USE OF BIO-FUNGICIDAL EXTRACTS IN MANAGING SEED-BORNE FUNGI FOR IMPROVED MAIZE (*Zea mays* L.) SEED GERMINATION IN MOROGORO

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A DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE, MOROGORO, TANZANIA.

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EXTENDED ABSTRACT

Maize (*Zea mays* L.) is an important crop in Tanzania for food, feed and source of household income. Recently, farmers reported the decline in maize productivity due to poor maize seed germination. The problem was linked to seed-borne fungal which have not been extensively studied in Tanzania. This study aimed at identifying the prevalent seed-borne fungi and developing a suitable bio-fungicide formulation for management of seed-borne. Certified and farmer-saved seeds were the matrix from which fungi were isolated and identified. Deep freezing blotter method was used to grow fungi. Thereafter, the produced fungal colonies were examined under microscope. On the management side, neem (*Azadirachta indica*), ginger (*Zingiber officinale*) and coffee (*Coffee arabica*) were used as the source of bio-fungicides. Both farmer-saved and certified seeds were found to be contaminated with *Fusarium verticillioides*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* spp, *Rhizopus* spp and *Curvularia* spp. The incidences of *A. flavus, F. verticillioides* and *A. niger* in farmer-saved TMVI were 93.5%, 55% and 24.5% respectively higher ($p< 0.01$) than in certified TMV1 (24.5%, 34.25% and 4.5% respectively). It was also found that, ethanol-extracted bio-fungicides caused 100% inhibition of mycelial growth. For water-extracted bio-fungicides, *A. indica* (55.88%) and *Z. officinale* (46.31%) Had were higher efficiency than *C.* a*rabica* (5.15%). From *in vivo* assay, seeds treated with water-extracted bio-fungicides had significantly higher mean percentages of seedling emergence (66.7% and 83.33% for certified STAHA treated with coffee and farmer-saved STAHA treated with neem respectively) than ethanol-extracted bio-fungicides (7.5% and 6.67% for certified STAHA treated with coffee and farmer-saved STAHA treated with neem respectively). Farmers are advised to use certified seeds. But when farmer-saved seeds have to be used, pre-treating them with bio-fungicides before sowing is crucial.

However, further studies on quantifying the bio-active compounds contained in bio-fungicides responsible for managing seed-borne fungi is recommended.

DECLARATION

I, **ERASTO REHEMA** do hereby declare to Senate of Sokoine University of Agriculture that the work presented here is my original work and that it has neither been submitted nor being concurrently submitted for degree award in any other Institution.

_____ _____

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LIST OF ABBREVIATIONS AND SYMBOLS

^{0}C	Degree Celsius
ANOVA	Analysis of Variance
ASA	Agricultural Seed Agency
CIMMYT	International Maize and Wheat Improvement Center
CV	Coefficient of variation
FAOSTAT	Food and Agriculture Organization Statistics
GIEWS	Global Information and Early Warning System
IITA	International Institute of Tropical Agriculture
ISTA	International Seed Testing Association
LSD	Least Significance Differences
m.a.s.l	Meters above sea level
P-value	Observed significance level
SAT	Sustainable Agriculture Tanzania
SUA	Sokoine University of Agriculture
URT	United Republic of Tanzania
WPRD	Workshop for Participatory Research Design

CHAPTER ONE

1.0 INTRODUCTION, JUSTIFICATION AND OBJECTIVES

1.1 Introduction

Maize (*Zea mays* L.), is the most important grain crop in Tanzania and is produced all over the country under varied environments (URT, 2017). It has a good potential due to its low production cost, wide adaptability and varied use (FAOSTAT, 2014; Mtaki, 2019). Maize is among the most substantial staple food crops in developing countries, feed for livestock and raw material to the evolving biofuel industry in developed world (FAOSTAT, 2014). About 65-80% of total maize produced is consumed within producing households and about 20-35% of it may enter commercial channel (Wilson and Lewis, 2015). Its production has significantly increased over past two years from 5,652 thousand tones to 6,300 thousand tones with average growth annual rate of 6.41% (GIEWS, 2020). Despite the increment in production, crop productivity is still below the expected yield potential. This is caused by both biotic and abiotic factors affecting crop productivity (Orsi *et al*., 2000).

In Tanzania, maize production is mostly under low-input rain-fed conditions with minimum use of mechanization, low use fertilizers, and the low use of quality seeds (Baijukya *et al*., 2020; Mghweno *et al*., 2020). Majority of the small holder farmers are still using their recycled farmer-saved seeds from previous seasons (Msuya and Stephano, 2010; Mghweno *et al*., 2020; Kansiime *et al*., 2021). This is partly due to low cost and timely accessibility of those seeds (Etten *et al*., 2017; SAT, 2019; Mghweno *et al*., 2020). Farmer-saved seeds have questionable health status because of potential vulnerability to biotic stresses including diseases. According to Castellarie *et al*. (2010), plant diseases caused by different pathogens are foremost constraint to expected crop productivity.

Some species within kingdom fungi constitute a group of most destructive pathogens responsible for several plant diseases. Some fungal pathogens occur in seeds as seed-borne fungi that associated with maize seeds in stores and cause seeds deterioration (Tsedaley, 2016). Sometimes the pathogens remain viable for long time and infect the germinating seeds or emerging seedlings in the field (Tsedaley, 2016).

It is believed that, about 40% of all maize diseases at seedling stage are caused by seed-borne fungi (Hussain *et al*., 2013). Fungal growth and development may be influenced by maize seed moisture content prior and during storage, degree of fungal contamination prior storage, insect and mite activities facilitating fungal dissemination, storage time and storage temperature (Suleiman and Omafe, 2013). According to Hussain *et al*. (2013), some of the maize diseases due to seed-borne fungi includes; Gibberela ear rot, stalk rot, seedling blight, seed rot, wilt and stunt cause by *Fusarium spp* and *Penicillium* spp., seedling blight caused by *Aspergillus* spp., *Penicillium* spp., Bipolaris leaf spot caused by *Bipolaris maydis* and Curvularia leaf spot due to *Curvularia lunata* infections.

Fusarium spp, can invade more than 50% of maize grain before harvest and produce mycotoxins (fumonisins) (Charity *et al*., 2010). But also, most of xerophytic fungi produce mycotoxins. When consumed, these mycotoxins may cause a number of health problems as well as death. For example, *A. flavus*, a food contaminant which produces aflatoxins responsible for liver damage (Charity *et al*., 2010).

If not managed, these pathogens will keep on surviving in maize crops and after harvest they are likely to be found in maize seeds hence perpetuation of their generations while causing crop losses.

In order to reduce incidence of seed-borne fungi for improved maize productivity, approaches such as seed health tests and seed treatments can be put into actions (Niaz and

Dawar, 2009). For a longtime now, commercially produced maize seeds are almost universally treated with chemical fungicides prior to selling in order to shield establishment of seed-borne fungi in stores and after planting. Regardless of their efficiency and reliability, continuous use of these chemical fungicides have been associated with several negative impacts including non-biodegradability and residual toxicity causing health hazards and pollution (Debnath *et al*., 2012; Perelló *et al*., 2013).

Due to negative impacts caused by chemical fungicides, bio-control agents including bio-fungicides are suggested to be used as alternative to chemical fungicides. These bio-fungicides are useful, cost-effective and environmentally friendly (Mbega *et al*., 2012; Hubert *et al*., 2015). Coffee, neem and ginger containing chlorogenic acid, triterpenoides with active ingredients nimbidine, nimbin and azadirachtin respectively and terpene compounds respectively are among the botanicals reported to have anti-fungal bio-activities. The bio-active compounds can mostly be found in coffee beans, neem plant parts (seeds, leaves) and in ginger rhizomes (Gyasi *et al*., 2020).

Availability of these raw materials for extraction of active ingredient, standardization of botanical extracts, rapid degradation and regulatory approval limit the use of botanicals in managing plant diseases although they are eco-friendly, cheap and promising disease managing materials (Usharani, 2019).

Poor maize seed germination resulting to low crop productivity has been reported in Mvomero district, Morogoro region (SAT, 2019). This was reported by farmers during the fifth workshop for participatory research design (6^{th} WPRD) held at the Sokoine University of Agriculture, as the major production constraint in Mvomero district (SAT, 2019).

1.2 Justification

Maize is among important cereal crops to small scale farmers in Mvomero, Morogoro. Nevertheless, its productivity is hampered by poor maize seed germination. This problem was reported by farmers during the workshop for participatory research design (6th WPRD) organized by SAT conducted at SUA (SAT, 2019). Similar problem was reported in other countries like Ethiopia and Pakistan, whereas prevalence of seed-borne fungi have been reported to be the major cause of the problem (Niaz and Dawar, 2009; Tsedaley, 2016).

These fungi cause seed deterioration hence poor germination and field epidemics (Mohamed *et al*., 2001). The problem dominates when poor quality seeds are used (Mathur and Kongsdal, 2003). Factors reported to aggravate the prevalence of seed-borne fungi include, insect pests, high moisture content and temperature (Suleiman and Omafe, 2013). Several studies on detecting and identifying seed-borne fungi have been done in several areas of the world where maize is grown and most of these studies recommended the use of chemical fungicides in seed treatment (Tsedaley, 2016). But in Mvomero, where most of farmers use farmer-saved seeds, health status of those seeds is questionable (SAT, 2019).

Although chemical fungicides help to reduce the prevalence and effects of seed-borne fungi on maize seeds, the prolonged use have been reported to pose several negative impacts including; resistance development especially when used at sub-lethal dose, reduced seed longevity, having hazardous effects to consumers and environment and increase production costs (Debnath *et al*., 2012). Due to those negative impacts, the use of bio-control agents including botanical fungicides seems to be alternative method to manage seed-borne fungi.

Most of the studies have been done *in vitro* and little *in vivo* for other crops' seeds (Mbega *et al*., 2012; Hubert *et al*., 2015; Tsedaley, 2016). Therefore, this study aimed at detecting and identifying seed-borne fungi found in/on maize seeds sourced from different seed producers and storage environments. But also establishing effective, eco-friendly and feasible management option through the use of bio-fungicidal extracts for improved maize productivity.

1.3 Objectives

1.3.1 Overall objective

Improvement of maize productivity in Morogoro by using healthy seeds.

1.3.2 Specific objectives

i. To identify seed-borne fungi responsible for deterioration of maize seeds
ii. To determine the effects of bio-fungicidal extracts on fungal isolates' growth and germination of maize seed under laboratory condition
iii. To evaluate the effects of bio-fungicidal extracts on maize seedlings emergence and growth under field condition

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CHAPTER TWO

2.0 PHYTOPATHOGENIC SEED-BORNE FUNGI RESPONSIBLE FOR LOW GERMINATION RATE OF FARMER-SAVED MAIZE SEEDS IN MVOMERO DISTRICT, MOROGORO

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Abstract

Seed health is an important attribute to be taken care of, since it is from healthy seed a healthy plant is regenerated. Seed-borne fungi are among the serious groups of pathogens causing significant losses in both quantity and quality of maize seeds. They cause low seed germination, poor seedling vigor and diseases to a growing crop plant. This study was designed to detect and identify seed-borne fungi associated with farmer-saved seeds in Mvomero district, Morogoro region. The primary seed samples were drawn using stick trier. For all the seed sources, composite samples were formed by combining and mixing all the primary samples taken from lots of respective seed variety. Then, each composite sample of seed variety was reduced to 1kg, labeled, packed, sealed and submitted for testing. A deep freezing blotter (DFB) method was used to grow seed-borne fungi. Thereafter, the produced fungal colonies were examined under microscope; stereomicroscope for morphological identification and compound microscope for identification of reproductive structures. The incidence, relative density and isolation frequency of seed-borne fungi were calculated.

F. verticillioides, *A. flavus*, *A. niger*, *Penicillium* spp, *Rhizopus* spp and *Curvularia* spp were detected in both farmer-saved and certified seeds. However, the incidences were significantly higher ($p<0.01$) in farmer-saved seeds than in certified seeds from ASA. The incidences of *A. flavus*, *F. verticillioides* and *A. niger* in farmer-saved TMVI were 93.5%, 55% and 24.5% respectively higher than those in certified TMV1 which were 24.5%, 34.25% and 4.5% respectively. Therefore, farmers should be encouraged to use certified seeds which were found to have good quality.

Keywords: Farmer-saved seeds, seed quality, seed health, seed-borne fungi

2.1 Introduction

Seed is the biological entity and basic agricultural input used in any crop production system. It is from seed where plant's life is perpetuated (Roopa and Wadje, 2012; ISTA, 2015). Seeds are produced through formal and informal seed production systems. The formal system is the one entrusted to give high quality and healthy seeds (ISTA, 2015; Etten *et al*., 2017).

Regardless of the advantages of using certified seeds, majority of the small holder farmers in Mvomero district, Morogoro region are still using recycled farmer-saved seeds from previous seasons (Msuya and Stephano, 2010; Mghweno *et al*., 2020; Kansiime *et al*., 2021). This is because they are of low cost, readily available and timely accessible (Etten *et al*., 2017; SAT, 2019; Mghweno *et al*., 2020). However, the farmer-saved seeds are of poor physiological quality and are usually contaminated with noxious weed seeds and seed-borne diseases leading to low field emergence, reduced crop vigor and low productivity (Mahender *et al*., 2015).

In order to obtain optimal germination, emergence, good seedling vigor and high crop yield, seeds should be of high quality. The quality of the seeds can be hampered by several factors, disease causing organisms being among them. Fungi, bacteria and viruses are pathogens responsible for interfered seed health, fungi being the leading group (Mathur and Kongsdal, 2003; Tsedaley, 2016). The diseases develop as the seeds provide natural substrate for the growth of associated fungi. The fungi grow either externally on the seed surface and seed coat or internally in the endosperm, cotyledons, plumule, radicle and embryo (Mathur and Kongsdal, 2003; Roopa and Wadje, 2012).

In either way, the fungi may cause both qualitative and quantitative losses of seeds leading to low seed germination and seedling vigor (Debnath *et al*., 2012; Al-Askar *et al*., 2013; Gyasi *et al*., 2020). The problem is ascribed to poor storage conditions characterized of conducive humidity, temperature for fungal growth and hence low seed germination and seedling vigor (Niaz and Dawar, 2009). To mention few, *Aspergillus* spp, *Fusarium* spp, *Penicillium* spp *Bipolaris maydis* and *Rhizopus* spp are the reported phyto-pathogenic seed-borne fungi (Tulin and Askun, 2006; Niaz and Dawar, 2009).

Knowledge and information on fungal species that can be associated with low seed germination and poor seedling vigor from farmer-saved seeds in Mvomero district (Morogoro) are lacking. Though the scant information on maize seed-borne pathogens in Tanzania has been recently highlighted in a review by Luzi-Kihupi *et al*. (2015). Therefore, this study was designed to detect the occurrence of seed-borne fungi in the farmer saved maize seeds used by smallholder farmers in Mvomero district, Morogoro region of Tanzania.

2.2 Materials and methods

2.2.1 Description of the study area and duration

Seed samples were collected from smallholder farmers in Mvomero district ($6^0$45′0″ S and $37^0$32′59″ E) and Agricultural Seed Agency (ASA), Morogoro. During sampling, information on storage facilities used in storing each maize seed variety were collected. Isolation of fungal pathogens from maize seeds was conducted at the laboratories of the International Institute of Tropical Agriculture (IITA)-Dar es Salaam. The Laboratories are located at 6.756523^0S and 39.234947^0E, Plot No 25 Mikocheni Light Industrial Area, Mwenge Coca-Cola Road, Mikocheni B.

2.2.2 Maize varieties

Maize varieties used in this study were certified seeds of STAHA, SITUKA-M1 and TMV1 sourced from ASA in Morogoro and farmer-saved STAHA, SITUKA-M1 and TMV1 from farmers in Mvomero district, Morogoro region. These are open pollinated varieties with ability to tolerate drought but also can breed true to type when recycled. The primary samples were drawn using stick trier (762mm trier with outside diameter 25.4mm and 6 slots). The trier was inserted diagonally into the bags. It was pushed into the bag in the closed position, then opened and turned a couple of times to allow it to fill completely. Thereafter, it was closed again, withdrawn and emptied into a cloth bags (Mathur and Kongsdal, 2003). For all the sources of seeds, composite samples were formed by combining and mixing all the primary samples taken from the lots of respective seed variety. Subsequently, each composite sample of seed variety was reduced to one kilogram (1kg), labelled (variety name, source and storage facility used) packed into cloth bags, sealed and submitted to the testing station (Mathur and Kongsdal, 2003; ISTA, 2015). The submitted seed samples were placed in thick paper bags of uniform size and stored in a refrigerator at a temperature of 5^0c until used.

2.2.3 Seed purity analysis

Purity test was conducted for both certified and farmer-saved seeds. A working sample of one kilogram of each variety was separated into pure seeds, inert matters, other seeds, insect bored and diseased seeds, and broken seeds.

After separating, weight of each part was measured separately using an electronic weighing balance to get percentage composition of each seed sample (ISTA, 2015).

2.2.3.1 Data processing: Was done as per ISTA (2015).

$$Purity\,(\%) = \frac{Weight\ of\ pure\ seeds}{Weight\ of\ seed\ lot} \times 100 \quad \text{..... i}$$

2.2.4 Germination test

Both certified and farmer-saved seeds were subjected to germination test. Four hundred (400) seeds of each seed sample were randomly taken from a working sample, then sown in containers with sand collected from IITA as growing media. Thereafter sown seeds were irrigated and left to imbibe until germination took place. Seven (7) days later data on germinated seeds, dead seeds, normal seedlings and abnormal seedlings (Fig. 2.1) were collected according to ISTA (2015).

