EFFECT OF INTERCROPPING MAIZE VARIETIES WITH DIFFERENT CROPS ON FUNGAL CONTAMINATION OF MAIZE GRAIN IN DOREBAFANA WOREDA SIDAMA REGION

MSc. THESIS

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HAWASSA UNIVERSITY,

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A THESIS SUBMITTED TO THE HORTICULTURAL SCIENCES, COLLEGE OF AGRICULTURE, SCHOOL OFGRADUATE STUDIES HAWASSA UNIVERSITY
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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE DEGREE IN CROP PROTECTION (SPECIALIZATION, PLANT PATHOLOGY)

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COLLEGE OF AGRICULTURE

SCHOOL OF PLANT AND HORTICULTURAL SCIENCE

ADVISORS' APPROVEL SHEET

(Submission sheet -1)

This is to certify that the thesis entitled **The effect of intercropping Maize varieties with different crops on fungal contamination of maize grain in Dore Bafana Woreda Sidama Region** submitted by Teshale Tafesse Lintamo in partial fulfillment for the requirements of the degree of **Master of Science** with specialization in **Crop Protection**, in Graduate Program of the School of Plant and Horticulture Science, College of Agriculture, and this thesis is my original work carried out under my supervision and no part of a thesis has been submitted for any other degree or diploma. The assistance and help received during the course of this investigation have been duly acknowledged. Therefore, I recommend that it be accepted as fulfilling the thesis requirements for the degree of **Master of Science** in plant science with specialization in **Crop Protection.**

____________________	____________________	____________
Name of major advisor	Signature	Date

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We, undersigned, members of the board of examiners of the final open defense by **Teshale Tefesse Lintamo** have read and evaluated his thesis entitled **Effect of intercropping Maize varieties with different crops on fungal contamination of maize grain in Dore Bafana Woreda (Sidama Region)** and examined the candidate. This is therefore, to certify that the thesis has been accepted in partial fulfillment for the requirements of the degree of **Master of Science** with specialization in **Crop Protection**.

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As members of the Examining Board of the Final MSc Open Defense, we certify that we have read and evaluated the thesis prepared by Teshale Tafesse Lintamo entitled **Effect of intercropping Maize varieties with different crops on fungal contamination of maize grain in Dore Bafana Woreda (Sidama Region)** that it be accepted as fulfilling the thesis requirement for the degree of the Master of Science in Crop Protection.

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…………………………………	..……………………..	…..……………….
Name of Thesis Advisor	Signature	Date

DEDICATION

I dedicate this thesis document to my wife Mrs. Miheret Samuel for treating me with love and for her dedicated partnership in the success of my life.

STATEMENT OF AUTHOR (DECLARATION)

I declare that this thesis is my genuine work and all sources of materials used for this thesis have been properly acknowledged. I seriously declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

Name: Teshale Tafesse **Signature __________**

Place: College of Agriculture, Hawassa University, Hawassa

Date of Submission _____________

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ACRONOMYS AND ABBERVIATIONS

FAO	Food and Agricultural Organization
FAOSTAT	Statistical Database of the Food and Agriculture of the United Nations
CSA	Central Statistical Agency
CIMMYT	International Maize and Wheat Improvement Center
TLB	Turcicum Leaf Blight
GLS	Grey Leaf spot
MCMV	Maize Chlorotic Mottle Virus
SCMV	Sugar Cane Mosaic Virus
MSV	Maize streak virus
MLND	Maize Lethal Necrosis Disease
WSMV	Wheat Streak Mosaic Virus
MDMV	Maize Dwarf Mosaic Virus
FAW	Fall Armyworm
AFB 1	Aflatoxin B 1
AFB 2	Aflatoxin B 2
AFG 1	Aflatoxin G 1
AFG 2	Aflatoxin G 2
RCBD	Randomized Complete Block Design
NPS	Nitrogen phosphorus sulfur
ISTA	International Seed Testing Association
PDA	Potato Dextrose Agar
SNA	Spezieller Nahrspoffarmer Agar
LSD	List Significant Deference
SAS	Statistical Analyses Software

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Effect of intercropping Maize varieties with different crops on fungal contamination of maize grain in Dore Bafana Woreda (Sidama Region)

