.27%	MW990049.1

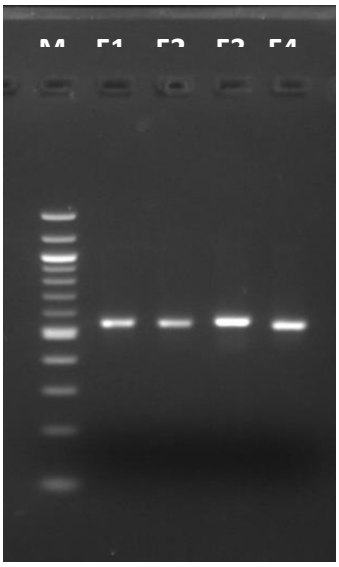

Fig. 2. Gel picture and resolution of amplified PCR products on agarose gel electrophoresis

M- 100bp DNA ladder, 1-F1, 2-F2, 3-F3 and 4- F4 which confirmed that ITS1 forward and ITS4 reverse primers amplified the internal transcribed spaced gene that is universally present in all fungi samples submitted for molecular identification (Fig. 2).

B. Fungicidal activities on the percent disease incidence

Higher doses of the fungicides showed greater inhibitory effects. Thiram (45.88 mm) at all doses showed greater inhibitory effects on mycelial growth than metalaxyl + Tebuconazole (24.25 mm) (Table 4).

When this was expressed in ratio of the inhibited zones to the total area of disease incidence, similar results was obtained. Although this study did not partition the effect of the fungicide on the individual pathogen species but it was observed that Metalaxyl + Tebucodazole resulted into 21.83% inhibition of the sclerotia growth of the fungi while Thiram resulted into 41.29% inhibition of the sclerotia growth of the pathogens (Table 5).

Correlation analysis performed on the data set also reveals that there is negative significant correlation between zone of

inhibition and percent disease incidence (r = -0.99***), but the relationship was positive with number of non-infected seeds (r = 0.99***). Similarly, significantly positive correlation was observed between percent disease incidence and non-inhibited disease zone (r = 0.99***) (Table 6).

In relation to this, percent non-infected seeds positively correlating with the zone of inhibition shows that, increasing the zone of inhibition will increase the number of non-infected seeds and the normal seedling (germination percentage) (Table 5).

TABLE 4. EFFECT OF FUNGICIDES FORMULATIONS (ZONE OF INHIBITION) ON THE MYCELIAL (RADIAL) GROWTH OF THE PATHOGEN

Dose	Mycelial growth inhibition (mm)	
	Metalaxyl + Tebuconazole	Thiram
1 (50%)	6.0a	36.50a
2 (100%)	21.0a	43.50a
3 (150%)	30.0b	48.50a
4 (200%)	40.0a	55.00a
Mean ± s.e	24.25±18.56	45.88±11.08

Means followed by different letters in a row are significantly different according to LSD p ⩽ 0.05. Values represents mean ± LSD.

TABLE 5. ANALYSIS OF ZONE OF INHIBITION AND PERCENT DISEASE INCIDENCE

	Metalaxy+ Tebuconazole						fungicide
Dose	Disease Incidence (%)	Number of non-infected seed (%)	Zone of Inhibition (%)	Non-Inhibited Zone (%)	Disease Incidence (%)	Number of non-infected seed (%)	Zone of Inhibition (%
1 (50%)	66.67	33.33	5.40	94.60	70.00	40.00	32.85
2(100%)	56.67	43.33	18.90				39.15
3(150%)	46.67	53.33	27.00				43.65
4(200%)	33.33	63.33	36.00				49.50
Mean ±s.e	50.84±7.12	48.33±6.46	21.83±6.49	78			41.29±3.5

TABLE 6. CORRELATION ANALYSIS FOR DISEASE INCIDENCE (%), NUMBER OF NON-INFECTED SEED (%), ZONE OF INHIBITION AND DISEASE RESISTANT ZONE (%)

	Percent Disease incidence	Number of non-infected seeds
Number of non-infected seeds	-0.99***	
Zone of inhibition	-0.99***	0.99***
Non-inhibited zone	0.99***	-0.99***

N = 8, *** significant at the p ⩽ 0.001 probability level, - = negatively correlated, + = positively correlated