Figure 2.1: Emerged maize seedlings and dead seeds after germination test (a) dead seeds (b) abnormal seedlings (c) normal seedlings

2.2.4.1 Data processing: Was done as per ISTA (2015).

Germination percent for each seed sample was calculated as follows;

$$Germination(\%) = \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ sown\ seeds} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$$

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2.2.5 Detection and identification of seed-borne fungi

The non-blocking experiment with six (6) treatments (maize samples) was laid down using completely randomized design (CRD). The maize samples were examined for the presence of seed-borne fungi using moist blotter method (ISTA, 2015). Maize seeds (400 seeds for each sample) were surface disinfected in 1% NaOCl for 1 minute, rinsed 2 times in sterile distilled water (SDW), then left to dry for 3 minutes before plating for incubation. The 10 surface sterilized seeds were placed on three layers of moistened sterilized blotter papers in sterile Petri dishes.

Forty Petri dishes (each with 10 seeds) with three layers of moistened sterilized blotter papers were used for each seed sample (Fig. 2.2a). Seeds were deep freezed in Petri dishes at -20^{0}c for 24 hours. Then, seeds were incubated at 25^{0}c under alternating cycles of 12/12 hours of light and darkness for seven days (Mathur and Kongsdal, 2003; ISTA, 2015). Each seed was examined thoroughly under a stereo microscope (x 50) (Leica, CLS 100, Germany) and many fungal colonies were observed on seed surface (Fig. 2.2b). Each fungal colony was separately picked with a slightly bent inoculating needle and transferred at the center of Potato Dextrose Agar (PDA) media (Mathur and Kongsdal, 2004). This was followed by incubation for five days at 28˚C - 32˚C in interchanging cycles of 12hours of light and darkness in order to induce fungal growth. Repeated sub-culturing were done to get a pure culture.

During each subculture, inoculating needles were sterilized by flaming to red hot after each cut, to prevent cross contamination. Pure cultures were exposed to UV light irradiation at wavelength of 350-500nm by alternating 12 hours of light and 12 hours of darkness to induce sporulation (Su *et al*., 2012). Examination of produced conidia was done under compound microscope (x 750) (Mathur and Kongsdal 2003). The Petri dishes containing pure fungal inocula were finally stored in the refrigerator at 5˚C for further use (Harlapur *et*.*al*., 2007).

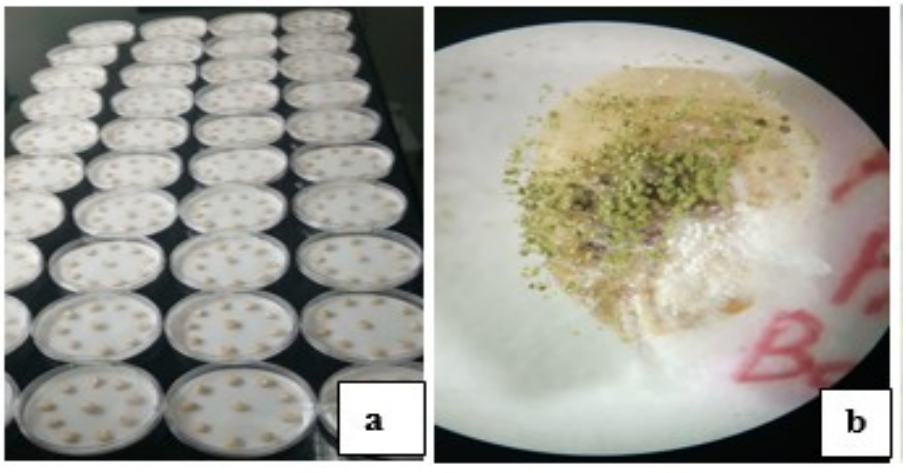

Figure 2.2: Plated maize seeds, 10 seeds/plate (a) ready to be incubated, Fungal growth on maize seeds as seen under light microscope (b, x 50)

2.2.5.1 Data collection

Number of isolated fungal genus, species, number of maize samples colonized by each species and number of maize samples with seed-borne fungal species were recorded according to Mathur and Kongsdal, (2004) and ISTA, 2015).

2.2.5.2 Data processing: Was done as per Marasas et al. (1988)

$$Fungal\ Incidence(\%) = \frac{Number\ of\ infected\ seeds}{Total\ number\ of\ seeds} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$$

iii

$$Relative\ density\ (\%) = \frac{Number\ of\ isolated\ fungal\ genus \vee species}{Total\ number\ of\ fungi} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots$$

iv

$$Isolation\ frequency(\%) = \frac{Number\ of\ samples\ with\ a\ fungal\ genus \vee species}{Total\ number\ of\ seed\ samples} \times 100 \ldots\ldots\ldots\ldots$$

v

2.2.6 Statistical analyses

Prior to analysis of variance (ANOVA), the collected and processed data were subjected to normality test using Shapiro-Wilk test. Those which were not normally distributed were transformed using either square-root or arcsine transformation depending on the type of data (Gomez and Gomez, 1984). Transformation was done to yield approximately normally distributed data. GenStat software (16^{th} version, VSN International) was used in data transformation and analysis. Tukey's test at $p<0.05$ was used to separate treatment' means.

2.3 Results

2.3.1 Maize storage facilities

The study found that, 67% of the collected farmer-saved seeds were stored in the woven polypropylene bags and 33% in plastic buckets. Seed samples collected from agricultural seed agency (ASA) were stored in woven polypropylene bags (Table 2.1).

Table 2.1: Materials used in storing maize seeds

Storage facility	Seed source (%)	
	ASA	**Farmers**
Plastic buckets	0	33
Woven polypropylene bags	100	67

2.3.2 Purity test

There was a significant difference between seed varieties and seed sources in terms of percentage of pure seeds ($p<0.001$). The percentages of pure seeds observed from seeds sourced from ASA, that is; STAHA (99.39%), SITUKA-M1 (98.86%) and TMV1 (98.83%) were higher than those from farmer-saved seeds, that is; STAHA (95.55%), TMV1 (93.62%) and SITUKA-M1 (91.83%) (Fig. 2.3).

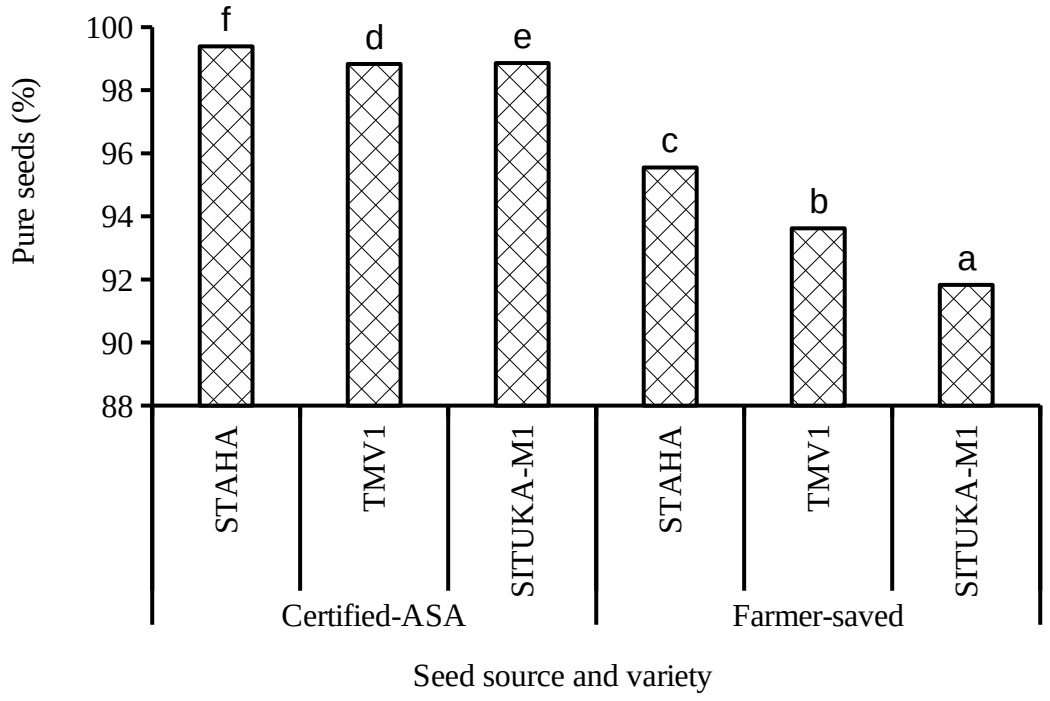

Figure 2.3: Percentages of pure seeds for maize seed varieties obtained from different sources ($p<0.001$)

The percentages of insect bored and diseased seeds varied significantly ($p<0.001$) between seed varieties and seed sources. Seeds sourced from farmers, that is; farmer-saved SITUKA-M1 (4.98%) followed by STAHA (4.14%) and TMV1 (3.58%) had higher percentages than those sourced from ASA, that is STAHA (0.41%), SITUKA-M1 (0.4%) and TMV1 (0.38%) (Table 2.2). The highest percentages of chuffs and broken seeds were recorded from farmer-saved TMV1 (2.17%) and farmer-saved SITUKA-M1 (1.98%) (Table 2.2). The highest percent of other crop seeds (0.52%) was noted in farmer-saved SITUKA-M1 (Table 2.2). The highest percent of shriveled seeds (0.69%) was recorded from farmer-saved SITUKA-M1 (Table 2.2).

Table 2.2: Seed purity based on percentage composition of maize seed lots

Source	Variety	IB&D (%)	C&B (%)	OCS (%)	SV (%)
Certified-ASA	STAHA	0.41 c	0.20 b	0.001 b	0.00 a
	TMV1	0.38 a	0.72 c	0.02 d	0.07 b
	SITUKA-M1	0.40 b	0.74 c	0.00 a	0.00 a
Farmer-saved	STAHA	4.14 e	0.04 a	0.018c	0.31 c
	TMV1	3.58 d	2.17 e	0.00 a	0.65 d
	SITUKA-M1	4.98 f	1.98 d	0.52 e	0.69 e
	CV	0	0.3	0.3	0.3
	p-value	<.001	<.001	<.001	<.001
	LSD	0.005673	0.005209	0.003112	0.00521

Key; IB&D=Insect bored and diseased, C&B= Chuffs and broken seeds, OCS= Other crop seeds, SV=Shriveled seeds. Means with the same letters along the same column are not significantly different ($p<0.05$)

2.3.3 Germination test

The general ANOVA revealed that there were significant differences ($p < 0.001$) among the seed varieties and seed sources in germination test. SITUKA-M1, STAHA and Farmer-saved STAHA seeds had the highest germination percent of 98.25%, 95.5% and 95.5% respectively (Fig. 2.4). Percentage of normal seedling were observed to be highest in SITUKA-M1 (96.75%), STAHA (91%) and Farmer-saved STAHA (87.75%) (Fig. 2.4). All the farmer-saved seeds had higher percent of abnormal seedlings than certified seeds from ASA (Fig. 2.5).

The percent of dead seeds was high in farmer-saved SITUKA-M1 (39.5%) followed by TMV1 (21.5%) (Fig. 2.5).

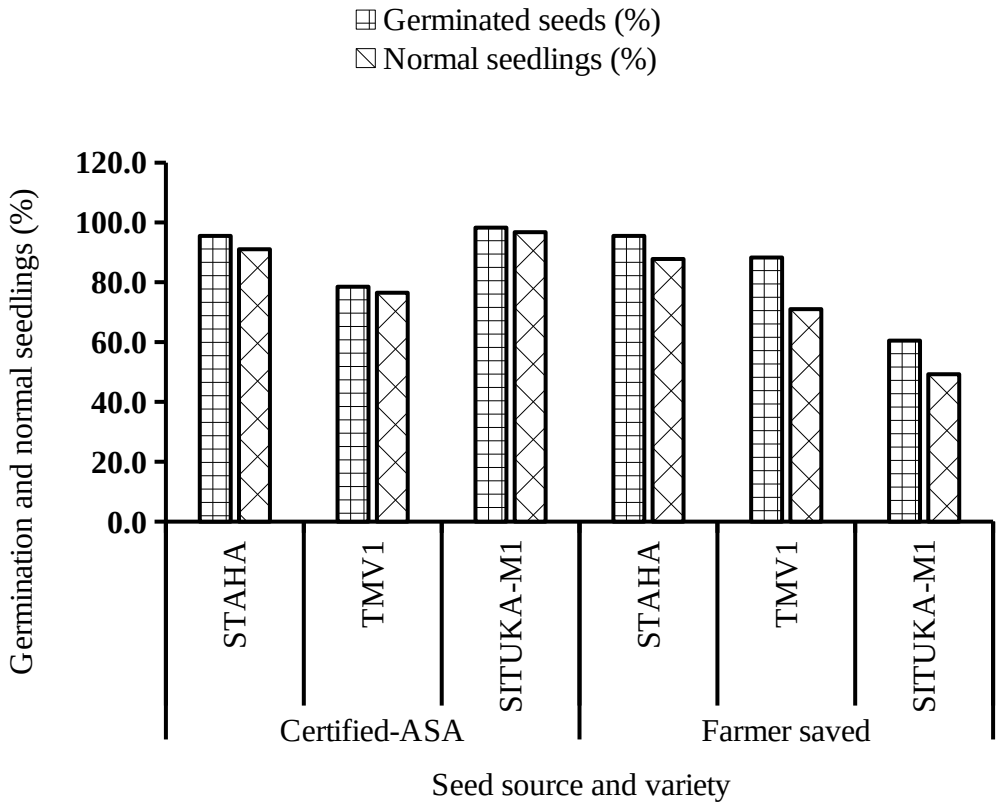

Figure 2.4: Germination capacity and seedling vigor of maize ($p<0.001$)

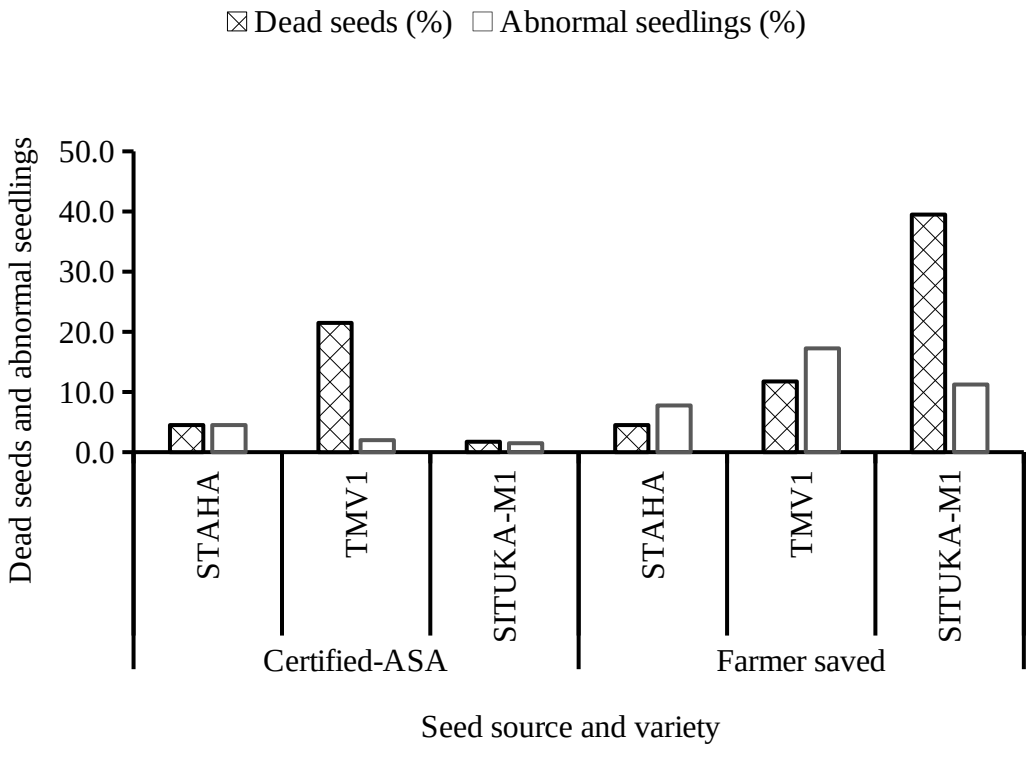

Figure 2.5: Effects of seed source and variety on seedling vigor of maize ($p<0.001$)

2.3.4 Relationship between germination and purity of seeds.

Germination of maize seeds and percentage of pure seeds were positively correlated (r=0.61). In contrast, the insect bored and diseased seeds, chuffs and broken seeds, other crop seeds and shriveled seeds were observed to have a significantly negative correlation with maize seeds germination (r= -0.47, -0.61, -0.86 and -0.59 respectively) (Table. 2.3).

Table 2.3: Correlation between maize seeds germination and seed purity components

Seed purity component (%)	Germination
	r
Pure seeds	0.61
Insect bored and diseased seeds	-0.47
Chuffs and broken seeds	-0.6
Other crop seeds	-0.86
Shriveled seeds	-0.59

Key; r = Correlation coefficient

2.3.5 Incidence of fungi identified from collected maize samples

The incidences of *F. verticillioides, A. flavus, A. niger, Penicillium* spp and *Rhizopus* spp varied significantly ($p<0.01$) between seed varieties and source. Highest incidence of *F. verticilioides* was observed in STAHA (85.75%) and Farmer-saved STAHA (70.25%) (Fig. 2.6). The incidence of *A. flavus* was highest in Farmer-saved TMV1 (93.5%) and Farmer-saved SITUKA-M1 (90.75%) (Fig. 2.6). The incidence of *A. niger* was higher in Farmer-saved seeds than in certified seeds from ASA (Fig. 2.7). Highest incidence of *Penicillium* spp was observed in Farmer-saved SITUKA-M1 (18.5%) and TMV1 (11.75%) (Fig. 2.7). Farmer-saved SITUKA-M1, SITUKA-M1 and TMV1 had high incidence of *Rhizopus* spp occurring at 10.5%, 10.25% and 9.5% incidences respectively (Fig. 2.7). Compared to other fungal species identified in this study, low incidences of *Curvularia* spp was observed in SITUKA-M1 (0.75%) (Fig. 2.7).