Teshale Tafesse

Major Advisor: Elfinesh Shikur (PhD), Hawassa University

Abstract

Maize (Zea mays) is attacked by many diseases in the field as well as in the storage. Fungi are among the principal causes of deterioration and yield loss on farmers' maize. The present study was aimed to identify the effect of different crop combinations in the intercropping systems and maize varieties on fungal contamination of maize grain. The experiment was conducted at dorebafana woreda of Sidama region at rukesa suke kebele and the laboratory analysis was conducted at Hawassa University, College of Agriculture, Plant Protection Laboratory. Three maize varieties (Limu, Kurtu and BH 540) and four cropping systems (Maize sole, Maize with haricot bean intercropping, Maize with mung bean intercropping, maize with sweet potato intercropping) were the treatments. The experiment was laid using completely randomized block design (RCBD) with three replications. For each treatment a sample of 100 seeds were tested for fungal contamination using the blotter technique in the laboratory. A total of six fungal genera consisting of nine species of fungi were isolated from maize grains collected from the experimental field. Fusarium spp were the most frequently isolated ones followed by Aspergillus spp. The fungal contaminations of maize grains were significantly different ($p<0.05$) between sol cropping and intercropping treatments. The sol cropping had higher fungal contamination level compared to intercropping treatments. On the other hand, intercropping maize with haricot bean has resulted in the lowest fungal contamination followed by intercropping maize with mung bean. Fusarium spp had resulted highest contamination level followed by Aspergillus spp, Penicillium spp, Alternaria spp, Trichoderma spp, and Rhizopus sp. Low level of fungal frequency was recorded on limu maize variety. Whereas the variety kurtu had the highest level of fungal contamination. The result of the present study revealed that maize cropping system affects the level of fungal contamination. In the present study it is also seen that maize varieties tested vary in their resistance to fungal contamination. Thus, good agronomic practices like intercropping and variety selection can play determinant role in reduction of fungal contamination in maize grain. Based on the finding of present study, intercropping maize with haricot bean and variety limu can be used to reduce effect of maize contamination with fungi.

Key words: Intercropping, fungi, maize, variety

1. INTRODUCTION

Maize (*Zea mays)* belongs to the tribe Maydae, family Poaceae. It was originated in Mexico and Central America (Schnable *et al*., 2009). Maize is cultivated on over 202 million hectares in the world, yielding about 116 million tones with a productivity of 5.75 tones ha^{-1} (FAOSTAT, 2022). It is one of the most important cereal crops cultivated in Ethiopia. The total land areas covered by cereals Ethiopia during 2020/21 wasabout 10 million hectares of this, maize covers 2.5 million hectares (CSA, 2020). The major maize producing regions of Ethiopia are southern, western and southwestern and some northern, northwestern and eastern parts where over 90% of the maize produced are used as food among the low income groups. Although maize is a principal food crop in Ethiopia, its average yield is 3.6 ton ha^{-1}which is lower than the world's average (5.6 t ha^{-1} in 2016) (Bekele *et al.,* 2022). A significant portion of this yield gap is attributable to biotic and abiotic stresses, slow turnover of varieties tolerant or resistant to these stresses and low-level use of improved management and other inputs (Worku *et al.,* 2012; Abate *et al.,* 2017).The low productivity of maize is attributed among others, to frequent occurrence of drought (Gezahegn *et al.,*2012*)* declining of soil fertility, poor agronomic practice, limited use of input, lack of credit facilities, poor seed quality (CIMMYT., 2004), diseases (Temesgen *et al.*, 2012; Ward *et al.,*1997*)* insect pests and weed (Tewabech *et al.,*2012). The moisture content of grain and temperature are the most important factors affecting the growth of potentially mycotoxigenic fungi and spread of infection to the maize grain before and after harvest (Kana *et al.,* 2013).

Many pests and diseases attack maize in the field and during the storage period. Fungi are important ones and rank second as the cause of deterioration and loss of maize (Suleiman *et al.,* 2013). Fungi could cause about 50 – 80 % of damage on farmers' maize during the storage period if conditions are favorable for their development (Dubale *et al*., 2014*)*. The major genera commonly encountered on maize in tropical regions are *Fusarium, Aspergillus* and *Penicillium* (Orsi *et al*., 2000).

Intercropping is the growing of two or more crops in proximity to promote synergism for increased productivity and cropping system diversity (Preston, 2003). Intercropping which is an ancient practice is still used in most of developing countries to maximize crop (Machado, 2009).

The aim of this cropping system is to optimize factors and environmental resources usage, thus leading to an increased yield or output of the mixture (Li w*, et al.,* 2005; Dwivedi *et al.*, 2015).Intercropping offers greater financial stability than sole cropping and is easily practiced by labor-intensive smallholder farms and it is traditional but often the agronomic aspects are less well understood compared with monoculture systems (Lithourgidis *et al*., 2011). Maize-legume intercropping can result in improved soil nutrient and water use, increased productivity, and greater yield stability with reduced risk of crop failure while enabling healthier diets (Elodie *et al*., 2010; Meighen and Marney, 2012). Legume intercropping with maize can be a way to grow a staple cereal crop while benefiting from the legume crop (Seran and Brintha, 2010).Intercropping can also help to reduce damage by pests and diseases by reducing the number of susceptible hosts (dilution effect), resistant plants acting as a physical barrier to susceptible plants (barrier effect), inducing resistance by increasing the diversity of pests and diseases, reducing the speed by pest adaption through disruptive selection, and compensation of one species that performs poorly (Borg *et al.,* 2017). Intercropping generally reduce fungal contamination of maize grain compared with sol cropping (Julian *et al.,* 1995).Varietal resistance plays a vital role in minimizing contamination of grains in different crops including maize. However the impact of intercropping and variety on fungal contamination in the study area has not been studied yet. The present study was therefore designed with the following objectives.