C. *Efficieny of the Fungicidal activities on the Pathogen Species*

Regression analysis conducted to evaluate the response of the pathogen to fungicides is presented in figure 1, 2, 3 and 4. For Sammaz 15 after been treated with Metalaxy+ Tebuconazole fungicides and Thiram, it was revealed that R. stolonifer (for Metalaxy+ Tebuconazole) and Aspergillus flavus (for Thiram fungicide) were more significantly affected with corresponding regression equation of Y = 0.5x2 - 5.6x + 20.2; R^2 = 0.98*** and Y = 0.8429x2 – 7.517x + 26.46; R^2 = 0.97** (Figure1 and 2). For Sammaz 52 treated with Metalaxy+ Tebuconazole, A. flavus with regression equation of Y = 0.67x2 - 6.37x + 25.26; R^2 = 0.99*** was more responsive when compared with F. solani (with regression equation of Y = 0.2857x2 – 5.6143x + 31.6; R^2 = 0.96*** which responded slowly to the Thiram Fungicide treatment (figure 3 and 4). Similarly when the seeds were treated with Thiram fungicide, F. solani (with regression equation of Y = 0.04x2 – 3.96x + 30.8; R^2 = 0.99***) responded slowly to the fungicidal activities when compared with A. niger with regression equation of Y = 1.41x2 – 13.55x + 50.36; R^2 = 0.91***.

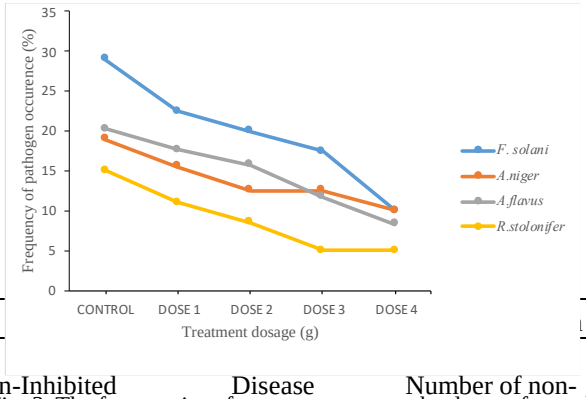

Fig. 3. The frequencies of occurrence across the dosage for each pathogen isolated from Sammaz 15 treated with Metalaxyl + Tebuconazole fungicide

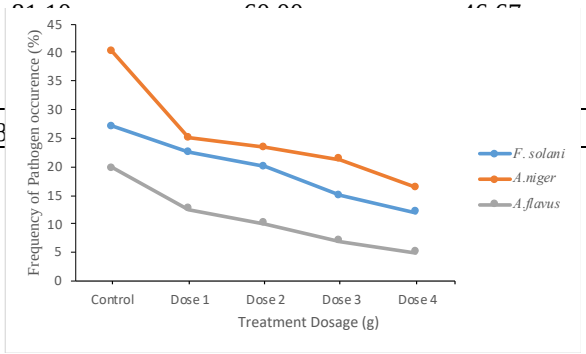

Fig. 4. The level of infection across the dosage for each pathogen isolated from Sammaz 15 treated with Thiram fungicide

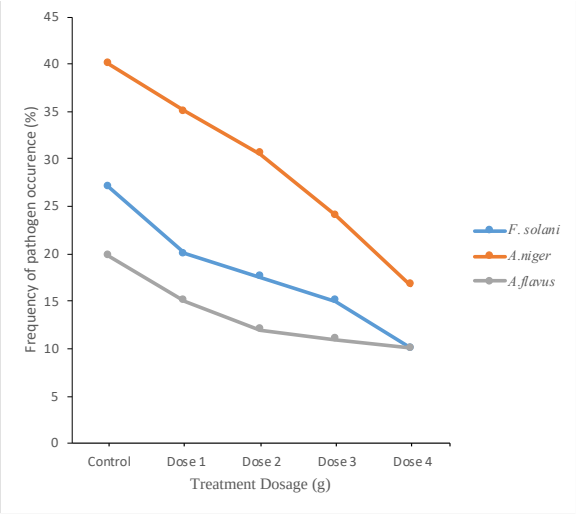

Fig. 5. The level of infection across the dosage for each pathogen isolated from Sammaz 52 treated with Metalaxyl + Tebucoldazole fungicide