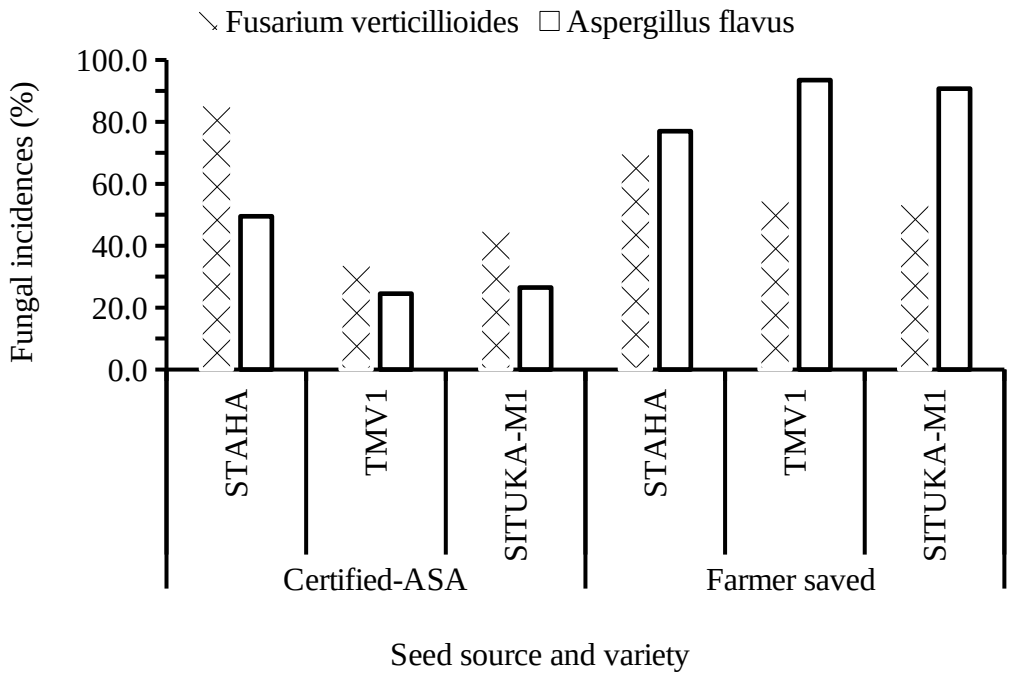

Figure 2.6: Incidence (%) of *F. verticillioides* and *A. flavus* (p<0.01)

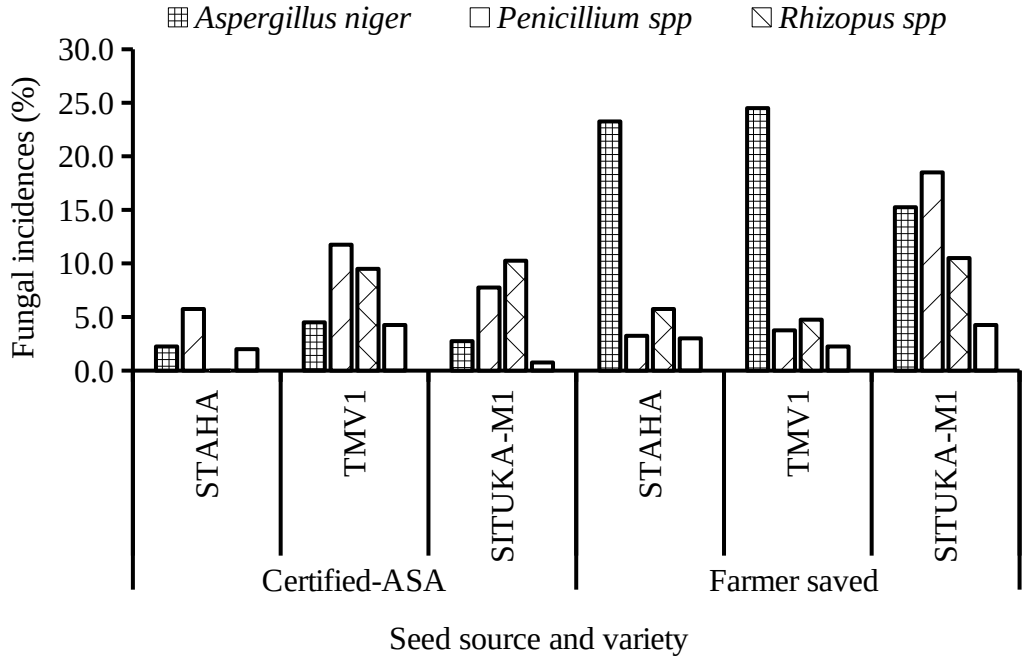

Figure 2.7: Incidence of *A. niger, Penicillium* spp, *Rhizopus* spp and *Curvularia* spp, seed-borne fungi (p<0.01)

2.3.6 Relationship between fungal incidence and germination of maize seeds

Table 2.4 shows the correlations between maize seeds germination and fungal incidences. It can be seen that the germination of maize seeds and incidence of *F. verticillioides* had no statistical correlation (r = 0.31). In contrast, *A. flavus* and *A. niger* (r = -0.34 and -0.11 respectively) were observed to have statistically no correlation. On the other hand, *Penicillium* spp, *Rhizopus* spp and *Curvularia* spp were observed to have significant negative correlation with maize seeds germination (r= -0.77, -0.47 and -0.52 respectively).

Table 2.4: Correlation between maize seeds germination and fungal incidences

Incidence of seed-borne fungi (%)	**Germination**
	r
F. verticillioides	0.31
A. flavus	-0.34
A. niger	-0.11
Penicillium spp	-0.77**
Rhizopus spp	-0.47*
Curvularia spp	-0.52*

Key; r = Correlation coefficient, * and ** = Significant correlation at 0.05 and 0.001 respectively

2.3.7 Relative density

Results show that, the relative density of fungi varied significantly ($p<0.001$) between varieties. The relative density of *F. verticillioides* was significantly higher in STAHA (59.15%) and SITUKA-M1 (48.21%) than in other seed varieties (Fig. 2.8). The relative density of *A. flavus* was higher in farmer-saved TMV1(50.89%), farmer-saved SITUKA-M1, (47.07%) and farmer-saved STAHA (42.3%) (Fig. 2.8). The relative density of *A. niger* was higher in farmer-saved TMV1 (13.353%) and farmer-saved STAHA (12.633%) than in other seed varieties (Fig. 2.9). The relative density of *Penicillium* spp was higher in TMV1 (13.069%), farmer-saved SITUKA-M1 (9.607%) and SITUKA–M1 (8.34%) than

in other seed varieties (Fig. 2.9). The relative density of *Rhizopus* spp was significantly higher in SITUKA-M1 (11.014%) and TMV1 (10.783%) than in other seed varieties (Fig. 2.9). The relative density of *Curvularia* Spp was higher in TMV1 (4.796%) and farmer-saved SITUKA-M1 (2.209%) than in other seed varieties (Fig. 2.9).

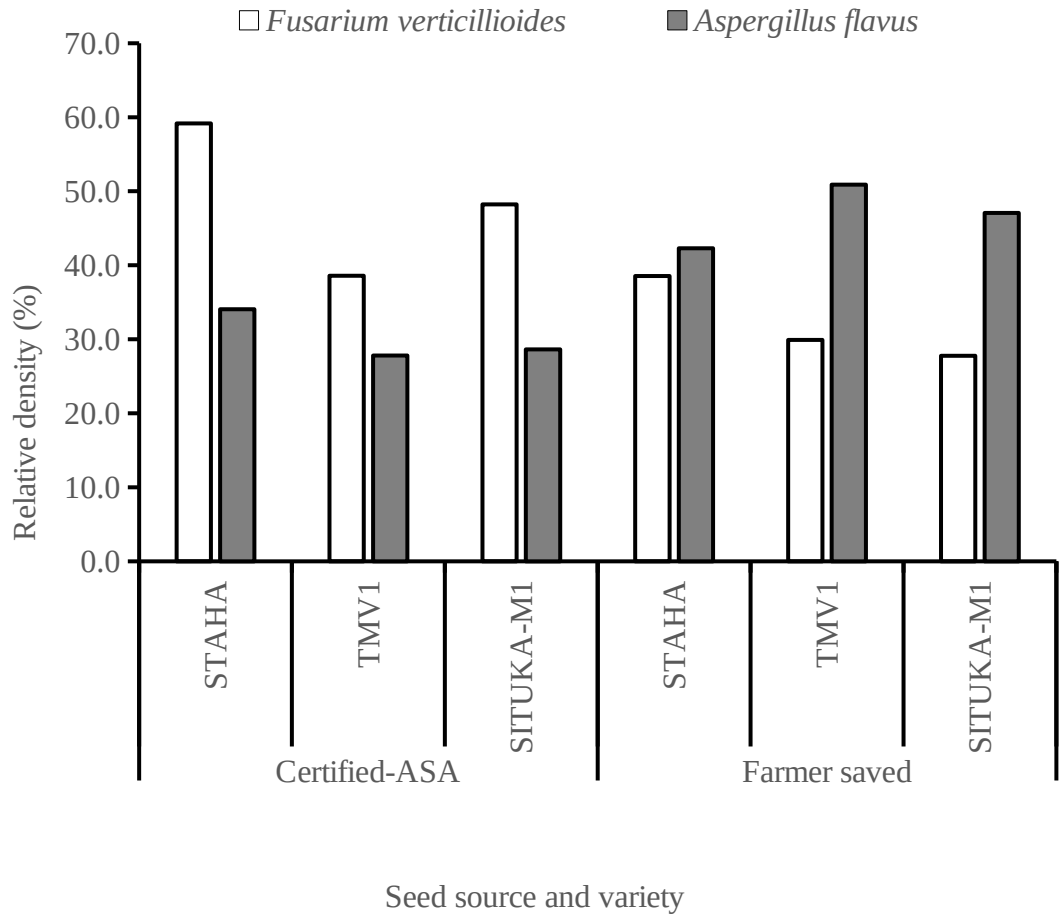

Figure 2.8: Relative density of *F. verticillioides* and *A. flavus* (p<0.001)

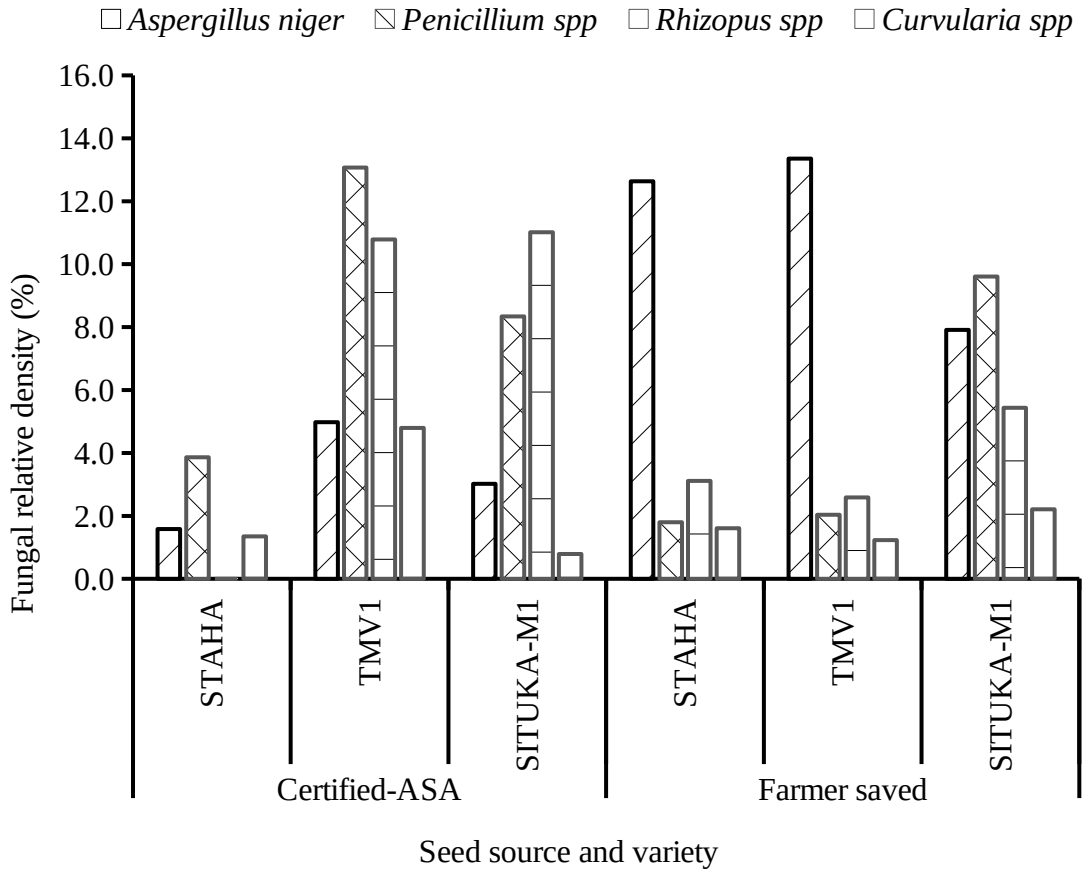

Figure 2.9: Relative density of *A. niger*, *Penicillium* spp, *Rhizopus* spp and *Curvularia* spp, seed-borne fungi (p<0.001)

2.3.8 Isolation frequency

All fungal species were present in all samples except *Rhizopus* spp which was isolated in 8 out of every 10 samples (Fig. 2.10).

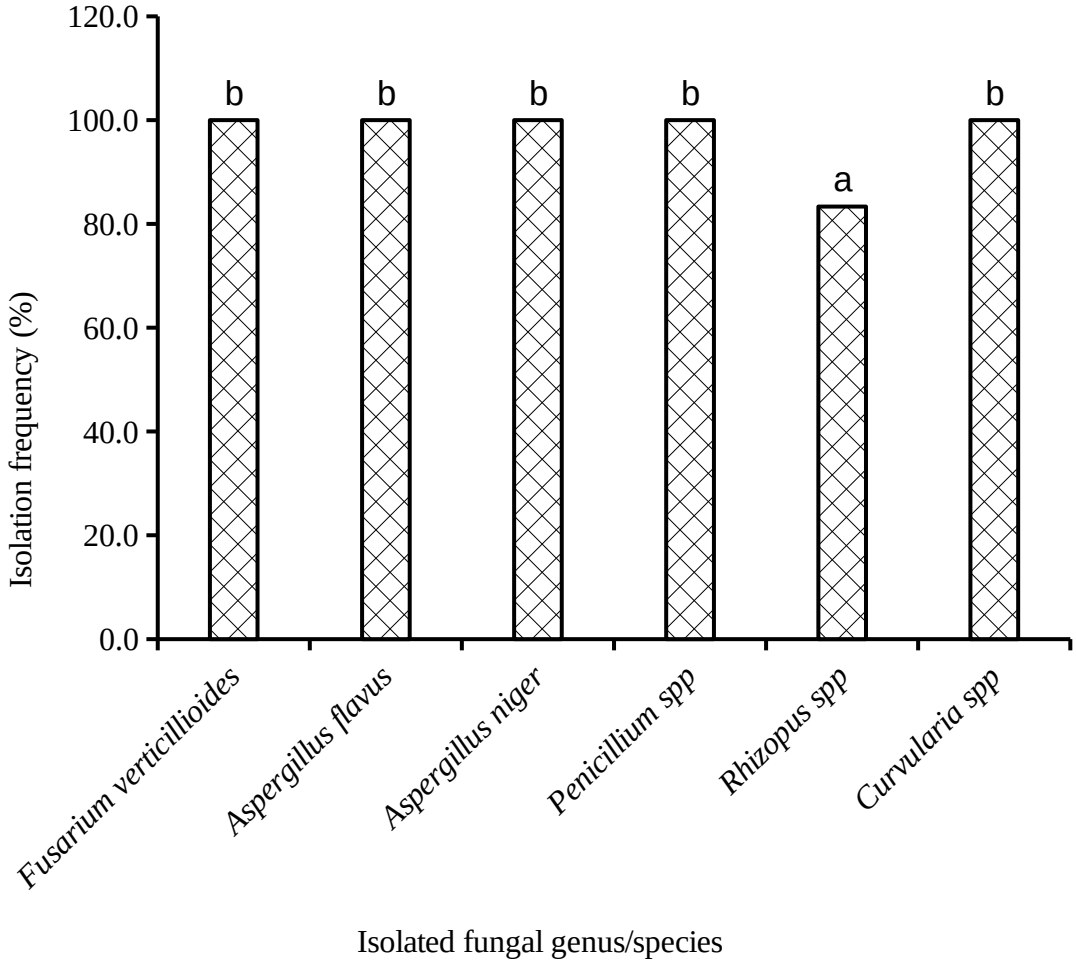

Figure 2.10: Isolation frequencies of fungi (p<0.001)

2.4 Discussion

2.4.1 Source and storage of maize seeds

The study found that, maize seeds sourced from farmers were poorly stored compared to certified maize seeds from ASA. Poor storage of seeds can be the reason for increasing incidence of seed-borne fungi in farmer-saved seeds which later can lead to poor germination performance and field epidemics of fungal diseases.

Topping on that, a preliminary study done by Rehema *et al*. (2019) to explore the reasons for poor maize seeds germination found that about 80% of farmers in Mvomero used farmer-saved maize seeds continuously while only 20% used certified seeds. Study by

Niaz and Dawar (2009) also reported that, the quality and quantity losses of maize seeds occur mainly because of improper storage.

2.4.2 Purity test

From the study, farmer-saved seeds had lower percentage of pure seeds than certified seeds multiplied and sold by ASA. But also, farmer-saved seeds had higher percentage of chuffs, insect bored seeds, shriveled seeds, and broken seeds than seeds from ASA. This disqualifies farmer-saved seeds to have practical planting (ISTA, 2015).