1.2. Objectives

1. To identify the effects of different crop combinations in the intercropping systems on fungal contamination of maize grain.
2. To study the scenario of maize varieties and fungal contamination in maize grain

2. LITERATURE REVIEW

2.1. Importance of maize in Ethiopia

Maize is cultivated in almost all countries of world. In nutritional terms, maize has a carbohydrate-rich composition, mainly in the form of starch, and also has proteins, lipids, vitamins and minerals (Oliveira *et al.,* 2014). One important feature of maize is that grains can be directly consumed, without the need for processing to remove the hull as it is done with other cereals, such as rice and wheat. In sub-Saharan Africa, maize is the most important agricultural commodity as it is the most widely cultivated crop and providing 40 to 50% of the calories and proteins consumed in the region (Prasanna, 2012; Cairns *et al.,* 2013). Further, it is a source of important vitamins and minerals to the human body. Along with rice and wheat, maize provides at least 30 % of the food calories to more than 4.5 billion people in 94 developing countries (Gautam *et al.,*2021).

The food security and economic wellbeing of Ethiopia, in general, depends on agriculture. Maize is considered amongst the top commodities contributing to food security in the country due to its wide adaptability, high production, productivity and relatively cheap calories compared to other cereals. As a result, it has been included in the national food security strategy via intensive agriculture systems (Abate *et al.,* 2015). The crop accounts for 29% of the national calorie intake (Berhane *et al*., 2011). Maize is also considered as a source of income, means of employment for all producers to business communities, while the stalk serves for feed and fuel (Preibisch *et al*., 2002). In agriculture productivity is a prevailing motive for farmers and a driving force in Ethiopia's agricultural policy. Maize is one of the most important cereal crops cultivated in Ethiopia ranking second after teff in area coverage and first in total production (Kassaye *et al*., 2021).

2.2. Production constraints of maize in Ethiopia

Despite the importance of maize as a principal food crop, its average yield in Ethiopia is 3.6 ton per hectare is still lower than that of worlds average (5.6 t /hr in 2016) (Bekele *et al*.,2022). A significant portion of this yield gap is attributable to biotic and abiotic stresses, slow turnover of varieties tolerant or resistant to these stresses and low-level use of improved management and other inputs (Worku *et al*., 2012; Abate *et al*., 2017).Among the biotic stresses, diseases are one of the most important limiting factors in maize production.From these diseases gray leaf spot, Turcicum leaf blight, leaf spot, common leaf rust, eyespot, brown spot, streak virus, and storage disease caused by *Fusarium* spp and *Aspergillus flavus* are the major maize disease in Ethiopia (Tegegne *et al.,* 2009).

2.3. Maize grain contaminating pathogens

Fungi can cause about 50 - 80 % of damage on farmers' maize during the storage period if conditions are favorable for their development(Dubale *et al*.,2014).During storage, several kinds of fungi can remain associated to maize seeds either causing their deterioration or simply remain viable to infect germinating seedlings. The fungi genera typically found in stored grains are *Aspergillus, Penicillium, Fusarium* (Orsi *et al*.,2000*;* Castlellarie *et al*.,2010) and several of them with capabilities of producing toxins (Castlellarie *et al .,*2010). Maize ear rot is one of the most important diseases wherever maize is grown (Gxasheka *et al*., 2015). The common ear rot diseases of maize are *Aspergillus* and *Fusarium* ear rot/kernel rot (Mesterházy *et al*., 2012; Gxasheka *et al*., 2015). Two types of maize ear rot are associated with *Fusarium* species, namely *Fusarium* ear rot, caused by *F. proliferatum*, *F. subgulitnans*, and *F. verticillioides*, and Gibberella ear rot caused by *F. avenaceaum*, *F. cerealis*, *F. culmorum*, and *F. graminearum* (Mesterházy *et al*., 2012).

Many ear rot fungi grow more vigorously, and produce higher levels of mycotoxins, under certain environmental conditions, which often include high temperatures and drought stress. It is thus suggested that increased temperatures and erratic rainfall due to global climate change may exacerbate the problem (Wu *et al*., 2011). Mycotoxins are low-weight molecular secondary metabolites mainly produced in the mycelial structures of certain filamentous fungi, during fungal growth (D'Mello and Macdonald 1997; Placinta *et al.* 1999; Leslie and Summerell, 2006; Bhat *et al*., 2010). These secondary metabolites are toxic to humans and animals when consumed and can even be carcinogenic, neurotoxic,nephrotoxic or immunosuppressive especially when chronic exposure occurs (Gelderblom *et al*., 1988; Hussein and Brasel, 2001; Lazicka and Orzechowski, 2010; FeijóCorrêa *et al*., 2018). The most common type of exposure in humans and animals is by consumption of contaminated feedstuffs or foods (Rheeder *et al*., 1992; Bhat *et* *al*.. 2010; Mwanza, 2011). Fungi known to produce mycotoxins are referred to as toxigenic fungi. This group is dominated by three genera, namely *Aspergillus, Penicillium* and *Fusarium*, and to a lesser extent other genera that include *Alternaria*, *Claviceps* and *Stachybotrys* (Bennett and Klich, 2003; Wambacq *et al*., 2016).*Fusarium* species represent a number of plant pathogens, and are responsible for infection before and during harvesting, while *Penicillium* and *Aspergillus* more commonly colonize commodities and foods during drying and storage (Miller, 2008; Osibona *et al*., 2018).