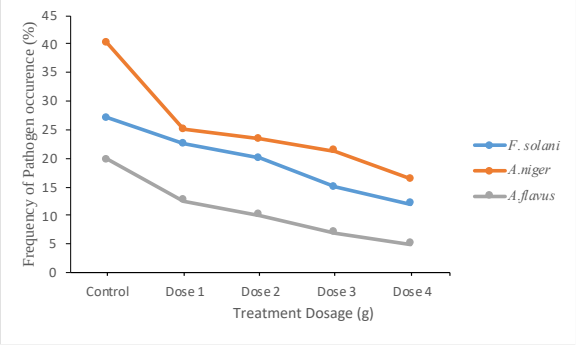

Fig. 6. The level of infection across the dosage for each pathogen isolated from Sammaz 52 treated with Thiram fungicide

D. Interaction among Fungicidal Activities, Percent Disease Incidence and Seedling Performance

Analysis on Sammaz 15 treated with either of Metalaxy+ Tebuconazole (Imidacloprid-20% + metalaxyl-M20% + Tebuconazole 2%WS) and Thiram fungicides (Imidacloprid-10% + Thiram-10%WS), shows that apart from the number of root and the shoot length, the mean values for other traits differs significantly across the levels of Doses (Table 7). However, among percentage the traits, the coefficient of variations (CVs) for normal seedling (germination), root length, number of root, shoot length and percent disease incidence were lower than 10%. The R2 for abnormal seedling, root length, dead seeds, normal seedlings and percent disease incidence were relatively high with corresponding values of 71.15, 72.80, 81.45, 89.49 and 95.20%.

Mean values for normal seedling ranged from 81.33% in Dose 3 to 94.00% in Dose 1. Number of Dead seeds ranged between 1.67% in Dose 1 to 14.67% in Dose 3. Root length ranged from 17.89 (Dose 4) to 26.00 (untreated seeds –Dose 0), Shoot length ranged between 8.24 in Dose 4 and 9.66 in Dose 2 while percent disease incidence ranged from 33.33% (Dose 4) to 83.33% on untreated seed lots (Table 8).

Similar trend was observed among seed lots from Sammaz 52 (Table 7), the mean values vary significantly in normal seedlings, dead seed, root length, shoot length and percent disease incidence among the Doses. Analysis of variance conducted on the treatment, for normal seedling, abnormal seedling and dead seeds shows significant difference. Also significant interaction was also observed between Dose and treatment for normal seedling, and dead seed. The CVs for normal seedling (germination percentage), root length, number of root and shoot length and percent disease incidence were lower than 10% while the R2 for root length, shoot length, abnormal seedlings, dead seeds, normal seedling and disease incidence was high with corresponding values of 70.36, 71.08, 78.22, 94.30 and 94.61%. For Metalaxy+ Tebuconazole, the mean values for normal seedling ranged from 88.00% in Dose 0 to 96.67% in Dose 2. Root length ranged from 19.11 (Dose 4) to 26.00 in Dose 0 for Metalaxy+ Tebuconazole. For Thiram fungicide, the mean values for normal seedling ranged from 86.00.00 % in Dose 4 to 93.33% in Dose 1; abnormal seedling ranged from 0.00 (Dose 1 and 2) to 4.00 (Dose 4), dead seed ranged from 8.00 (Dose 0) to 10.00 (Dose 1 and 2) while percent disease incidence ranged from 33.33% (Dose 4) to 86.68% on untreated seed lots form Dose 0 (Table 9).

TABLE 7. ANALYSIS OF VARIANCE FOR THE SEEDLING PERFORMANCE AND DISEASE INCIDENCE FOR SAMMAZ 15 WHEN TREATED WITH FUNGICIDES THIRAM AND METALAXYL.