2.4.3 Seed germination and fungal infection

Seeds from farmers appeared to have lower germination percentage compared to seeds from ASA. This might be attributed to harvesting and subsequent storage conditions of those seeds, that is, poor conditions for farmer-saved seeds and good conditions for seeds from ASA. This agrees with the study by Godefroid *et al*. (2010) which concluded that pre-maturely harvested and poorly stored seeds usually shrivel and succumb to easily attack by fungi hence reduced germination capacity. It is also in agreements with the notion of Quezada *et al*. (2006) that seeds with high rates of fungal infection have very low germination rates, which can be as low as 28% of original potential. Again, this disqualifies farmer-saved seeds to have practical planting value (ISTA, 2015).

2.4.4 Detection and identification of seed-borne fungi

All the seed samples were found to be contaminated with seed-borne fungi. Except *F. verticillioides* which was found to be of higher incidence in certified STAHA seed, other detected fungi (*A. flavus, A. niger, Penicillium* spp, *Curvularia* spp and *Rhizopus* spp) were of higher incidence in farmer-saved seeds. Higher incidence of these fungi seem to endanger the seeds germination due to the fact that their presence in or on seeds contribute

to quality and quantity losses of seeds. This agrees with studies by Niaz and Dawar (2009) and Gyasi *et al.* (2020) which reported seed-borne fungi to be responsible in reducing seed quality and quantity hence poor seed germination, infects seedlings to cause root rot, reduces seedling vigor by weakening the plant at its initial growth, cause field epidemics and may contribute to contamination of mycotoxins in maize grains. Mycotoxins (fumonisins and aflatoxins produced by *F. verticillioides* and *A. flavus* respectively) are responsible for health hazards in human and animals (Debnath *et al*., 2012; Madege *et al*., 2016).

2.4.5 Relationship between seed purity, fungal infection and seed germination

Correlation between seed purity and seed germination found a significant positive relationship between percentage of pure seeds and seed germination. In contrast, there were significant negative relationships between seed germination and other seed lot compositions (shriveled seeds, chuffs and broken seeds, insect bored and diseased and other crop seeds). The reduced maize seeds germination can be linked with the increased percentage of non-pure seeds compositions of seed lot. This links well with facts on relationship between seed purity and seed germination by ISTA (2015).

For the case of relationship between fungal infection and seed germination, results found significant negative relationships between *Penicillium* spp, *Rhizopus* spp and *Curvularia* spp incidences and seed germination. But there was insignificant positive relationship between *F. verticillioides* incidence and seed germination. Also, there were insignificant negative relationships between *A. flavus* and *A. niger* incidences and seed germination. The notion obtained from this study is that, not all the detected seed-borne fungi can be responsible for poor seed germination. For instance, a negative relationship between fungal incidences and seeds germination imply negative impacts of fungi on seed germination. Meaning that, a significant increase in fungal incidences reduced seed

germination. This agrees with the study by Niaz and Dawar (2009) which reported the reduced seed quality and quantity due to seed-borne fungi, hence reduced seed germination.

On the other side, insignificant negative relationship between fungal incidences and seed germination bring a message that, there might be a contribution of fungi in hampering seed germination even if the impact is not statistically significant. This can be seen when antagonistic fungi co-exist, as one fungal species can be the reason for reduced fungal growth and aggressiveness hence reducing its impact on seed germination. This is in agreement with the study by Giorni *et al*. (2019) which reported reduction of *F. verticillioides* growth by *A. flavus* when co-occurred. These two fungi have different efficiency and rapidity of using carbon source and invade the substrate substrate at high temperature (25–30°C) and dry conditions (0.87a_w) (Camardo *et al*., 2019; Giorni *et al*., 2019). Optimal water activity for *F. verticillioides* to grow is 0.97 a_w (Samapundo *et al*., 2005).

2.5 Conclusion and recommendation

Findings indicated that most of farmers use their non-treated farmer-saved seeds recycled from previous harvests and stored in woven polypropylene bags. These seeds contained numerous seed-borne fungi predominantly *F. verticillioides*. This implies the farmers use improper storage techniques leading to high infection by seed-borne fungi which in turn deteriorate seeds leading to poor seed germination, low seedling vigor and crop stand. When farmers have to recycle their farmer-saved seeds, storage facilities must be good enough to reduce build-up of seed-borne fungi. Also treating seeds with eco-friendly fungicides before sowing is recommended.

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CHAPTER THREE

3.0 EFFICACY OF BIO-FUNGICIDES AND THEIR EXTRACTION METHODS AGAINST PHYTOPATHOGENIC SEED-BORNE FUNGI

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Abstract

Seed-borne fungi are solemn and deleterious pathogens capable of causing significant losses of maize seeds and grains. They infect the crop at all points of the production chain from farm to stores. To identify more effective management options, we evaluated the efficacy of water-extracted and ethanol-extracted bio-fungicides from 3 plant species *in vitro* and *in vivo* assays for antifungal activity against *F. verticillioides*, one of the seed-borne fungus of maize. In the *in vitro* assay, mycelial growth inhibition was determined. In terms of mycelial growth inhibition, the most effective bio-fungicides were the extracts from *A. indica* (55.88%) and *Z. officinale* (46.31%) while *C. arabica* caused the least performance (5.15%). For the case of *in vivo* assay, farmer-saved STAHA seeds treated with water-extracted plant extracts had significantly ($p<0.001$) higher germination percentages of 90, 90 and 80 for ginger, neem and coffee respectively. But those treated with ethanol-extracted had low germination percentages of 2.5, 2.5 and 0 for ginger, coffee and neem respectively. These results indicate that the tested water extracted bio-fungicides were potential candidates for seed treatment against maize seed-borne fungi. Reasons why ethanol extraction caused least performance could not be established in this study.

Keywords: Bio-fungicidal extracts, antifungal activity, *F. verticillioides*, seed-borne fungi, seed treatment.

3.1 Introduction

Seed-borne fungi, *F. verticillioides* being among them, are the deleterious and serious pathogens capable of causing both qualitative and quantitative losses of maize seeds (Castellari *et al*., 2010; Madege *et al*., 2018; Gyasi *et al*., 2020). These pathogens not only cause seed deterioration, but can also remain viable for long time to infect the germinating or emerging seedlings and later cause disease epidemics in crop fields (Tsedaley, 2016). About 40% of all maize diseases at seedling stage are caused by seed-borne fungi (Hussain *et al*., 2013). The use of farmer-saved maize seeds facilitates perpetuation and multiplication of seed-borne fungi within the seeds and become a source of inoculum for new infection (Hubert *et al*., 2015).

In Tanzania, the use of farmer-saved maize seeds has been a common practice among smallholder farmers. For instance, most of smallholder farmers in Mvomero district, Morogoro region use maize seeds for sowing in the subsequent season (SAT, 2019) that are recycled from the previous cropping cycle. This practice contributes highly to build up of seed-borne fungal diseases hence their subsequent negative impacts, poor seed germination being the foremost impact. However, before harvest the pathogens can invade more than 50% of maize grains and produces mycotoxins (Charity *et al*., 2010). These mycotoxins, include fumonisins produced by *F*. *verticillioides* and aflatoxins produced by *A. flavus.* These toxins are the most worrisome because can cause health problems to humans and animals when they ingest contaminated food and feed respectively (Niaz and Dawar, 2009; Charity *et al*., 2010; Madege *et al*., 2018). A review by Ismaiel and Papenbrock (2015) established that previous studies had confirmed some mycotoxins are associated with seedling mortality. Seed treatment is an important approach to be used in managing seed-borne fungi (Mbega *et al*., 2012). It reduces survival chances of the

pathogens in or on maize seeds, their transmission to maize crops and other negative impacts expected from fungi (Niaz and Dawar, 2009).

Seed treatment using chemical fungicides has been a common practice for a long time in Tanzania but also worldwide. Fungicides such as Benomyl, Benomyl + Copper sulphate, Mancozeb, probenazole, Metalaxyl and thiabendazole have been commonly used (Mbega *et al*., 2012; Hubert *et al*., 2015). The continuous use of chemical fungicides regardless of their efficiency and reliability, is characterized of non-biodegradability, rapid pathogen's resistance development, increased production cost, residual toxicity causing health hazards and environmental pollution (Debnath *et al*., 2012; Perelló *et al*., 2013; Hubert *et al*., 2015).

Due to that, bio-fungicidal extracts are known to be useful, cost-effective, environmentally friendly, non-toxic to mammals, have very low or no residuals on plants and have antifungal properties. Hence they are recommended for use as alternatives to chemical fungicides (Mbega *et al*., 2012; Hubert *et al*., 2015).

Since ancient time, botanical extracts are known to contain anti-microbial property (Lalitha *et al*., 2010). Coffee, neem and ginger are among botanicals with known anti-fungal bio-active compounds. Bioactive compounds found in coffee, neem and ginger are chlorogenic acid, triterpenoides (azadirachtin, nimbidine, nimbin nimonol, nimocinol and nimocinolide) and terpene compounds respectively.

These bio-compounds are mostly found in coffee beans, neem plant parts (seeds, leaves) and in ginger rhizomes respectively (Gyasi *et al*., 2020).

Different solvents can be used in extraction of biocides from plant materials. A study by Amadioha (2000), found that the leaf extracts of neem extracted using water and ethanol

were effective in reducing fungal growth *in vitro* and their development in plants. Most of these products have been used to manage seed-borne diseases *in vitro* (Mbega *et al*., 2012; Hubert *et al*., 2015) and managing plant diseases under field condition.

Generally, botanical extracts are becoming a promising source of agricultural chemicals to manage seed-borne fungi and plant diseases. These *in vitro* and *in vivo* studies were conducted to determine the efficacy of selected bio-fungicides in managing the growth of phyto-pathogenic seed-borne fungi.

3.2 Materials and methods

3.2.1 Study area and duration

The study area is as described in section 2.2.1 of this dissertation.

3.2.2 Isolation and preparation of *F. verticillioides* inoculum

Isolation of fungi from seeds and identification was done as per section 2.2.5 of this document.

3.2.3 Preparation of bio-fungicide plant materials

About 5kg of fresh neem leaves, fresh ginger rhizomes and dried coffee beans were collected from Sokoine University of Agriculture (SUA), Morogoro market and Mbeya respectively (Table. 3.1).

Fresh neem leaves were harvested using a knife and put in perforated sack. Ginger rhizomes and neem leaves were washed with running tape water to remove soil materials and other debris then rinsed three times with sterile distilled water (SDW). The leaf and rhizome were then cut into small pieces (5mm diameter) and placed on screen house benches at 25˚C - 28˚C for one month to dry. The dried neem leaves, ginger rhizomes and

coffee beans were ground into powder separately using a milling machine (Foss Tecator Cyclotec 1093 Sample Mill) and then sieved with one millimeter sieve. The powder of each plant species was packed in water proof plastic bags and labeled appropriately and stored at 4˚C until used (Hasan *et al*., 2005; Akinbode and Ikotun, 2008; Hubert *et al*., 2015).

Tables 3.1: Botanicals, sources of bio-fungicides

Common name	Scientific name	Used part	Source
Coffee	*C. arabica*	Beans	Mbeya
Ginger	*Z. officinale*	Rhizomes	Morogoro market
Neem	*A. indica*	Leaves	SUA

3.2.4 Preparation of bio-fungicide extracts

3.2.4.1 The extraction using water

50g powder of each type of plant material was dissolved in 100mls of SDW resulting to 50%w/v in a 500mls conical flask. The mixture was thoroughly stirred and left for 24h. The extracts were separately filtered through a muslin cloth and re-filtered again through Whatman No. 1. filter paper into a sterile 500ml beaker.

The obtained solutions were collected into sterilized conical flasks (Fig.3.1) and stored at 25˚C - 28˚C until used (Mamiro and Royse, 2004).

3.2.4.2 The extraction using ethanol

This was done by dissolving 50g of powdery plant materials in 100mls ethanol (70%) to make a concentration of 50% w/v. The mixtures were thoroughly agitated and placed in a refrigerator at 25˚C - 28˚C for 24hrs (Nduagu *et al*., 2008; Zida *et al*., 2008). Each solution was individually filtered firstly using muslin cloth and lastly passed through filter

paper (Whatman filter No. 1). The filtrates obtained were concentrated by evaporation of solvent (ethanol) in a water bath at 50°C according to Rauha *et al*. (2000). The solutions were collected into sterilized conical flasks and stored at 4°C until used (Rauha *et al.*, 2000; Mamiro and Royse, 2004).

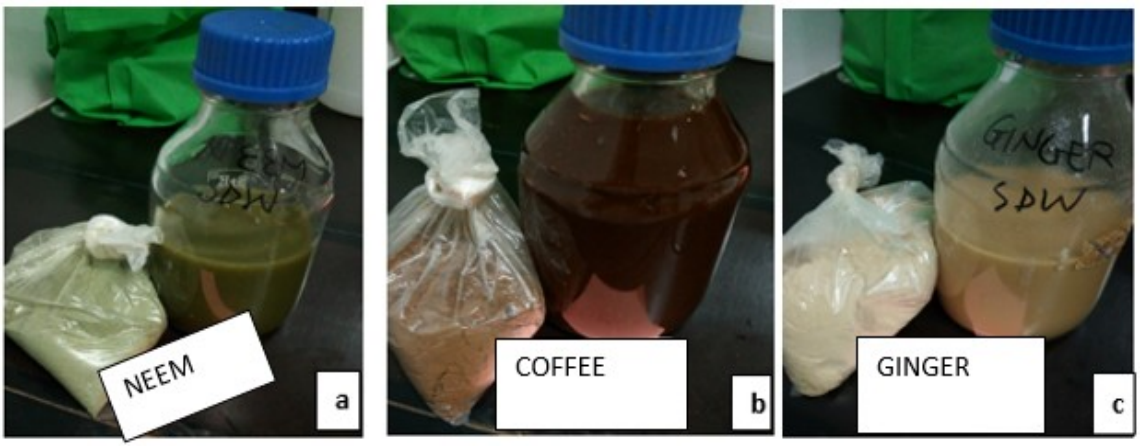

Figure 3.1: Plant extracts in powdery form (in plastic bags) and extracts dissolved in sterile distilled water (in glass bottles). (a) neem, (b) coffee and (c) ginger

3.2.5 Experiment 1: Efficacy of water and ethanol extracted bio-fungicides against *F. verticillioides* under in vitro culture

3.2.5.1 Media preparation

PDA media was prepared by dissolving 39g of Agar powder into 1000mls of SDW (Fig. 3.2) according to Degraeve *et al*. (2016). Then the media was autoclaved at 121^0c for 30 minutes, it was then allowed to cool to 50-55^0c.

Figure 3.2: PDA growth media for in vitro culture of fungi

3.2.5.2 Amendment of PDA media with bio-fungicides

The prepared bio-fungicides (50ml of each stock solution) were added to the cool autoclaved molten PDA (150 ml) using sterile micro-filters in the laminar flow chamber. This made a 25% concentration of PDA-BF (PDA mixed with bio-fungicide) for each type of bio-fungicide. Thereafter, 20 ml of each PDA-BF was poured into separate Petri dish and allowed to solidify. They were stored in refrigerator (upside down) until used (Kamalakannan and Shanmugam, 2005; Japtap *et al*., 2012).

3.2.5.3 Bio-fungicide inhibition of *F. verticillioides* mycelial growth

The experiment was laid down as factorial in CRD replicated 3 times. It involved two (2) factors; factor A (Fungicides) and factor B (Extraction solvents) hence a 5x2 treatment combination. The PDA-BF were inoculated at the center with 2mm inoculum disc of the 1week old pure culture of *F. verticillioides* (Harlapur, 2007).

This was followed by incubating at 28°C for 5 days and the radial growth diameter of the fungal colony was measured every day for 5 days after inoculation. Untreated cultures served as negative control while the cultures inoculated with chemical fungicide (Apron Star® 42 WS with 20% Thiamethoxam, 20% Metalaxyl-M and 2% Difenoconazole), served as positive control (Fig. 3.3). The fungal mycelial growth diameters in the PDA media with bio-fungicides (PDA-BF) was measured along the two diagonal lines using a 30 cm plastic ruler (Hubert *et al*., 2015). Calculation of percent inhibition of fungal growth was estimated based on Ogbeborand and Adekunle methods (2005).

$$\%\, Mycelial\ growth\ inhibition = \frac{Mycelial\ growth\ diameter\ (control - treatment)}{Mycelial\ growth\ diameter\ control} \times 100 \ldots\ldots i$$

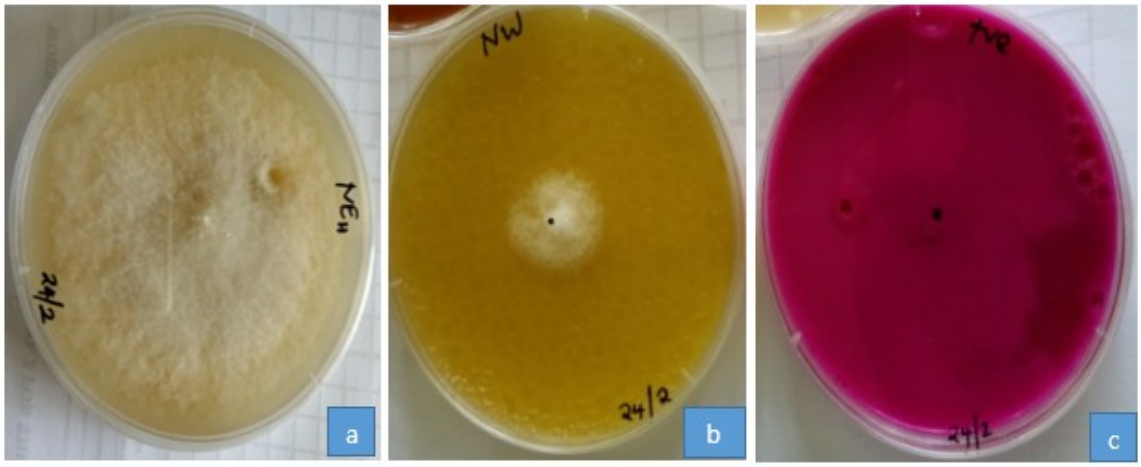

Figure 3.3: Fungal mycelial growth in (a) negative control, (b) PDA-BF(neem) and (c) positive control

3.2.6 Experiment 2: Efficacy of bio-fungicides in controlling seed-borne *F. verticillioides* in maize grown under screen house condition

The experiment was laid down as factorial in CRD replicated 4 times. It involved three (3) factors; factor A (2 Seed types), factor B (5 Fungicides) and factor C (2 Extraction solvents) hence a 2x5x2 treatment combination.