2.4. Management of grain contaminating pathogens

The prevention of mycotoxin contamination of agricultural products is an important priority in human and animal safety. Strategies to control mycotoxin producing fungi at pre-harvest stages include improved crop management and agronomic practices, control of insects that favor fungal infection,host plant resistance and biological control (e.g. non aflatoxigenic antagonistic strains) (Mauro *et al*., 2018).

2.4.1. Cultural methods

Intercropping

Intercropping which is an ancient practice still used in most of developing countries to maximize crop productivity (Machado, 2009). It basically consists growing of two or more crops in the same area of land at the same time. Maize-legume intercropping can result in improved soil nutrient and water use, increased productivity, and greater yield stability with reduced risk of crop failure while enabling healthier diets (Elodie *et al*., 2010; Meighen and Marney, 2012). These advantages are important in low-input, risk vulnerable, and land scarce semi-subsistence farming systems (Rezaei- Chianeh *et al*., 2011). Legume intercropping with maize can be a way to grow a staple cereal crop while benefiting from the legume crop (Seran and Brintha, 2010). Intercropping, i.e., the mixed cultivation of two (or more) crop species on the same field Li L *et al*., 2002), is a crop diversification strategy which allows lowering inputs while achieving higher crop yields than expected based on the sole crop yields of the constituent species (Tamburini *et al*., 2020).More generally, intercrops can diminish the damage by pests and diseases by reducing the number of susceptible hosts (dilution effect), resistant plants acting as a physical barrier to susceptible plants (barrier effect), inducing resistance by increasing the diversity of pests and diseases, reducing the speed by pest adaption through disruptive selection, and compensation of one species that performs poorly (Borg *et al.,* 2017).

Mung bean (*Vigna radiata* (L.)Wilczek) is an eco-friendly food grainleguminous crop of dry land areas with rich source of proteins, vitamins, and minerals (Keatinge *et al.*, 2011).According to CSA (2020) report, the estimated area under mungbean in Ethiopia during the main cropping season of 2019/2020 cropping season was 49,123.52 ha with productivity of 1136 Kg ha^{-1}.Mung bean is a quick crop, requiring 75-90 days to mature. It is a useful crop in drier areas and has a good potential for crop rotation, relay cropping and intercropping with cereals (Singh, 2014). Maize and mung bean intercropping can reduce the risk of crop failure that could result from terminal moisture deficit, as mung bean matures early relative to maize. However, the yield in intercropping system depends on selection of compatible genotypes with suitable characters for establishment of minimum competition and maximum complementarily (Mutungamiri *et al.,* 2001).

Haricot bean (*Phaseolus vulagris* L.) is a very important legume crop and it is an annual crop. Haricot bean is grown predominantly under smallholder producers as an important food crop and source of cash.It is one of the fast expanding legume crops that provide an essential part of the daily diet and foreign earnings for most Ethiopians (Girma, 2009).Haricot bean is a principal food crop particularly in Southern and Eastern part of Ethiopia, where it is widely intercropped with maize and sorghum, respectively, to supplement farmers' income (EPPA, 2004). It also used for different cropping systems and is compatible for intercropping with maize and sorghum and for alley cropping with perennial leguminous shrubs which can also improve soil fertility. This system can produce an additional biomass, which can be used for fodder or for mulching or for green manuring without any significant grain yield reduction of haricot beans (Daniel et al., 2012).Maize haricot bean intercropping is one of the commonly practiced agronomic practices in Ethiopia. Maize and haricot bean plays key role in human nutrition as a food and economic value for small and large scale farmers. Maize contains many nutrient factors and is highly nutritious (Dahmardeh *et al*., 2009).

Hybrid maize-sweet potato intercropping is compatible as they possess different photosynthetic pathways, different growth habit and requirement of different growth resources. In the production of sweet potato most farmers practice relay cropping with maize where sweet potato is planted when maize is approaching physiological maturity. Rarely do they practice intercropping to maximize on time and space (Islam *et al*., 2007).