Source of variation	Df	Mean square						
		Normal Seedling	Abnormal seedling	Dead	Root Length	Number of Root	Shoot Length	Disease Incidence
Rep	2	0.40	4.63	3.23	0.43	0.38	0.37	23.33
Dose	4	82.97***	3.87	65.72***	43.93***	0.39	0.95*	2053.33***
Treatment	1	124.03***	32.03***	22.50*	0.44	0.10	0.28	53.33
Dose *Treatment	4	26.03***	2.70	34.11***	6.94	0.01	0.34	3.33
CV		2.15	41.59	28.16	9.18	9.32	5.81	8.23
R^2(%)		89.49***	71.15***	81.45***	72.80**	39.89	56.15	95.20***

*, ** and *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively

TABLE 8. ANALYSIS OF VARIANCE FOR THE SEEDLING PERFORMANCE AND DISEASE INCIDENCE FOR SAMMAZ 52 WHEN TREATED WITH FUNGICIDES THIRAM AND METALAXYL

Source of variation	Df	Mean square						
		Normal Seedling	Abnormal seedling	Dead	Root Length	Number of Root	Shoot Length	Disease Incidence
Rep	2	2.10	0.63	0.40	11.30	0.83	0.24	30.00
Dose	4	249.80***	16.00***	146.00***	31.98**	0.20	0.26	2328.33***
Treatment	1	104.53***	36.30***	20.83*	35.21*	0.86	15.95***	83.33
Dose *Treatment	4	118.89***	2.80	125.67***	17.55*	0.48	1.42	8.33
CV		2.63	46.62	23.17	11.29	8.37	8.71	9.60
R^2(%)		94.30***	78.22***	92.71***	70.36***	37.24	71.08	94.61***

*, ** and *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively

TABLE 9. MEAN VALUES FOR THE SEEDLING PERFORMANCE AND DISEASE INCIDENCE FOR SAMMAZ 15 AFTER BEEN SUBJECTED TO THE FUNGICIDES METALAXY+ TEBUCONAZOLE; AND THIRAM FUNGICIDE

Variety	Dose	Metalaxyl + Tebuconale							Thiram						
		NOR	ABN	DEAD	RLT	NRT	STL	PDI	NOR	ABN	DEAD	RTL	NRT	STL	PDI
Sam 15	0	88.00b	4.33a	7.67b	26.00a	4.67a	9.22abc	83.33a	88.00c	4.33a	7.67ba	26.00a	4.67a	9.22a	83.33a
	1	94.00a	4.33a	1.67c	23.94bc	4.67a	9.33ab	66.67b	93.33ab	0.67b	7.33ba	21.33bc	4.44a	8.67a	70.00b
	2	89.00b	3.33a	7.67b	23.91bc	5.12a	9.66b	56.67bc	94.67a	1.33b	4.00b	21.78bc	5.00a	8.94a	60.00c
	3	81.33c	4.00a	14.67a	21.13bc	5.17a	8.52bc	46.67c	90.00bc	2.00ab	8.00ba	23.67ab	5.11a	8.56a	50.00d
	4	82.00c	4.00a	14.00a	17.89c	5.06a	8.24c	33.33d	88.67c	1.33b	10.00a	18.89c	4.89a	8.61a	36.67e
Average		86.87	4.00	9.13	22.58	4.94	8.99	57.33	90.93	1.93	7.40	22.33	4.82	8.80	60.00

*Values in a column with different letters are significantly different at $P<0.05$
NOR = Normal seedling (%); ABN = Abnormal Seedling; DEAD = Dead seed; STL = Shoot length (cm)
NRT = Number of Root; RTL = Root length (cm); PDI= Percent Disease Incidence

IV. CONCLUSIONS

In conclusion, *Fusarium solani*, *Aspergillus flavus*, *Apergillus niger*, and *Rhizopus stolonifera* were isolated and identified from the two maize seed varieties. It is apparent that *F. solani* was more predominant when compared with the remaining pathogen. The fungicides reduce the percent disease incidence by inhibiting the mycelia growth of the fungi. Thiram fungicide is more effective in reducing the disease incidence than metalaxyl fungicide. The analysis of the zone of inhibition reveals that approximately 21.83% and 41.29% of the zone of disease incidence were inhibited by metalaxyl + Tebuconazole, and Thiram fungicides respectively. The fungicides affect the frequencies of pathogen species disease incidence differently. Across the doses, Thiram fungicide enhance physiological performance of maize seed in terms of germination and seedling vigour to a threshold compared to metalaxyl + Tebuconazole fungicide formulations. In both, the fungicidal activities were lethal to the physiological performance of the seeds.

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