3.2.6.1 Seed inoculation with *F. verticillioides*

Four hundred (400) untreated seeds of each maize seed category (certified-STAHA and farmer-saved STAHA) were inoculated by spraying 1×10^5spores/ml *F. verticillioides* strain following procedures of Namai and Ehara (1986). Inoculated seeds were dried in the laminar flow chamber on three layers of blotter papers in Petri dishes for 2h. Seeds were stored at 4˚C until used.

3.2.6.2 Treatment of inoculated seeds with bio-fungicides

The 50% w/v of ethanol-extracted and water-extracted bio-fungicides were used in *in vivo* assay. Maize seeds that were pre-inoculated with *F. verticillioides* were placed in a beaker and 40 ml of each bio-fungicides suspension was added. The seeds were gently stirred by stirring glass rod to ensure a complete immersion and even distribution. To lessen exterior contamination, beakers with already inoculated seeds

were enclosed by aluminum foil. The beakers were then placed at 25˚C for 20h. Thereafter, the seeds were dried on sterile blotter papers for 2h in the laminar flow chamber (Hubert *et al*., 2015).

3.2.6.3 Evaluating efficacy of bio-fungicides

Eighty (80) maize seeds in 4 replicates (20 seeds per replicate) were tested for each bio-fungicide. Treated seeds were planted in pots (10 seeds/pot) containing sand and kept under screen house conditions. The efficacy of bio-fungicides against effects of *F. verticillioides* was evaluated based on seed germination, seedling growth and seedling vigor where the number of germinated seeds/emerged seedlings, dead seeds, shoot length, and weight of seedlings were evaluated 7 days after sowing. The shoots were cut from the soil surface and their lengths were determined using a ruler by measuring aerial parts (Fig.

3.4a) (ISTA, 2015). Maize seedlings were carefully cut at the bottom then the fresh weight was determined using weighing balance (Fig. 3.4b).

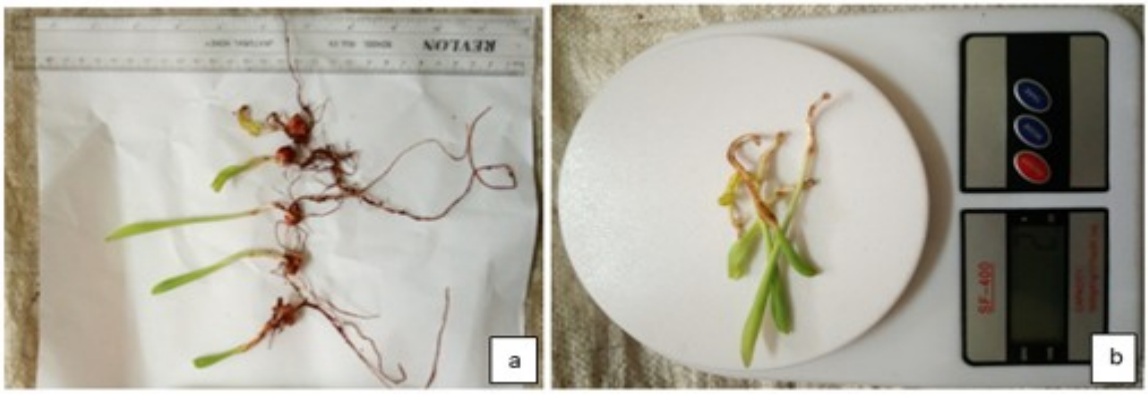

Figure 3.4: Measuring seedlings vigor based on (a) height and (b) weight

3.2.7 Data analyses

Shapiro-Wilk test was done to see if the collected data were normally distributed. The square-root and arcsine data transformations were performed prior data analysis. Data analysis was performed based on the factorial experiment's arrangement in a CRD analysis of variance (ANOVA) model. GenStat (16^{th} version, VSN International) was the software used in data transformation and analysis. Tukey's test ($p<0.05$) was used in mean separation.

The statistical models used were;

$$Y = \mu + A + B + AB + \varepsilon \quad \text{……..……..……ii}$$

$$Y = \mu + A + B + C + AB + AC + BC + ABC + \varepsilon \text{……... iii}$$

Where μ is grand mean, ε is a random error term and the uppercase letters represent the main effects and interactions. ii) and iii) are models for *in vitro* assay and *in vivo* assay respectively. For equation ii, A=Fungicides and B=Extraction solvents; for equation iii, A=Seed types, B=Fungicides, C=Extraction solvents.

3.3 Results

3.3.1 Inhibition of *F. verticillioides* mycelial growth

Results in Table 3.2 show that, both main factors (fungicides and extraction solvents) and the two-way interaction (A×B) had highly significant effects ($p<0.001$) on inhibition of *F. verticillioides* mycelial growth. The inhibition due to application of Neem (77.94%) followed by ginger (73.16%) were found to have significantly higher inhibition efficiency than coffee (52.58%) and Apron Star® 42 WS (50.00%). But also the results in Table 3.2 show that effects due to interaction of bio-fungicide and extraction solvents were highly significant ($p<0.001$).

Fungal mycelial growth was effectively inhibited by bio-fungicides that were extracted by using ethanol (extraction solvent). The ethanol-extracted bio-fungicides had 100% inhibitory effect on both observations while inhibitory effects of water-extracted bio-fungicides varied with type of plant extract. For water-extracted bio-fungicides, it was established that the mycelial growth inhibition caused by neem (55.88%) followed by ginger (46.31%) were higher than that of coffee (5.15%).

On the other hand, Apron Star® 42 WS (positive control) had 100% mycelial growth inhibition while water (negative control) had 0% inhibition.

Tables 3.2: Effects of fungicides and extraction solvents on inhibition of mycelial growth of *F. verticillioides*

	Inhibition
Factor: Fungicides (A)	**%**
Neem	77.94 d
Ginger	73.16 c
Coffee	52.58 b
Apron Star® 42 WS	50.00 a
p-value	**<0.001**
Factor: Extraction solvents (B)	
Water	51.84
Ethanol	75
p-value	**<0.001**
Fungicides × Extraction solvents (A×B)	
Neem×Water	55.88 d
Neem×Ethanol	100.00 e
Ginger×Water	46.31 c
Ginger×Ethanol	100.00 e
Coffee×Water	5.15 b
Coffee×Ethanol	100.00 e
Apron Star® 42 WS	100.00 e
No fungicide (Water)	0.00 a
p-value	**<0.001**

Means with the same letters along the same column are not significantly different ($p<0.001$)

3.3.2 Effects of bio-fungicides on mycelial growth rate of *F. verticillioides*

Table 3.3 below shows that, main factors (fungicides and extraction solvents) had highly significant ($p<0.001$) effects on the fungal mycelial growth rate. Considering the effects of fungicides on mycelial diameters, significantly different mycelial growth rates were observed one day after inoculation whereby 0.12cm, 0.20cm, 0.50cm, and 0.80cm colony diameter were measured in cultures treated with neem, ginger, coffee and Apron Star® 42 WS respectively.

Throughout the five days for which growth rate of *F. verticillioides* was monitored, a similar trend was noted except in the fifth day which the colony diameter in culture treated with coffee extract (2.15cm) was not significantly different from colony diameter in

cultures treated with Apron Star® 42 WS (2.27cm). Also the impact of extraction solvents on growth rate of *F. verticillioides* was significant throughout the five days' measurements after bio-fungicide treatments. In this respect, the colony diameter in cultures treated with water extracted plant bio-fungicides was consistently higher than the same in cultures treated with ethanol extracted bio-fungicides (Table 3.3). Results on the interaction of the two factors (A×B) (Table 3.3), showed a highly significant different ($p<0.001$) effects on the growth rate of *F. verticillioides*. With exception of Apron Star® 42 WS and ethanol-extracted bio-fungicides which resulted to 0 mycelial growth, water-extracted bio-fungicides had significantly varying effects on colony diameters.

One day after inoculation, the colony diameter of *F. verticillioides* in cultures treated with water-extracted neem (0.23cm) was the lowest followed by ginger (0.4cm) then coffee (1.0cm). On the other hand, cultures that were not treated with fungicide had the highest colony diameter (1.6cm) of *F. verticillioides*. The effects due to interaction of the two factors (A×B) remained significant ($p<0.001$) throughout the five days of measurements (Table 3.3).

Tables 3.3: Effects of fungicides and extractions solvents on mycelial growth rate of *F. verticillioides*

	Mycelial diameter (cm)				
Factor: Fungicides (A)	**1DAI**	**2DAI**	**3DAI**	**4DAI**	**5DAI**
Neem	0.12 a	0.25 a	0.67 a	0.77 a	1.00 a
Ginger	0.20 b	0.53 b	0.78 b	1.17 b	1.22 b
Coffee	0.50 c	1.00 c	1.72 c	2.08 c	2.15 c
Apron Star® 42 WS	0.80 d	1.78 d	2.20 d	2.23 d	2.27 c
p-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Factor: Extraction solvents (B)					
Water	0.41	0.89	1.58	2.01	2.18
Ethanol	0.4	0.89	1.1	1.12	1.13
p-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Fungicides×Extraction solvents (A×B)					
Neem×Water	0.23 b	0.50 b	1.33 b	1.53 b	2.00 b
Neem×Ethanol	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
Ginger×Water	0.40 c	1.07 c	1.57 c	2.33 c	2.43 c
Ginger×Ethanol	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
Coffee×Water	1.00 d	2.00 d	3.43 d	4.17 d	4.30 d
Coffee×Ethanol	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
Apron Star® 42 WS	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
Water	1.60 e	3.57 e	4.40 e	4.47 e	4.53 d
p-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Means with the same letters along the same column are not significantly different ($p<0.001$), DAI=Days after inoculation

3.3.3 Efficacy of bio-fungicides against *F. verticillioides* in maize grown under screen house condition

The Table 3.4 shows that there were significant differences in the effects of seed types ($p=0.036$), fungicides ($p<0.001$) and extraction solvents ($p<0.001$) on percentages of germination and dead seeds.

Tables 3.4: Effects of seed types, fungicides and extraction solvents on seed germination and dead seeds under screen house condition

Factor: Seed types (A)	Germination (%)	Dead seeds (%)
Certified STAHA	51.25	48.75
Farmer-saved STAHA	47.5	52.5
p-value	**0.036**	**0.036**
Factor: Fungicides (B)		
Neem	45.00 b	55.00 c
Ginger	48.75 b	51.25 b
Coffee	40.00 a	60.00 d
Apron Star® 42 WS	63.75 d	36.25 a
p-value	**<0.001**	**<0.001**
Factor: Extraction solvents C		
Water	73.44	26.56
Ethanol	25.31	74.69
p-value	**<0.001**	**<0.001**

Means with the same letters along the same column are not significantly different at ($p<0.05$)

Significant effects on percentages of germination and dead seeds due to interaction of experimental factors were observed for seed types and bio fungicides ($p=0.041$), seed types and extraction solvents ($p= 0.001$) as well as bio-fungicides and extraction solvents ($p=0.001$). Highest germination percentage of up to 51.25% was observed in certified STAHA seeds treated with ginger extract as well as 70.00% germination in certified STAHA seeds which were treated with Apron Star® 42 WS. Effects of all other treatment interactions on seed germination were not significantly different except the germination of seeds under the combination of certified STAHA seeds treated with ginger. Due to interaction between maize seed types and extraction solvents (A×C), a highly significant difference ($p<0.001$) was observed in seed germination and the proportion of dead seeds.

Influence of maize seed type on seed germination was significantly dependent the type of solvent used to extract bio-fungicides for seed treatment. We observed that certified

STAHA and farmer-saved STAHA seeds which were treated with bio-fungicides that were extracted using water had highest germination of 78.12% and 68.75% respectively. Lowest germination percentages of the same seed types were observed in seeds treated with bio-fungicides that were extracted using ethanol (Table 3.5).

It was observed that, there was highly significant difference ($p<0.001$) in proportions of dead seeds due to interaction between fungicides and the extraction solvents (B×C). Bio-fungicides extracted by using water gave higher percentages of seed germination than those extracted by using ethanol. On the other hand, when water (negative control) was used, higher percentage of seed germination (95.0%) was observed while Apron Star® 42 WS resulted to lower germination percentage (32.5%) (Table. 3.5).

Tables 3.5: Effects of two-way interaction among maize seed types, fungicides and extraction solvents on percentage germination and dead seeds

Seed types*Fungicides (A*B)	Germination (%)	Dead seeds (%)
Certified STAHA*Neem	45.00 a	55.00 c
Certified STAHA*Ginger	51.25 b	48.75 b
Certified STAHA*Coffee	38.75 a	61.25 c
Certified STAHA* Apron Star® 42 WS	70.00 c	30.00 a
Farmer-saved STAHA*Neem	45.00 a	55.00 c
Farmer-saved STAHA*Ginger	46.25 a	53.75 c
Farmer-saved STAHA*Coffee	41.25 a	58.75 c
Farmer-saved STAHA* Apron Star® 42 WS	57.50 c	42.50 a
p-value	**0.041**	**0.041**
Seed types*Extraction solvents (A*C)		
Certified STAHA*Water	78.12 c	21.88 a
Certified STAHA*Ethanol	24.38 a	75.62 c
Farmer-saved STAHA*Water	68.75 b	31.25 b
Farmer-saved STAHA*Ethanol	26.25 a	73.75 c
p-value	**<0.001**	**<0.001**
Fungicides*Extraction solvents (B*C)		
Neem*Water	90.00 d	10.00 b
Neem*Ethanol	0.00 a	100.00 e
Ginger*Water	95.00 e	5.00 a
Ginger*Ethanol	2.50 a	97.50 e
Coffee*Water	76.25 c	23.75 c
Coffee*Ethanol	3.75 a	96.25 e
Apron Star® 42 WS	32.50 b	67.50 d
Water	95.00 e	5.00 a
p-value	**<0.001**	**<0.001**

Means with the same letters along the same column are not significantly different ($p<0.05$)

There were significant effects ($p<0.001$) on germination and dead seeds due to interactions among seed types, fungicides and extraction solvents. The certified STAHA that was treated with water-extracted ginger had 100% germination followed by 90% germination in certified STAHA treated with water-extracted neem, farmer-saved STAHA treated with water-extracted neem and the farmer-saved STAHA treated with water-extracted ginger. The farmer-saved STAHA treated with water-extracted coffee had 80% germination followed by 72.5% germination of certified STAHA, STAHA treated with water-extracted coffee. Lowest germination percent was observed in certified STAHA seeds treated with

ethanol-extracted coffee (5%) followed by 2.5% germination in certified STAHA treated with ethanol-extracted ginger, farmer-saved STAHA treated with ethanol-extracted coffee and farmer-saved STAHA treated with ethanol-extracted ginger. Certified STAHA treated with ethanol-extracted neem and farmer-saved STAHA treated with ethanol-extracted neem had 0% germination. For the dead seeds, results show that, seeds treated with ethanol-extracted bio-fungicides had significantly higher percentage of dead seeds than those water-extracted bio-fungicides. The effect of ethanol-extracted bio-fungicides were statistically not different, caused almost 100% seedling death. (Tab. 3.6). The dead seeds recorded in certified STAHA treated with water-extracted coffee was 27.5% followed by farmer-saved STAHA treated with water-extracted coffee (20%), farmer saved STAHA treated with water-extracted ginger (10%) and farmer saved STAHA treated with water-extracted neem (10%) were statistically similar but differed with that from STAHA treated with water-extracted ginger (0%) (Tab. 3.6).

When Apron Star® 42 WS (positive control) was used, germination percentage of STAHA (50%) was higher than that of farmer-saved STAHA (15%). When water (negative control) was used, germination percentage of STAHA (90%) was lower than that of farmer-saved STAHA (100%). But also, percentages of dead seeds for STAHA (10%) was higher than that of farmer-saved STAHA (0%).

Tables 3.6: Effects of a three-way interaction among seed types, fungicides and extraction solvents on percentages of germination and dead seeds

Seed types*Fungicides*Extraction solvents (A*B*C)	**Germination (%)**	**Dead seeds (%)**
Certified STAHA*Neem*Water	90 d	10 b
Certified STAHA*Neem*Ethanol	0.00 a	100 e
Certified STAHA*Ginger*Water	100 e	0.00 a
Certified STAHA*Ginger*Ethanol	2.5 a	97.5 e
Certified STAHA*Coffee*Water	72.5 d	27.5 b
Certified STAHA*Coffee*Ethanol	5.00 a	95 e
Farmer-saved STAHA*Neem*Water	90 d	10 b
Farmer-saved STAHA*Neem*Ethanol	0.00 a	100 e
Farmer-saved STAHA*Ginger*Water	90 d	10 b
Farmer-saved STAHA*Ginger*Ethanol	2.5 a	97.5 e
Farmer-saved STAHA*Coffee*Water	80 d	20 b
Farmer-saved STAHA*Coffee*Ethanol	2.5 a	97.5 e
STAHA* Apron Star® 42 WS	50 c	50 c
STAHA*Water	90 d	10 b
Farmer-saved STAHA* Apron Star® 42 WS	15 b	85 d
Farmer-saved STAHA*Water	100 e	0.00 a
p-value	**<.001**	**<.001**

Means with the same letters along the same column are not significantly different ($p<0.001$)

There were highly significant effects ($p<0.001$) between main treatments (seed types, fungicides and extraction solvents) on percentages of normal seedlings. On the other hand, there were significant effects observed between fungicides ($p<0.001$) and extraction solvents ($p=0.035$) while insignificant difference ($p=0.609$) for seed types was observed on percentages of abnormal seedlings (Table. 3.7).