Use of resistant varieties

It is well known that the use of resistant varieties is the cheapest, environmental friendly and sustainable method of disease management. Breeding programs(in South Africa)have reported sources of resistance against *A. flavus* preventing aflatoxin contamination and against *F. verticillioides* preventing fumonisin production, but varieties resistant to all mycotoxigenic fungi are currently not available(Small *et al.,* 2012*)*. Insect resistance is also a valuable trait to avoid infection, because insect damage facilitates the entry of fungi into and subsequent infection of maize plants (Kamika *et al*., 2016*)*.The farmers' systems of seed supply and crop development form by far the most important source of seed in most farming systems of the world. Despite the efforts of large seed programms to replace the farmers' seed systems for a system in which farmers use seed as an external input, the major part of agricultural land in the world is still sown with seed that is informally produced by farmers. Aiming for a formal seed sector that supplies 100% of the seed for planting is only realistic for a small number of crops and in few countries(Almekinders, and Louwaars, 2002),as well as increase adoption of improved maize varieties by smallholder farmers(Tanzania) (Mmbando, & Baiyegunhi, 2016).

Biological

Biological control is the use of biological agents for the control as pests. It is ent to the development of pest resistance than the conventional chemical and has advantages such as minimum or no residual toxicity and environmental pollution (Lazarovits *et al*., 2014).The production of volatile and non-volatile antibiotics, cell wall-degrading enzymes, hyper parasitism on pathogenic organisms, promotion of plant growth, and induction of systemic resistance in the host plant have been reported as their action mechanisms (Al Raish *et al.,* 2021). The effectiveness of two *Streptomyces* spp. strains to control pathogenic fungi was studied in stored maize grain. The treatments included seed disinfection and inoculation with *Streptomyces* spp. strains previously isolated from maize rhizosphere. Treatments with *Streptomyces* spp. strains alone effectively suppressed the development of *Aspergillus* spp., *Curvularia lunata*, and

Drechslera maydis and significantly (p< 0-05) reduced the incidence of *Fusarium subglutinans*and *Cephalosporium acremonium*. Among the inoculation treatments, non-disinfested seed inoculated with filtered suspension was the only treatment that did not suppress the development of *Penicillium s*pp. Maize seed inoculation with total suspension of strains was the most effective treatment to control the incidence of seed pathogenic fungi (Bressan, 2003).

Chemical

Chemical management of several plant diseases remains the main strategy to reduce the incidence of many plant pathogens in most crops. The use of fungicides has become an essential part of modern agriculture (Brent and Hollomon, 2007). However, control of mycotoxin producing fungi with fungicides is not widely used, because of the cost, the difficulty of applying agrochemicals at the right crop stage to restrict colonization and dispersal by mycotoxigenic fungi to avoid mycotoxin contamination, and because extensive field evaluation has not been carried out. However, if fungicides are applied as pre-harvest treatments at the appropriate crop growth stages(i.e. during anthesis), these can be an efficient, cost effective strategy for preventing mold growth, and consequent mycotoxin production (Santos, 2011).Several previous studies have shown that fungicides from several groups (including anilinopyrimidines, benzimidazoles, dicarboximides, phenylpyrroles,strobilurins and triazoles) can inhibit the growth and the mycotoxin production in *Aspergillus, Fusarium* and *Penicillium* species, although most of these studies report in vitro results. Field studies that report effective chemicals for control of aflatoxins in maize are lacking (Mateo *et al*., 2017).

Integrated approach

Methods for reduction **of fumonisins** in maize are applied pre-harvest or during harvesting and processing (Wild and Gong, 2010). These include several existing strategies to reduce *Fusarium* growth and production of fumonisins in food sources, i.e., controlled agricultural practices, ensiling strategies, breeding for insect and fungal resistance in maize cultivars, various physical-, chemical-, and biological treatment methods and genetic engineering approaches. Good agricultural management and hazard analysis and critical control point practices promote the general condition of crops, reducing but not eliminating fungal growth, and mycotoxin contamination, while resistance breeding strives to achieve a balance between developing resistant crops and maintaining high quality crop yield (Cleveland et al., 2003; Wild and Gong, 2010).The prevention of mycotoxin contamination of agricultural products is an important priority in human and animal safety. Strategies to control mycotoxin producing fungi at pre-harvest stages include improved crop management and agronomic practices, control of insects that favor fungal infection, host plant resistance and biological control (e.g. non-aflatoxigenic antagonistic strains) (Smith, 1997; Abbas *et al.*, 2007; Mauro *et al.*, 2018).

3. MATERIALS AND METHODS

3.1. Description of study area

The experiment was conducted in Dore Bafana Woreda, Sidama region during 2021 cropping season. The study area is located between 7° 00' 0.00" N latitude and 38° 29' 59.99" E longitude, at distance of about 21Km from Hawasa with an altitude range of 560–2300 meters above sea level. The area is characterized by moist to sub humid warm subtropical climate. Annual precipitation ranges from 750 to 1200mm in a bimodal distribution pattern, expected in March to April and June to August (Dessie and Kleman, 2007). Maize is the common crop produced in the area. The experiment was conducted from May-October, 2021.

3.2. Treatments and Experimental Procedures

The experiment consisted of the commonly used maize varieties in the study area, Limu, BH540 and Kurtu as varietal treatment and four cropping systems as intercropping treatments. The treatment combinations are presented in Table 1. The experimental design for the experiment was Randomized Complete Block Design (RCBD) with three replications. Blocks and plots were separated by 1m and 0.5m, respectively. The plot size was 4x1.25m and the total experimental area was 60mx20m (1200m^2). The varietal treatments were planted at inter and intra-row spacing of 80 cm and 30 cm, respectively, both in sole and intercropping systems. Mung bean and Haricot bean were planted within the available space between maize rows at inter-row spacing of 40x10 and Sweet potato was planted within the available space between maize rows at maize.

NPS Blended fertilizer (N 32%; P2O5 46%; S 8.5%) was applied at a rate of 121Kg/ha (0.0605kg/plot) at planting with 89kg (0.0445kg/plot) of urea, (N 41%). The time of planting, weeding and harvesting was as per the recommendation for the area.

Table 1. Treatments and treatment combinations used for the experiment

Treatment No	**Treatment combination**
1	Limu with Haricot bean
2	Limu with Mungbean
3	Limu with Sweet potato
4	Kurtu harcot been
5	Kurtu with mungbean
6	Kurtu with Sweet potato
7	BH540 with Haricot bean
8	BH540 with Mung bean
9	BH540 with Sweet potato
10	Limu sol
11	Kurtu sol
12	BH540 sol

3.3. Sample Collection

For data collection, five plants were randomly selected from the net plot area by leaving the border rows. The grain samples were collected from central five maize plants in each plot and total of 36 samples were collected for each treatment and composite sample was made by mixing the grains collected from each plot. The samples were properly labeled with the relevant information and transported to the laboratory.

3.4. Laboratory studies

3.4.1. Assessment of fungal frequency associated with maize grain

Fungi associated with the maize seeds were studied by employing the blotter techniques recommended by the International Seed Testing Association (ISTA, 2001). The maize samples were surface sterilized with 70% ethanol for 2 minutes and rinsed three times in sterile water. In order to assess fungal infestation surface sterilized maize grain were placed on moist blotter paper in metallic trays which were covered with transparent plastic sheets and kept in microbiological incubator at 28±1 C° for 7-14 days. For each sample, 100 seeds were tested for fungal contamination using the blotter technique. Fungi grown on diseased maize seeds were observed under stereomicroscope to have a hint on the type of fungal genera growing on the seed. For further identification, fungal colonies were transferred to Potato Dextrose Agar (PDA) and kept at in an incubator at a temperature of 25±1C° for five to seven days. Sub culturing to new PDA was carried out to get fungal pure culture for identification. Those fungal cultures showing typical *Fusarium* mycelium characteristics (white orange or pink mycelium color) were transferred to SNA (Spezieller Nahrspoffarmer Agar) for further identification based on conidial structure. After identification of the type of fungi growing on the maize grain, the frequency of fungi genus was calculated based on the following formula.

3.4.2 Identification of fungi

Identification of fungi was done based on cultural and microscopic morphological characteristics using identification manuals (Humber, 1997; Klich, 2007; and Leslie and Summerell, 2006). Some of the morphological features used for identification of fungus include growth pattern, color of aerial mycelia, reverse colony color, macroconidia presence and morphology, microconidia presence or absence and morphology. Pieces of fungal culture was mounted on microscope slide containing a drop of water and covered with cover slip. Observation of the cultures was made at ×40 objective lenses and all morphological structures were recorded by photo camera and the frequency of fungi genus was calculated based on the following formula.

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Fungal Frequency (%) $= \frac{no.\ of\ isolates\ of\ a\ geuns\ with \in a\ sample}{total\ no.\ of\ isolates\ of\ all\ genera\ within\ a\ samle}\ x\ 100$

3.5. Data Analysis

Data collected was analyzed using SAS (2002) version 9.1.0. Treatment means were separated using LSD at 5 % level of significance. The results are presented in the form of Tables and Figures.

4. RESULTS AND DISCUSSION

4.1. Effect of intercropping on fungal contamination of maize grain

The result of effect of intercropping system on fungal contamination is presented in Table 2. Fungi belonging to six different genera at varying degree of contamination were recorded in the present study. These are *Fusarium* spp., *Aspergillus* spp., *Alternaria* sp. *Penicillium* sp. *Rhizopus* sp. and *Trichoderma* sp. The analysis of variance indicated that contamination of most of the fungi identified in the present study varied significantly (P<0.05) with cropping system. Significantly (P<0.05) higher level of fungal contamination was recorded from maize sole cropping system as compared to the intercropping treatments. However difference in level of contamination of maize grain by *Aspergillus flavus* and*Penicillium citrianum* was non-significant (P>0.05) between maize sole and Maize with sweet potato intercropping systems. In addition level of *Fusarium graminearum, Alternaria infectoria* and *Rhizopus stolonifer* level did not vary significantly for maize with mung bean and maize with haricot bean intercroppings. The lowest fungal contamination was observed in maize with haricot bean intercropping system.The genus *Fusarium* was the most predominant maize grain contaminating fungal genera in this study. The level of maize grain contamination was highest *Fusarium verticillioides*(Table 2)followed by *F. graminearum* and *F. proliferatum*in all the intercropping systems. On the other hand *Trichoderma harzianum* was the least frequent maize grain contaminating fungi in all the cropping systems evaluated in the present study.