Tables 3.7: Effects of seed types, fungicides and extraction solvents on normal and abnormal seedlings

Factor: Seed types (A)	Normal seedlings (%)	Abnormal seedlings (%)
Certified STAHA	49.06	2.19
Farmer-saved STAHA	44.38	3.12
p-value	**<0.001**	**0.609**
Factor: Fungicides (B)		
Neem	45.00 a	0.00 a
Ginger	46.88 b	1.88 a
Coffee	38.12 a	1.88 a
Apron Star® 42 WS	56.88 c	6.88 b
p-value	**<0.001**	**<0.001**
Factor: Extraction solvents C		
Water	71.88	1.56
Ethanol	21.56	3.75
p-value	**<0.001**	**0.035**

Means with the same letters along the same column are not significantly different ($p<0.05$)

The interaction between seed types and fungicides (AxB) had significant effects ($p<0.001$) on percentage of normal seedlings. It shown that, higher percentages of normal seedlings for all the seed types were found when seeds interacted with ginger and neem than when interacted with coffee. But also, higher percentages were observed when seed types interacted with Apron Star® 42 WS. For the interaction between seed types and extraction solvents, a highly significant difference ($p<0.001$) was observed on percentage of normal seedlings. Percentages of normal seedlings were found to be higher when seed types interacted with water than when interacted with ethanol (Table 3.8). From the interaction between fungicides and extraction solvents, a highly significant difference ($p<0.001$) was also observed on percentage of normal seedlings. It was found that, higher percentages of normal seedlings were observed when bio-fungicides interacted with water than when interacted with ethanol. On the other hand, water (negative control) gave higher percentage of normal seedlings than Apron Star® 42 WS (positive control) (Table 3.8).

Tables 3.8: Effects of two-way interaction among seed types, fungicides and extraction solvents on percentages of normal and abnormal seedlings

Seed types*Fungicides (A*B)	Normal seedlings (%)	Abnormal seedlings (%)
Certified STAHA*Neem	45.00 c	0
Certified STAHA*Ginger	50.00 d	1.25
Certified STAHA*Coffee	36.25 a	2.5
Certified STAHA* Apron Star® 42 WS	65.00 e	5
Farmer-saved STAHA*Neem	45.00 c	0
Farmer-saved STAHA*Ginger	43.75 bc	2.5
Farmer-saved STAHA*Coffee	40.00 ab	1.25
Farmer-saved STAHA* Apron Star® 42 WS	48.75 d	8.75
p-value	**<0.001**	**0.542**
Seed types*Extraction solvents (A*C)		
STAHA*Water	75.62 c	2.50 ab
STAHA*Ethanol	22.50 a	1.88 ab
Farmer-saved STAHA*Water	68.12 b	0.63 a
Farmer-saved STAHA*Ethanol	20.62 a	5.63 b
p-value	**<0.001**	**0.004**
Fungicides*Extraction solvents (B*C)		
Neem*Water	90.00 d	0
Neem*Ethanol	0.00 a	0
Ginger*Water	93.75 e	1.25
Ginger*Ethanol	0.00 a	2.5
Coffee*Water	76.25 c	0
Coffee*Ethanol	0.00 a	3.75
Apron Star® 42 WS	27.50 b	5
Water	86.25 d	8.75
p-value	**<0.001**	**0.37**

Means with the same letters along the same column are not significantly different ($p<0.05$)

The results in Table 3.9 below show that, the effects of interactions (seed types, fungicides and extraction solvents) on proportion of normal and abnormal seedlings were significantly different ($p<0.001$). Seed types treated with ethanol-extracted bio-fungicides had lower percentages of normal seedlings (both had 0%) than seed types treated with water-extracted bio-fungicides. The certified STAHA treated with water-extracted ginger had 100% normal seedlings followed by certified STAHA treated with water-extracted neem and farmer-saved STAHA treated with water-extracted neem which had 90%, farmer saved STAHA treated with water-extracted ginger (87.5%), farmer saved STAHA

treated with water-extracted coffee (80%) and STAHA treated with water-extracted coffee (72.5%).

For the abnormal seedlings, the interaction between seed types, fungicides and extraction solvents (AxBxC) was highly significant ($p<0.001$). The results in Table 3.9 show the proportion of abnormal seedlings in certified and farmer-saved maize seeds treated with water and ethanol extracted bio fungicides. Except farmer-saved STAHA treated with water-extracted ginger which had 2.5% of abnormal seedlings, other seeds treated with water-extracted bio-fungicides had 0% of abnormal seedlings. The proportion in certified STAHA treated with ethanol-extracted coffee (5%) and 2.5% for farmer saved STAHA treated with ethanol-extracted coffee, STAHA treated with ethanol-extracted ginger and farmer saved STAHA treated with ethanol-extracted ginger were significantly the same. When Apron Star® 42 WS (positive control) was on untreated seeds used, percentage of normal seedlings for STAHA (40%) was higher than that of farmer-saved STAHA (15%). But also, percentage of abnormal seedlings for STAHA (10%) was higher than that of farmer-saved STAHA (0%).

When water (negative control) was used, percentage of normal seedlings for STAHA (90%) was higher than that of farmer-saved STAHA (82.5%). But also, percentage of abnormal seedlings for STAHA (0%) was lower than that of farmer-saved STAHA (17.5%).

Tables 3.9: Effects of a three-way interaction among seed types, fungicides and extraction solvents on percentages of normal and abnormal seedlings

Seed types*Fungicides*Extraction solvents (A*B*C)	Normal seedlings (%)	Abnormal seedlings (%)
Certified STAHA*Neem*Water	90 g	0.00 a
Certified STAHA*Neem*Ethanol	0.00 a	0.00 a
Certified STAHA*Ginger*Water	100 h	0.00 a
Certified STAHA*Ginger*Ethanol	0.00 a	2.5 ab
Certified STAHA*Coffee*Water	72.5 d	0.00 a
Certified STAHA*Coffee*Ethanol	0.00 a	5 ab
Farmer-saved STAHA*Neem*Water	90 g	0.00 a
Farmer-saved STAHA*Neem*Ethanol	0.00 a	0.00 a
Farmer-saved STAHA*Ginger*Water	87.5 fg	2.5 ab
Farmer-saved STAHA*Ginger*Ethanol	0.00 a	2.5 ab
Farmer-saved STAHA*Coffee*Water	80 de	0.00 a
Farmer-saved STAHA*Coffee*Ethanol	0.00 a	2.5 ab
STAHA* Apron Star® 42 WS	40 c	10 bc
STAHA*Water	90 g	0.00 a
Farmer-saved STAHA* Apron Star® 42 WS	15 b	0.00 a
Farmer-saved STAHA*Water	82.5 ef	17.5 c
p-value	**<.001**	**<.001**

Means with the same letters along the same column are not significantly different ($p<0.001$)

Table 3.10 below show that main treatments (fungicides and extraction solvents) had significant effects ($p<0.001$) on both shoot length and shoot weight. Seed types had insignificant effect ($p= 0.564$ for shoot length and $p=0.375$ for shoot weight).

Tables 3.10: Effects of seed types, fungicides and extraction solvents on shoot length and shoot weight

Factor: Seed types (A)	Shoot length (cm)	Shoot weight (g)

Certified STAHA	10.16	4.27
Farmer-saved STAHA	11.4	4.79
p-value	**0.564**	**0.375**
Factor: Fungicides (B)		
Neem	9.33 a	4.50 b
Ginger	10.96 a	5.38 c
Coffee	9.56 a	2.89 a
Apron Star® 42 WS	13.28 b	5.34 c
p-value	**<0.001**	**<0.001**
Factor: Extraction solvents C		
Water	15.82	6.54
Ethanol	5.74	2.51
p-value	**<0.001**	**<0.001**

Means with the same letters along the same column are not significantly different ($p<0.05$)

Interaction between seed types and extraction solvents (A×C) had significant effects ($p = 0.018$) on shoot weight. Seed types interacted with water solvent to cause significantly higher shoot weight than those interacted with ethanol extraction solvent (Table. 3.11). Again interaction between fungicides and extraction solvents (B×C) had highly significant effects ($p < 0.001$) on both shoot length and shoot weight. It was found that, bio-fungicides extracted by using water resulted to higher shoot lengths and shoot weights than those extracted by using ethanol. Again, water (negative control) gave higher shoot lengths and shoot weights than Apron Star® 42 WS (positive control) (Table 3.11).

Tables 3.11: Effects of two-way interaction among seed types, fungicides and extraction solvents on shoot length and shoot weight

Seed types*Fungicides (A*B)	Shoot length (cm)	Shoot weight (g)
Certified STAHA*Neem	8.55	4
Certified STAHA*Ginger	9.94	5.1
Certified STAHA*Coffee	8.94	2.21
Certified STAHA* Apron Star® 42 WS	13.23	5.75
Farmer-saved STAHA*Neem	10.1	5
Farmer-saved STAHA*Ginger	11.98	5.66
Farmer-saved STAHA*Coffee	10.19	3.56
Farmer-saved STAHA* Apron Star® 42 WS	13.33	4.93
p-value	**0.911**	**0.075**
Seed types*Extraction solvents (A*C)		
Certified STAHA*Water	14.26	5.88 b
Certified STAHA*Ethanol	6.06	2.66 a
Farmer-saved STAHA*Water	17.38	7.21 b
Farmer-saved STAHA*Ethanol	5.42	2.36 a
p-value	**0.122**	**0.018**
Fungicides*Extraction solvents (B*C)		
Neem*Water	18.65 c	9.00 d
Neem*Ethanol	0.00 a	0.00 a
Ginger*Water	19.79 c	10.50 d
Ginger*Ethanol	2.13 a	0.26 a
Coffee*Water	16.69 c	5.50 c
Coffee*Ethanol	2.44 a	0.28 a
Apron Star® 42 WS	8.15 b	1.18 b
Water	18.40 c	9.50 d
p-value	**<0.001**	**<0.001**

Means with the same letters along the same column are not significantly different ($p<0.05$)

Table 3.12 for the interaction between seed types, fungicides and extraction solvents, shows that, the effects of water and ethanol extracted-bio fungicides on maize seedling weights were significantly different ($p=0.025$).

Seedling shoot weights of maize treated with water-extracted bio-fungicides were higher than of maize treated with ethanol-extracted bio-fungicides. Farmer- saved STAHA treated with water-extracted ginger (11g), farmer saved STAHA treated with water-extracted neem (10g), STAHA treated with water-extracted ginger (10g), STAHA treated with water-extracted neem (8g) and farmer saved STAHA treated with water-extracted

coffee (7g) were statistically similar and higher than that of STAHA treated with water-extracted coffee (4g). Shoot weight of STAHA treated with ethanol-extracted coffee (0.43g), farmer saved STAHA treated with ethanol-extracted ginger (0.33g), STAHA treated with ethanol-extracted ginger (0.2g), farmer-saved STAHA treated with ethanol-extracted coffee (0.13g), STAHA treated with ethanol-extracted neem (0g) and farmer saved STAHA treated with ethanol-extracted neem (0g) were statistically similar. STAHA and farmer-saved STAHA treated by Apron Star® 42 WS (Positive control) had statistically similar shoot weights, 1.5g and 0.85g respectively. Also STAHA and farmer-saved STAHA treated by water (negative control) had statistically similar shoot weights, 9g and 10g respectively.

Tables 3.12: Effects of a three-way interaction among seed types, fungicides and extraction solvents on shoot length and shoot weight

Seed types*Fungicides*Extraction solvents (A*B*C)	**Shoot length (cm)**	**Shoot weight (g)**
Certified STAHA*Neem*Water	17.1	8 ef
Certified STAHA*Neem*Ethanol	0	0.00 a
Certified STAHA*Ginger*Water	18.38	10 ef
Certified STAHA*Ginger*Ethanol	1.5	0.2 a
Certified STAHA*Coffee*Water	14.28	4 d
Certified STAHA*Coffee*Ethanol	3.6	0.425 ab
Farmer-saved STAHA*Neem*Water	20.2	10 ef
Farmer-saved STAHA*Neem*Ethanol	0	0.00 a
Farmer-saved STAHA*Ginger*Water	21.2	11 f
Farmer-saved STAHA*Ginger*Ethanol	2.75	0.325 ab
Farmer-saved STAHA*Coffee*Water	19.1	7 e
Farmer-saved STAHA*Coffee*Ethanol	1.28	0.125 a
Certified STAHA* Apron Star® 42 WS	7.3	1.5 c
Certified STAHA*Water	19.15	10 ef
Farmer-saved STAHA* Apron Star® 42 WS	9	0.85 bc
Farmer-saved STAHA*Water	17.65	9 ef
p-value	**0.641**	**0.025**

Means with the same letters along the same column are not significantly different ($p<0.05$)

3.4 Discussion

3.4.1 Inhibition of *F. verticillioides* mycelial growth

It was generally observed that, ethanol-extracted bio-fungicides were more effective in inhibiting the *F. verticillioides* mycelial growth than water-extracted bio-fungicides. This can be related to differences in concentration of bioactive compounds extracted by different extraction solvents.

For instance, most of bioactive compounds which are potent biocides are organic in nature and ethanol (organic solvent) has higher ability to extract those organic bioactive compounds than water, hence its higher fungicidal effect.

This is in agreement with the study by Mondall *et al*. (2009) which concluded that, ethanolic extracts of neem leaves had higher fungicidal effects on *Aspergillus* spp and *Rhizopus* spp than crude extracts of neem. In addition to that, this can be related to inhibitory effects of ethanol (which is assumed to be partially removed from the bio-fungicides solution) on fungal growth. This conforms to the study by Hallsworth *et al*. (1998) which reported ethanol to reduce water availability for fungal growth.

It was found that about 31% of fungal growth inhibition by ethanol at 25^0c was caused by water stress, but at temperatures lower than 25^0C, the inhibitory effect due to water stress could exceed 31% since other non-water stress effects of ethanol become less severe. However, for water-extracted bio-fungicides, it was established that neem *(A. indica)* followed by ginger *(Z. officinale)* had higher inhibitory effect than coffee (*C. Arabica).* This may be due to differences in amount of bioactive compounds found in these potential medicinal plants. This is consistent with studies by Brahmachari (2004); Ghasemzadeh *et al*. (2010); Mahaptara *et al*. (2014); Keta *et al*. (2019) which reported neem and ginger to have many bioactive compounds (Alkaloids, flavonoids, saponins, tannins, phenols,

terpenoids, glycoside, anthraquinones and steroid) and (6-gingerol, flavonoids and phenolic acids) respectively than coffee which contained alkaloids alone.

Furthermore, it was observed that, when water-extracted bio-fungicides were used, the mycelial diameters increased with an increase in number of days after inoculation. This can be linked to quick degradation of bio-fungicides as compared to chemical fungicides. This relate to study by Usharani (2019) which concluded that rapid degradation of botanicals limits their use in managing plant diseases.

3.4.2 Efficacy of bio-fungicides in controlling seed-borne *F. verticillioides* in maize grown under screen house condition

The study found ethanol-extracted bio-fungicides to have negative impacts on seed germination and seedling vigor. It was observed that, percentages of germinated seeds, percentages of normal seedlings and shoot weight were higher from seeds treated with water-extracted bio-fungicides than those treated with ethanol-extracted bio-fungicides. It can be linked to the ability of bio-fungicides to inhibit fungal activities leading to seed deterioration and poor seedling vigor. This is consistent with the study by Keta *et al*. (2019) which reported higher amounts of bioactive compounds in neem and ginger. These bioactive compounds manage seed-borne fungi which could limit seed germination and seedlings vigor. On the other side, percentages of dead seeds and abnormal seedlings were observed to be higher in seeds treated with ethanol-extracted bio-fungicides than in those treated with water-extracted bio-fungicides. This might be attributed to the ability of ethanol (assumed to be upheld in bio-fungicides solution) to kill the germinating embryo and their negative effect on plant growth and development. This agrees with study by (Zida *et al*., 2008) which reported ethanol to have phytotoxic effects on seed embryo and the growing seedling.

3.5 Conclusion and recommendation

This study has revealed that, ethanol-extracted bio-fungicides were more effective than water-extracted bio-fungicides in inhibiting the mycelial fungal growth. In contrast, seed germination and seedling vigor were more improved when water-extracted bio-fungicides were used than when ethanol-extracted bio-fungicides were used.

For the bio-fungicides, *A. indica* and *Z. officinale* were more effective than *C. arabica* in managing seed-borne fungi and improving seed germination and seedling vigor. The study findings reflect the potentiality of bio-fungicides in managing seed-borne fungi. This study recommends the use of bio-fungicides as useful management approach against seed-borne fungi. This will minimize the use of chemical fungicides. But studies on quantification of extracted bioactive compounds are needed. Also for alcoholic extraction of bio-fungicides, more studies are recommended to come up with best method to be used in evaporation of organic solvents used in extraction of bioactive compounds from plant materials.

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CHAPTER FOUR

4.0 EFFICACY OF BIO-FUNGICIDES AGAINST PHYTOPATHOGENIC SEED-BORNE FUNGI FOR IMPROVING MAIZE SEED GERMINATION AND SEEDLING VIGOR UNDER FIELD CONDITION

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Abstract

Farmer-saved maize seeds are commonly used by farmers in Mvomero and several areas of the world. Such seeds are usually contaminated with seed-borne pathogens, fungi being the dominant kingdom. Seed-borne fungi are responsible for both pre and post-emergence death of grains, reduce seedlings vigor hence poor crop productivity. Seed treatment using chemical fungicides have been a common practice, but rather difficult to achieve a reasonably good control and has negative impacts caused by these chemicals. The use of biocontrol agents has an increasing interest as one of ecofriendly option for controlling seed-borne pathogens. Type of bio-pesticide to use and the extraction method to employ has been disturbing many researchers. This study was conducted to determine the efficacy of bio-fungicides (*A. indica, C. arabica* and *Z. officinale*) and extraction solvents in managing seed-borne fungi for improving maize seed germination and seedling vigor under field conditions. Maize seeds were treated by bio-fungicides and chemical fungicide then sown. Seed germination, seedling growth and vigor tests were used to evaluate efficacy of fungicides in managing seed-borne fungi.