The result of this study clearly showed that intercropping maize with mung bean and haricot bean which are legume crops has reduced fungal contamination in maize grain than sole cropping treatment. This might be due to the fact that intercropping with legumes helps to improve soil nutrient and hence boots maize plant's resistance to pathogen attack. The other reason may be inter cropping protect the main crop from insect damage, which intern can lower fungal contamination. Similarly, Borg *et al.* (2017) reported that intercrops can diminish the damage by pests and diseases by reducing the number of susceptible hosts, resistant plants acting as a physical barrier to susceptible plants, inducing resistance by increasing the diversity of pests and diseases, reducing the speed by pest adaption through disruptive selection, and compensation of one species that performs poorly. Maitra *et al.* (2020) also reported intercropping as a disease management strategy because in mixtures of crops functional diversity is created that reduces pathogen population. In another study, it is indicated that crops grown as intercrops can enhance the abundance of predators and parasites, which intern prevents the buildup of pests and diseases (Dwivedi *et al*., 2015). May study is the same to Elodie *et al*., 2010 that Maize-legume intercropping can result in improved soil nutrient and water use, increased productivity, and greater yield stability with reduced risk of crop failure while enabling healthier diets that means inter cropping empower plant to get more nutrient and healthier.

Table2. Effect of intercropping on fungal contamination of maize grain at Dore bafana.

		frequency%							
Inter crop	**Fv**	**Fg**	**Fx**	**An**	**Af**	**Pt**	**Ai**	**Th**	**Rs**
Maize alone	46.0^{a}	12.33^{a}	9.0^{a}	6.0^{a}	6.11^{a}	11.55^{a}	4.66^{a}	1.77^{a}	2.88^{a}
Maize with haricot been	27.7^{d}	7.44^{c}	5.99^{bc}	4.4^{b}	3.11^{d}	7.66^{c}	2.55^{c}	0.88^{c}	1.11^{c}
Maize with mung been	30.5c	7.77^{c}	5.11^{c}	6.2^{a}	4.55^{c}	9.0^{b}	2.66^{c}	1.44^{b}	1.22^{c}
Maize with sweet potato	33.6^{b}	9.33^{b}	6.44^{b}	6.1^{a}	5.33^{d}	11.0^{a}	3.44^{b}	1.88^{b}	2.22^{b}
CV	6.9	12.7	18.8	20.3	16.8	9.8	21.04	29.3	26.1
LSD	2.30	1.13	1.19	1.12	0.77	0.93	0.67	0.49	0.46

Means followed by the same letter (s) within a column are not significantly different from each other at 5% level of significance*,* LSD = least significance difference, CV=coefficient of variation; Fv: *Fusarium verticillioides,* Fg: *F. graminearum,* Fp: *F. Proliferatum,* Af: *Aspergillus flavus*, Pc: *Penicillium citrianum*; An: *A. niger, Ai: Alternaria infectoria*, Rs:*Rhizopus stolonifera* and Th:*Trichoderma harzianum,*

4.2. Effect of variety on fungal contamination of maize grain

Effects of variety on fungal contamination of maize grains are presented in Table 3.The frequency of maize gain contaminating fungal species was significantly influenced by maize varieties ($p < 0.05$) for some of the fungi genera isolated from the maize grain. The result of the present study indicated that the variety Kurtu was the most susceptible maize variety for fungal contamination among the varieties tested and had higher level of contamination for except *Penicillium cintrianum*, *Trichoderma harzianum*, and *Rhizopus stolonifer*. Whereas variety Limu had the lowest level of fungal contamination indicating its better resistance to fungal attack. Fungal contamination significantly ($P<0.05$) varied among the three varieties tested for *Fusarium verticillioides.* However the level of fungal contamination of *Fusarium proliferatum*, *Aspergillus niger, Aspergillus flavus, Penicillium citrianum* and *Alternaria infectoria* for variety Kurtu and BH 540 was non-significant ($P>0.05$)(Table 3).

The present finding indicated that the lower level of fungal contamination in Limu variety might be attributed to the existence of potential resistance genes in the variety against grain contaminating fungal species of which most of them are mycotoxigenic. This information can serve as a baseline information for the national breeding programs for maize breeding for resistance against grain contaminating pathogens. The result of this study has shown that variety selection is very important for disease management. The observed variation among maize cultivars for resistance to maize grain fungal contamination agree well with previous studies by Small *et al.* (2012) in South Africa, Afolabi *et al.* (2007) in Nigeria and Clements *et al.* (2004) in US.