Results found that, seeds treated with water-extracted bio-fungicides had significantly higher mean percentages of seedling emergence (66.7% and 83.33% for STAHA treated with coffee and farmer-saved STAHA treated with neem respectively) than ethanol-

extracted bio-fungicides (7.5% and 6.67% for STAHA treated with coffee and farmer-saved STAHA treated with neem respectively). But also minimum number of days to first emergence were lesser for seeds treated with water-extracted bio-fungicides (3 days for farmer-saved STAHA treated with coffee) than those treated with ethanol-extracted bio-fungicides (4.33 days for farmer-saved STAHA treated with coffee). Bio-fungicides are potential candidates in managing seed-borne fungi and improving seed germination and seedling vigor.

Keywords; Farmer-saved maize seeds, seed-borne fungi, bio-fungicides

4.1 Introduction

Maize (*Zea mays* L.) is the most important grain crop in Tanzania and is produced throughout the country under diverse environments (URT, 2017). Maize production in the country is mostly under low-input rain-fed conditions with minimum use of mechanization, low use fertilizers, and the low use of quality seeds (Baijukya *et al*., 2020; Mghweno *et al*., 2020).

Regardless of the advantages of using high quality certified seeds, majority of the small holder farmers are still using their recycled farmer-saved seeds from previous seasons (Msuya and Stephano, 2010; Mghweno *et al*., 2020; Kansiime *et al*., 2021). This is due to low cost and timely accessibility of those seeds (Etten *et al*., 2017; SAT, 2019; Mghweno *et al*., 2020). As a propagation material, seeds must have all the good quality attributes so that it gives healthy and vigorous seedlings or crop stand (ISTA, 2015). Unfortunately, most of the seeds used by farmers (local seed types and farmer-saved seeds) are of poor health status (Tsedaley, 2016).

To manage diseases affecting seeds, seed treatments have been a common practice to seed producing companies, and most of them use chemicals in managing seed-borne pathogens (Hubert *et al*., 2015). A large number of chemicals have been developed for the managing plant diseases especially those starting from seeds (Mbega *et al*., 2012; Hubert *et al*., 2015). But due to overgrowing consciousness of the hazardous side effects of these chemicals, more emphasis is being given to the use of safe and eco-friendly biocontrol agents (Lalitha *et al*., 2010; Gyasi *et al*., 2020).

Recently, there have been mentioned several biocontrol agents including plant extracts, but the challenge remains there to check for their safety on environment and human/animal health. Several plants have already emerged to be potential candidates in managing plant diseases, neem (*A. indica*) and ginger (*Z. officinale)* being among them. The current study aimed at determining the efficacy of selected bio-fungicides with their extracts obtained using different solvents for managing seed-borne diseases to improve maize seed germination and seedling vigor under field condition.

4.2 Materials and methods

4.2.1 Study area and duration

The field study was conducted during the long rain season (March to May) at Sokoine University of Agriculture (SUA) located at latitude 6^0 49′ 27″ S, longitude 37^0 39′ 48″ E and elevation of 509 m above sea level.

4.2.2 Experimental design

The experiment was laid down as factorial in RCBD replicated 3 times. It involved three (3) factors; factor A (Seed types), factor B (Fungicides) and factor B (Extraction solvents) with 2x5x2 treatment combination. Maize types, bio-fungicides, inoculum and inoculation procedures were done as in chapter three.

4.2.3 Evaluating efficacy of bio-fungicides

Treated seeds were planted. The efficacy of bio-fungicide treatments in the control of *F. verticillioides* effects was evaluated based on seed germination, seedling growth and vigor tests where the number of emerged seedlings (first and second seedling emergences), number of days to first emergence, plant height, leaf length, leaf width and weight of seedlings were evaluated (CIMMYT, 1985).

4.2.4 Data processing: Was done as per CIMMYT (1985).

$$Emergence(\%) = \frac{Number\ of\ emerged\ seedlings}{Number\ of\ sown\ seeds} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{i.}$$

$$Leaf\ area(cm2) = Leaf\ length \times leaf\ width \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{ii.}$$

4.2.5 Data analysis

Shapiro-wilk test was performed for normality test. The arcsine data transformation was done prior data analysis. Data analysis was performed based on the factorial experiment's arrangement in a RCBD analysis of variance (ANOVA) model. GenStat (16^{th} version, VSN International) was the software used in data transformation and analysis. Tukey's test ($p<0.05$) was used to separate means. Correlation analysis was also performed using Microsoft excel (2016).

The statistical model used was;

$$Y = \mu + A + B + C + AB + AC + BC + ABC + \varepsilon \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{iii}$$

Where μ is grand mean, ε is a random error term A=Seed types, B=Fungicides, C=Extraction solvents.

4.3 Results

4.3.1 Days to first seedling emergence

In Table 4.1 below, results show that there were highly significant differences between fungicides ($p<0.001$) and extraction solvents ($p<0.001$) while seed types had insignificant effects ($p=0.114$) on number of days to first seedling emergence.

Table 4.1: Effects of seed types, fungicides and extraction solvents on number of days to first emergence

Factor: Seed types (A)	**Days to first emergence**
Certified STAHA	4.08
Farmer-saved STAHA	4.38
p-value	**0.114**
Factor: Fungicides (B)	
Neem	4.67 b
Ginger	4.33 b
Coffee	4.42 b
Apron Star® 42 WS	3.50 a
p-value	**<0.001**
Factor: Extraction solvents C	
Water	3.54
Ethanol	4.92
p-value	**<0.001**

Means with the same letters along the same column are not significantly different ($p<0.05$)

From Table 4.2 below, it was found that, the interaction between seed types and fungicides (A*B) had significant effect ($p=0.005$) on number of days to first seedling emergence. Seedlings of farmer-saved STAHA treated with coffee emerged 3.67 days after sowing while certified STAHA seeds treated with coffee emerged 5.17 days after sowing. On the other hand, both seed type treated with Apron Star® 42 WS used the same number of days to first seedling emergence (3.50 days). When fungicides (B*C) interacted with extraction solvents, a significant difference ($p<0.001$) in number of days to first seedling emergence was observed (Table 4.2). Number of days were higher in ethanol-extracted bio-fungicides (6.00 days, 5.00 days and 5.67 days for neem, ginger and coffee respectively) than in

water-extracted bio-fungicides (3.33 days, 3.67 days and 3.17 days for neem, ginger and coffee respectively). When Apron Star® 42 WS and water were used, the number of days were 4.00 days and 3.00 days respectively. The A×C treatment combination was not significantly different (p=0.254).

Table 4.2: Effects of two-way interaction among maize seed types, fungicide and extraction solvents on number of days to first emergence

Seed types*Fungicides (A*B)	**Days to first emergence**
Certified STAHA*Neem	4.50 ab
Certified STAHA*Ginger	4.33 ab
Certified STAHA*Coffee	5.17 b
STAHA*Apron Star® 42 WS	3.50 a
Farmer-saved STAHA*Neem	4.83 b
Farmer-saved STAHA*Ginger	4.33 ab
Farmer-saved STAHA*Coffee	3.67 a
Farmer-saved STAHA*Apron Star® 42 WS	3.50 a
p-value	**0.005**
Seed types*Extraction solvents (A*C)	
Certified STAHA*Water	3.58
Certified STAHA*Ethanol	5.17
Farmer-saved STAHA*Water	3.5
Farmer-saved STAHA*Ethanol	4.67
p-value	**0.254**
Fungicides*Extraction solvents (B*C)	
Neem*Water	3.33 a
Neem*Ethanol	6.00 c
Ginger*Water	3.67 a
Ginger*Ethanol	5.00 bc
Coffee*Water	3.17 a
Coffee*Ethanol	5.67 c
Apron Star® 42 WS	4.00 ab
Water	3.00 a
p-value	**<0.001**

Means with the same letters along the same column are not significantly different (p<0.05)

There was a significant difference (p=0.031) in number of days to first emergence of maize seedlings\z due to three-way interaction among seed types, fungicides and extraction solvents (A*B*C). (Table 4.3).

For instance, minimum numbers of days to first emergence for seedlings from farmer-saved STAHA seeds treated with water-extracted coffee were 3 days and those treated with ethanol-extracted coffee were 4.33 days. In contrast, the maximum number of days to first emergence for seedlings from both certified STAHA and farmer-saved STAHA treated with water-extracted ginger were 3.67 days and STAHA treated with ethanol-extracted coffee were 7 days. Number of days to first seedling emergence for both the seed types was significantly the same when Apron Star® 42 WS (4 days) and water (3 days) were used.

Table 4.3: Effects of a three-way interaction among seed types, fungicides and extraction solvents on number of days to first emergence

Seed types*Fungicides*Extraction solvents (A*B*C)	Days to first emergence
Certified STAHA*Neem*Water	3.33 ab
Certified STAHA*Neem*Ethanol	5.67 cde
Certified STAHA*Ginger*Water	3.67 ab
Certified STAHA*Ginger*Ethanol	5.00 bcd
Certified STAHA*Coffee*Water	3.33 ab
Certified STAHA*Coffee*Ethanol	7.00 e
Farmer-saved STAHA*Neem*Water	4.00 abc
Farmer-saved STAHA*Neem*Ethanol	3.00 a
Farmer-saved STAHA*Ginger*Water	3.33 ab
Farmer-saved STAHA*Ginger*Ethanol	6.33 de
Farmer-saved STAHA*Coffee*Water	3.67 ab
Farmer-saved STAHA*Coffee*Ethanol	5.00 bcd
Certified STAHA*Apron Star® 42 WS	3.00 a
Certified STAHA*Water	4.33 abc
Farmer-saved STAHA*Apron Star® 42 WS	4.00 abc
Farmer-saved STAHA*Water	3.00 a
p-value	**0.031**

Means with the same letters along the same column are not significantly different ($p<0.05$)

4.3.2 First, second and third seedling emergences

There were significant differences ($p<0.001$) between main factors (seed types, fungicides and extraction solvents on both the seedling emergence counts (Table 4.4). The first, second and third counts of emerged seedling were consistently higher for farmer-saved

STAHA than certified seeds of the same variety. Influence of all the bio-fungicides on the 1st, 2nd and 3rd counts of seedling emergence were significantly lower than the chemical fungicide, Apron Star. The 1st, 2nd, and 3rd counts of seedling emergence were significantly influenced by type of extraction solvent whereby the values due water solvent were higher than values for ethanol (Table 4.4).

Table 4.4: Effects of seed types, fungicides and extraction solvents on seedling emergence counts

Factor: Seed types (A)	**1st Count**	**2nd Count**	**3rd Count**
Certified STAHA	14.37	16.92	18.58
Farmer-saved STAHA	18.42	20.92	22.5
p-value	**<0.001**	**<0.001**	**<0.001**
Factor: Fungicides (B)			
Neem	12.42 a	14.25 a	16.33 a
Ginger	13.08 a	15.42 a	17.42 a
Coffee	12.33 a	14.58 a	16.83 a
Apron Star® 42 WS	27.75 b	31.42 b	31.58b
p-value	**<0.001**	**<0.001**	**<0.001**
Factor: Extraction solvents C			
Water	25.08	28.12	28.88
Ethanol	7.71	9.71	12.21
p-value	**<0.001**	**<0.001**	**<0.001**

Means with the same letters along the same column are not significantly different ($p<0.05$)

From Table 4.5 below, a difference was observed for the interaction between seed types and fungicides (A*B) ($p=0.046$) in number of seedlings emerged on the first count was observed. Lower numbers of emerged seedlings were found in farmer-saved STAHA treated with neem (14.67) and coffee (13.00) and certified STAHA treated with neem (10.17), ginger (10.50) and coffee (11.67). on the other side, higher numbers were observed in all two seed types treated with Apron Star® 42 WS (25.17 and 30.33 for STAHA and farmer-saved STAHA) followed by farmer-saved STAHA treated with ginger (15.67). A significant effect ($p<0.001$) on first seedling emergence was also

observed due to fungicide-extraction solvent interactions (B*C). Water-extracted bio-fungicides had higher number of emerged seedlings (24.83, 23.33 and 24 for neem, ginger and coffee respectively) than ethanol-extracted-bio-fungicides (0, 2.83 and 0.67 for neem, ginger and coffee respectively). Apron Star® 42 WS (positive control) and water (negative control) had significantly the same number of emerged seedlings (28.17 and 27.33 respectively) (Table 4.5). The interaction between seed types and extraction solvents (A×C) was insignificantly different on the first count. For the second count, the seed types and extraction solvents combination (A×C) was insignificantly different ($p=0.144$). But there were significant differences ($p=0.008$ and $p<0.001$) for the interactions (A*B and B*C respectively) in second seedling emergence. Except seed treatment with Apron star, all the seed types treated with bio-fungicides had significantly the same seedling counts (Table 4.5). Water-extracted bio-fungicides resulted into larger number of emerged seedlings than ethanol-extracted bio-fungicides. But also, Apron Star® 42 WS and water gave significantly large number of emerged seedlings. On the other hand, there was a significant difference for the interaction between fungicides and extraction solvents (B*C) on third seedling emergence ($p<0.001$).

Numbers of emerged seedlings were significantly larger and similar when water-extracted bio-fungicides, Apron Star® 42 WS and water were used (Table 4.5). But treatment combinations (A×C and A×B) were insignificantly different ($p=0.215$ and $p=0.052$ respectively).

Table 4.5: Effects of two-way interaction among maize seed types, fungicides and extraction solvents on seedling emergence counts

Seed types*Fungicides (A*B)	1st Count	2nd Count	3rd Count
Certified STAHA*Neem	10.17 a	12.33 a	14.67
Certified STAHA*Ginger	10.50 a	12.67 ab	15.17
Certified STAHA*Coffee	11.67 a	13.17 a	14.83
Certified STAHA*Apron Star® 42 WS	25.17 c	29.50 d	29.67
Farmer-saved STAHA*Neem	14.67 a	16.17 a	18
Farmer-saved STAHA*Ginger	15.67 b	18.17 c	19.67
Farmer-saved STAHA*Coffee	13.00 a	16.00 bc	18.83
Farmer-saved STAHA*Apron Star® 42 WS	30.33 c	33.33 d	33.5
p-value	**0.046**	**0.008**	**0.052**
Seed types*Extraction solvents (A*C)			
Certified STAHA*Water	22.33	25.58	26.17
Certified STAHA*Ethanol	6.42	8.25	11
Farmer-saved STAHA*Water	27.83	30.67	31.58
Farmer-saved STAHA*Ethanol	9	11.17	13.42
p-value	**0.363**	**0.144**	**0.215**
Fungicides*Extraction solvents (B*C)			
Neem*Water	24.83 c	28.00 c	29.00 cd
Neem*Ethanol	0.00 a	0.50 a	3.67 a
Ginger*Water	23.33 c	26.33 c	27.33 c
Ginger*Ethanol	2.83 b	4.50 b	7.50 b
Coffee*Water	24.00 c	27.00 c	27.83 cd
Coffee*Ethanol	0.67 a	2.17 a	5.83 ab
Apron Star® 42 WS	28.17 c	31.17 c	31.33 cd
Water	27.33 c	31.67 c	31.83 d
p-value	**<0.001**	**<0.001**	**<0.001**

Means with the same letters along the same column are not significantly different ($p<0.05$)

There was a significant difference ($p=0.003$) between treatment combination (seed types, fungicides and extraction solvents) on first seedling emergence. Seed types treated with water-extracted bio-fungicides had significantly higher number of first counts than those treated with ethanol-extracted bio-fungicides. No significant differences were observed among seed types treated with water-extracted bio-fungicides. But for seed types treated with ethanol-extracted bio-fungicides, only farmer-saved STAHA treated with ginger had higher number of emerged seedlings (5.33), others had similarly lower numbers. Again, no difference was found when seed types were treated with Apron Star® 42 WS and water

(Table 4.6). Results in Table 4.5 below also found highly significant difference ($p<0.001$) between treatment combinations (seed types, fungicides and extraction solvents) in second count of seedling emergence. Percentages of emerged seedlings from seeds treated with water-extracted bio-fungicides (regardless of the seed type) were observed to be significantly higher than from seeds treated with ethanol-extracted bio-fungicides. The percentage emergences of farmer-saved STAHA seeds treated with water extracted bio-fungicides of 32%, 29% and 27.67% for neem, ginger and coffee respectively were higher than those treated with ethanol-extracted bio-fungicides of 0.33% followed by 4.33% and 7.33% for neem, coffee and ginger respectively. On the other hand, percentage emergences for STAHA seeds treated with water-extracted bio-fungicides of 24%, 23.67% and 26.33% treated with neem, ginger and water respectively were higher than those treated with ethanol-extracted bio-fungicides of 0%, 0.67% and 1.67% treated with coffee, neem and ginger respectively.

Both STAHA and farmer-saved STAHA had statistically the same percentage emergences of 28.33% and 34% respectively when treated with Apron Star® 42 WS (positive control) and 30.67% and 32.67% respectively when treated with water (negative control). Moreover, due to interaction among seed types, fungicides and extraction solvents interacted, it was found that there was a significant influence on the third count seedling emergence ($p<0.001$). Highest counts were seen from seed types treated with water-extracted bio-fungicides, Apron Star® 42 WS and water. In contrast, lowest seedling emergence counts were seen from seed types treated with ethanol-extracted bio-fungicides (Table 4.6).

Table 4.6: Effects of a three-way interaction among type of seeds, fungicides and extraction solvents on seedling emergence counts

Seed types*Fungicides*Extraction solvents (A*B*C)	1st Count	2nd Count	3rd Count
Certified STAHA*Neem*Water	20.33 c	24.00 e	24.67 d
Certified STAHA*Neem*Ethanol	0.00 a	0.67 ab	4.67 ab
Certified STAHA*Ginger*Water	20.67 c	23.67 e	24.67 d
Certified STAHA*Ginger*Ethanol	0.33 a	1.67 bc	5.67 bc
Certified STAHA*Coffee*Water	23.33 cd	26.33 ef	26.67 de
Certified STAHA*Coffee*Ethanol	0.00 a	0.00 a	3.00 ab
Farmer-saved STAHA*Neem*Water	25.00 cd	28.33 ef	28.67 def
Farmer-saved STAHA*Neem*Ethanol	25.33 cd	30.67 ef	30.67 def
Farmer-saved STAHA*Ginger*Water	29.33 cd	32.00 ef	33.33 ef
Farmer-saved STAHA*Ginger*Ethanol	0.00 a	0.33 ab	2.67 a
Farmer-saved STAHA*Coffee*Water	26.00 cd	29.00 ef	30.00 def
Farmer-saved STAHA*Coffee*Ethanol	5.33 b	7.33 d	9.33 c
Certified STAHA*Apron Star® 42 WS	24.67 cd	27.67 ef	29.00 def
Certified STAHA*Water	1.33 a	4.33 cd	8.67 c
Farmer-saved STAHA*Apron Star® 42 WS	31.33 d	34.00 f	34.00 f
Farmer-saved STAHA*Water	29.33 cd	32.67 ef	33.00 ef
p-value	**0.003**	**<0.001**	**<0.001**

Means with the same letters along the same column are not significantly different ($p<0.05$)

4.3.3 Final seedling emergence

Table 4.7 below shows that, there were highly significant differences ($p<0.001$) between main factors (seed types, fungicides and extraction solvents) on the final seedling emergence.

Table 4.7: Effects of type of seeds, fungicides and extraction solvents on final seedlings emergence

Factor: Seed types (A)	% Emergence
Certified STAHA	46.46
Farmer-saved STAHA	56.25
p-value	**<0.001**
Factor: Fungicides (B)	
Neem	40.83 a
Ginger	43.54 a
Coffee	42.08 a
Apron Star® 42 WS	78.96 b
p-value	**<0.001**
Factor: Extraction solvents C	
Water	72.19
Ethanol	30.52
p-value	**<0.001**

Means with the same letters along the same column are not significantly different ($p<0.05$)

For the interactions, A×C and A×B there were no significantly differences (p=0.054 and p=0.81 respectively) in percent seedlings emergence. Highly significant difference (p<0.001) was observed on the final seedling emergence due to fungicides extraction interactions. Higher percent emergence was observed when water-extracted bio-fungicides, Apron Star® 42 WS and water were used in seed treatments (Table 4.8).

Table 4.8: Effects of two-way interaction among seed types, fungicides and extraction solvents on final emerged seedlings

Seed types*Fungicides (A*B)	**% Emergence**
Certified STAHA*Neem	36.67
Certified STAHA*Ginger	37.92
Certified STAHA*Coffee	37.08
Certified STAHA*Apron Star® 42 WS	74.17
Farmer-saved STAHA*Neem	45
Farmer-saved STAHA*Ginger	49.17
Farmer-saved STAHA*Coffee	47.08
Farmer-saved STAHA*Apron Star® 42 WS	83.75
p-value	**0.81**
Seed type*Extraction solvents (A*C)	
Certified STAHA*Water	65.42
Certified STAHA*Ethanol	27.5
Farmer-saved STAHA*Water	78.96
Farmer-saved STAHA*Ethanol	33.54
p-value	**0.054**
Fungicides*Extraction solvents (B*C)	
Neem*Water	72.50 c
Neem*Ethanol	9.17 a
Ginger*Water	68.33 c
Ginger*Ethanol	18.75 b
Coffee*Water	69.58 c
Coffee*Ethanol	14.58 ab
Apron Star® 42 WS	78.33 c
Water	79.58 c
p-value	**<0.001**

Means with the same letters along the same column are not significantly different (p<0.05)

Table 4.9 below show that, significant difference (p=0.003) was observed between interaction effects (seed types, fungicides and extraction solvents) in percentage of emerged seedlings. Generally, the percentages of seedlings emergence from seeds treated

with water-extracted bio-fungicides were higher than those from seeds treated with ethanol-extracted bio-fungicides.

For instance, the percentages (66.7%, 61.67% and 61.67 for coffee, ginger and neem respectively) of seedling emergence for certified STAHA treated with water-extracted bio-fungicides were higher than those (7.5%, 14.17% and 11.67% for coffee, ginger and neem respectively) from seedlings treated with ethanol-extracted bio-fungicides. Again, seedling emergence percentages (83.33%, 75% and 72.5% for neem, ginger and coffee respectively) for farmer-saved STAHA treated with water-extracted bio-fungicides were higher than the percentage (21.67%, 23.33% and 6.67% for coffee, ginger and neem respectively) seedling emergence of seeds treated with ethanol-extracted bio-fungicides. On the other hand, there were insignificant differences between certified STAHA and farmer-saved STAHA treated with Apron Star® 42 WS (71.67% and 85% respectively) and treated with water (76.67% and 82.5% respectively).

Table 4.9: Effects of a three-way interaction among seed types, fungicides and extraction solvents on percentage of final emerged seedlings

Seed types*Fungicides*Extraction solvents (A*B*C)	**% Emergence**
Certified STAHA*Neem*Water	61.67 b
Certified STAHA*Neem*Ethanol	11.67 a
Certified STAHA*Ginger*Water	61.67 b
Certified STAHA*Ginger*Ethanol	14.17 a
Certified STAHA*Coffee*Water	66.67 bc
Certified STAHA*Coffee*Ethanol	7.50 a
Farmer-saved STAHA*Neem*Water	71.67 bcd
Farmer-saved STAHA*Neem*Ethanol	76.67 bcd
Farmer-saved STAHA*Ginger*Water	83.33cd
Farmer-saved STAHA*Ginger*Ethanol	6.67 a
Farmer-saved STAHA*Coffee*Water	75.00 bcd
Farmer-saved STAHA*Coffee*Ethanol	23.33 a
Certified STAHA*Apron Star® 42 WS	72.50 bcd
Certified STAHA*Water	21.67 a
Farmer-saved STAHA*Apron Star® 42 WS	85.00 d
Farmer-saved STAHA*Water	82.50 cd
p-value	**0.003**

Means with the same letters along the same column are not significantly different ($p<0.05$)

4.3.4 Seedling vigor parameters

Results in Table 4.10 indicate that there were highly significant differences ($p<0.001$) between main factors (seed types and extraction solvents) and insignificant difference ($p=0.07$) between fungicides in seedling height. Except fungicides with $p=0.733$, the remaining main factors (seed types and extraction solvents) had significant differences ($p<0.001$) in leaf area. Moreover, seed types and extraction solvents were the only factors with significant differences ($p=0.002$ and $p<0.001$ respectively) in seedling weight (Table 4.10).

Table 4.10: Effects of seed types, fungicides and extraction solvents on seedling height, leaf area and seedling weight

Factor: Seed types (A)	**Seedling height (cm)**	**Leaf area (cm^2)**	**Seedling weight (g)**
Certified STAHA	16.94	254	39.4
Farmer-saved STAHA	22.29	361	59.2
p-value	**<0.001**	**<0.001**	**0.002**
Factor: Fungicides (B)			
Neem	19.97	318.5	51.08
Ginger	19.38	301.4	46.25
Coffee	18.02	290.1	49.88
Apron Star® 42 WS	21.09	320.6	50.08
p-value	**0.07**	**0.733**	**0.941**
Factor: Extraction solvents C			
Water	22.21	365	61.7
Ethanol	17.02	250	37
p-value	**<0.001**	**<0.001**	**<.001**

There was a significant difference ($p=0.002$) for the interaction between fungicides and extraction solvents on seedling height. It was found that, seedling height was higher when water-extracted bio-fungicides, Apron Star® 42 WS and water were used in seed treatment (Table 4.11). But interactions (A×B and A×C) had no significant difference ($p=0.162$ and $p=0.988$ respectively). All the interactions (A×B, A×C and B×C) were insignificant difference ($p=0.857$, $p=0.742$ and $p=0.068$) on leaf area. But also, they were. insignificant difference ($p=0.432$, $p=0.868$ and $p=0.183$) on seedling weight.

Table 4. 11: Effects of two-way interaction among maize seed types, fungicides and extraction solvents on plant height, leaf area and plant weight

Seed types*Fungicides (A*B)	Seedling height (cm)	Leaf area (cm^2)	Seedling weight (g)
Certified STAHA*Neem	18.72	280	46.67
Certified STAHA*Ginger	16.93	250.2	40.67
Certified STAHA*Coffee	14.55	225.5	35.15
Certified STAHA*Apron Star® 42 WS	17.55	262.2	35.17
Farmer-saved STAHA*Neem	21.22	357.1	55.5
Farmer-saved STAHA*Ginger	21.83	352.6	51.83
Farmer-saved STAHA*Coffee	21.48	354.7	64.62
Farmer-saved STAHA*Apron Star® 42 WS	24.63	379	65
p-value	**0.162**	**0.857**	**0.432**
Seed types*Extraction solvents (A*C)			
Certified STAHA*Water	19.54	308.1	52.24
Certified STAHA*Ethanol	14.33	200.9	26.58
Farmer-saved STAHA*Water	24.88	421.8	71.08
Farmer-saved STAHA*Ethanol	19.7	299.9	47.39
p-value	**0.988**	**0.742**	**0.868**
Fungicides*Extraction solvents (B*C)			
Neem*Water	21.55 bc	408.8	66
Neem*Ethanol	14.48 a	228.2	36.17
Ginger*Water	23.23 c	363.4	62.17
Ginger*Ethanol	15.53 a	239.3	30.33
Coffee*Water	23.33 c	360.5	67.48
Coffee*Ethanol	16.60 ab	219.7	32.28
Apron Star® 42 WS	20.73 bc	327	51
Water	21.45 c	314.2	49.17
p-value	**0.002**	**0.068**	**0.183**

Means with the same letters along the same column are not significantly different ($p<0.05$)

From the interaction (seed types, fungicides and extraction solvents), it was found that there was a slight difference ($p=0.047$) in leaf area (Table 4.12). Maize seedlings from seeds treated with ethanol-extracted bio-fungicides were observed to have lower mean leaf area than those treated with water-extracted bio-fungicides.

For instance, STAHA treated with ethanol-extracted coffee had lower leaf area (154.2 cm^2) than that of farmer-saved STAHA treated with water-extracted neem which had

495.3 cm^2 (Table 4.12). But insignificant differences were observed on seedling height (p=0.669) and seedling weight (p=0.828).

Table 4. 12: Effects of a three-way interaction among seed types, fungicides and extraction solvents on plant height, leaf area and plant weight

Seed types*Fungicides*Extraction solvents (A*B*C)	Plant height (cm)	Leaf area (cm^2)	Plant weight (g)
Certified STAHA*Neem*Water	21.4	322.3 abc	58.33
Certified STAHA*Neem*Ethanol	16.03	237.7 ab	35
Certified STAHA*Ginger*Water	20.53	300 abc	57
Certified STAHA*Ginger*Ethanol	13.33	200.3 ab	24.33
Certified STAHA*Coffee*Water	18.57	296.8 abc	57.3
Certified STAHA*Coffee*Ethanol	10.53	154.2 a	13
Farmer-saved STAHA*Neem*Water	25.27	495.3 c	73.67
Farmer-saved STAHA*Neem*Ethanol	17.17	218.8 ab	37.33
Farmer-saved STAHA*Ginger*Water	25.93	426.7 bc	67.33
Farmer-saved STAHA*Ginger*Ethanol	17.73	278.4 abc	36.33
Farmer-saved STAHA*Coffee*Water	24.53	424.3 bc	77.67
Farmer-saved STAHA*Coffee*Ethanol	18.43	285.2 abc	51.57
Certified STAHA*Apron Star® 42 WS	17.67	313.2 abc	36.33
Certified STAHA*Water	17.43	211.3 ab	34
Farmer-saved STAHA*Apron Star® 42 WS	23.8	340.9 abc	65.67
Farmer-saved STAHA*Water	25.47	417 bc	64.33
p-value	**0.669**	**0.047**	**0.828**

Means with the same letters along the same column are not significantly different (p<0.05)

4.3.5 Correlation analysis

There were negative correlations between numbers of days to first emergence of maize seedlings with the seedling vigor parameters (Table 4.13).

Table 4.13: Correlation between days to first emergence and seedling vigor parameters

Seedling vigor parameter	Days to first seedling emergence
	r
Plant height	-0.5063
Leaf area	-0.4319
Seedling weight	-0.4088

Key; r = Correlation coefficient

4.3.6 Visual observation

From visual observation, most of chlorosis symptoms and drying of seedlings were found to dominate in seedlings emerged from seeds treated with ethanol-extracted bio-fungicides than those treated with water-extracted bio-fungicides (Fig. 4.1).

Figure 4.1: Seedlings under field conditions. **(a, b and c) Seedlings from seeds treated with ethanol-extracted bio-fungicides and (d) from seeds treated with water-extracted bio-fungicides.**

4.4 Discussion

Results pertaining to emergence of maize seedlings indicate that water-extracted bio-fungicides were not phytotoxic to maize seeds and seedlings. This is evidenced by the number of days to first emergence being lower for seeds treated with water-extracted bio-fungicides than those treated with ethanol-extracted bio-fungicides. But also the final emergences being higher for seeds treated with water-extracted bio-fungicides than those treated with ethanol-extracted bio-fungicides. In contrast, ethanol-extracted bio-fungicides and chemical fungicide (Apron Star® 42 WS) were possibly having phytotoxic effect on maize seeds and seedlings. That is, the bio-fungicides might have contained phytotoxins possibly ethanol residues which could have remained in the bio-fungicides solution due to incomplete evaporation responsible for impaired seed germination and seedlings emergence. This agrees with previous studies (Zida *et al*., 2008; Hubert *et al*., 2015) which reported ethanol and Apron Star® 42 WS to be responsible for reduced seedling emergence.

For the seedling vigor, it was also observed that, seedlings from seeds treated with water-extracted bio-fungicides were vigorously growing than those treated with ethanol-extracted bio-fungicides. This might be related to negative impacts caused by amount of ethanol residues suspected to remain in bio-fungicides possibly due to incomplete evaporation after extraction. Ethanol residues could be responsible for poor seedling growth as the observed chlorosis in seedling could be associated to it. But also compounds found in bio-fungicides had positive effects by promoting seed germination and seedling growth. These are consistent with the study by Keta *et al*. (2019) which reported higher amounts of bioactive compounds in plant extracts. These bioactive compounds not only

manage seed-borne fungi which could limit seedlings growth, but also stimulate seed germination and growth of seedlings (Mbega *et al*., 2012).

Deduction from correlation analysis is that, number of days for seeds to germinate or seedlings to emerge can be reduced by seed treatment with water-extracted bio-fungicides. This can be due to the fact that, seed-borne fungi responsible for reduced seed germination and seedling vigor are managed. But also bio-fungicides might have containing compounds responsible for stimulating plant growth. This agrees with study by Mbega *et al*. (2012).

4.5 Conclusion and recommendation

Study found that, maize seed types treated with water-extracted bio-fungicides from *Z. officinale*, *A. indica* and *C. arabica* had significantly higher emergence percentages and vigorous seedlings than those treated with ethanol-extracted bio-fungicides. The ability of these bio-fungicides to manages seed-borne fungi and improves seed germination without causing negative effects on seedling vigor, point to the potential of using them against seed-borne fungi. The study also found that, seed germination and seedling vigor for maize seeds treated with ethanol-extracted bio-fungicides were poor. Assumption was that, there might be amount of ethanol remained aside after evaporation. Further studies are recommended to identify bioactive compositions contained in bio-fungicides responsible for managing seed-borne diseases, stimulating plant growth as well as their mechanisms of action.

Moreover, proper methods of removing extraction solvents from the extracted bio-fungicides are needed, since bio-fungicides extracted using ethanol or other organic solvents have been reported to contain higher percentages of bioactive compounds (hence highly effective in managing pathogens) than those extracted using water.

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CHAPTER FIVE

5.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Most of farmers use their non-treated farmer-saved seeds recycled from previous harvests. These seeds were found to be contaminated with numerous seed-borne fungi predominantly *F. verticillioides*. But also, high percent of farmer-saved seeds are improperly stored leading to high infection by seed-borne fungi which in turn deteriorate seeds leading to poor seed germination, low seedling vigor and crop stand.

In vitro assay showed that, ethanol-extracted bio-fungicides were more effective than water-extracted bio-fungicides in inhibiting the mycelial fungal growth. But *in vivo* assay found, maize seed germination and seedling vigor to be more improved when water-extracted bio-fungicides were used than when ethanol-extracted bio-fungicides were used. For the bio-fungicides, *A. indica* and *Z. officinale* were more effective than *C. arabica* in managing seed-borne fungi and improving seed germination and seedling vigor. This reflect the potentiality of bio-fungicides in managing seed-borne fungi. Moreover, seed germination and seedling vigor for maize seeds treated with ethanol-extracted bio-fungicides were poor. Assumption behind was that, there might be amount of ethanol assumed to remain aside after evaporation.

5.2 Recommendations

When farmers have to recycle their farmer-saved seeds, handling of seeds from field to stores must be good enough to reduce build-up of seed-borne fungi. Also treating seeds with eco-friendly fungicides before sowing is recommended. On seed treatment, the study recommends the use of bio-fungicides as useful, cost effective and environmentally

friendly management approach against seed-borne fungi. This will minimize the use of chemical fungicides. But also researches to identify bioactive compositions contained in bio-fungicides responsible for managing seed-borne diseases, stimulating plant growth as well as their mechanisms of action are recommended. Moreover, proper methods of removing extraction solvents from the extracted bio-fungicides are needed, since bio-fungicides extracted using ethanol or other organic solvents have been reported to contain higher percentages of bioactive compounds for managing pathogens than those extracted using water.