Table 3. Effect of variety on fungal contamination of maize grain at Dore bafana

Varieties	Fungal Frequency%								
	Fv	**Fg**	**Fp**	**An**	**Af**	**Pc**	**Ai**	**Th**	**Rs**
Kurtu	43.25^{a}	10^{a}	7.25^{a}	6.3^{a}	5.3^{a}	10.16^{a}	4.16^{a}	1.58^{b}	1.9^{b}
BH 540	35.5^{b}	9^{b}	6.3^{ab}	5.75^{ab}	5^{a}	10.41^{a}	4.08^{a}	2.41^{a}	12.41^{a}
Limu	24.75^{c}	8.6^{b}	6.16^{b}	5^{b}	4^{b}	8.8^{b}	1.75^{b}	1.25^{b}	1.25^{c}
CV	6.9	12.7	18.8	20.3	16.8	9.8	21.04	29.3	26.1
LSD	2.04	0.98	1.03	0.97	0.67	0.8	0.58	0.42	0.40

Means followed by the same letter (s) within a column are not significantly different from each other at 5% level of significance,LSD = least significance difference, CV=coefficient of variation; Fv : *Fusarium verticillioides,* Fg: *F. graminearum,* Fp *F. proliferatum* Af: *Aspergillus flavus*, Pc; *Penicillium citrianum*; An:*A. niger, Ai: Alternaria infectoria*, Rs: *Rhizopus stolonifer* and Th: *Trichoderma harzianum,.*

4.3. Interaction of intercropping and variety on maize grain fungal contamination

The result of interaction of intercropping and variety on fungal contamination maize grain is presented in Table 4. The analysis of variance indicated that fungal contamination of maize grain were significantly ($p<0.05$) affected by the interaction between intercropping system and maize variety. The highest level of fungal contamination was recorded on variety Kutru with sole planting followed by BH540 sole planting for all fungal species detected in the present study except *Alternaria infectoria*, *Trichoderma harzianum* and *Rhizopus stolonifer*. On the other hand, the lowest frequency of fungal species *Penicillium* was recorded from Limu maize variety intercropped with haricot bean followed by Limu mungbean intercropping (Table 4).*Fusarium verticillioides* was the most frequently isolated fungal pathogen from all treatment combinations followed by *Fusarium graminearum*. The remaining fungal species presented in Table 4 are detected at lower level in all treatment combinations.

Generally, from the present study it is clearly seen that combination of maize variety with some degree of resistance against grain contaminating fungi and use of appropriate intercropping system can play a significant role in minimizing fugal contamination in maize grain. The study also showed that not only postharvest treatment but also Pre-harvest agronomic practice like variety selection and intercropping can be used as important management option to reduce fungal contamination and hence improve the quality and quantity of maize production.

Table 4. Interaction of intercropping and variety on maize grain fungal contamination at Dore bafana

	Fungal Frequency (%)								
Treatment	Fv	Fg	Fp	An	Af	Pc	Ai	Th	Rs
LSo	39^{d}	10.66^{bc}	7.66^{b}	6^{ab}	5.33^{bc}	10.66^{b}	3d^{e}	2.33^{bc}	2.33^{bcd}
LHB	17^{i}	7.66^{efg}	6^{cd}	3^{c}	1.66^{e}	5.33^{d}	1^{f}	0.66^{e}	0.33^{h}
LMB	20.33^{h}	7.66^{efg}	5^{d}	5.33^{ab}	3.66^{d}	8.66^{c}	1^{f}	0.66^{e}	0.66^{gh}
LSP	22.66^{h}	8.66^{def}	6^{cd}	5.66^{ab}	5.33^{bc}	10.66^{b}	2^{ef}	1.33^{ed}	1.66^{def}
KSo	51^{a}	14.33^{a}	11.66^{a}	6^{ab}	6.66^{a}	12.33^{a}	5.33^{ab}	2.66^{ab}	3^{ab}
KHB	38.33^{d}	8^{efg}	5.66^{cd}	6^{ab}	4.33^{cd}	8.33^{c}	3.66^{cd}	0.66^{e}	1.33^{efg}
KMB	40^{d}	8.33^{defg}	5.33^{d}	6.66^{a}	5^{c}	9^{c}	3.66^{cd}	1.33^{de}	1^{fgh}
KSP	43.66^{c}	9.33^{cde}	6.33^{bcd}	6.66^{a}	5.33^{bc}	11^{b}	4^{cd}	1.66^{cd}	2.33^{bcd}
BSo	48^{b}	12^{b}	7.66^{b}	6^{ab}	6.33^{ab}	11.66^{ab}	5.66^{a}	3.33^{a}	3.33^{a}
BHB	28^{g}	6.66^{g}	5.66^{cd}	4.33^{bc}	3.33^{d}	9.33^{c}	3de	1.33^{de}	1.66^{def}
BMB	31.33^{f}	7.33^{fg}	5^{d}	6.66^{a}	5^{c}	9.33^{c}	3.33^{cd}	2.33^{bc}	2^{cde}
BSP	34.66^{e}	10^{cd}	7^{bc}	6^{ab}	5.33^{bc}	11.33^{ab}	4.33^{bc}	2.66^{ab}	2.66^{abc}
CV	5.01	10.9	13.41	20.43	15.63	7.5	22.6	31.0	27.9
LSD	2.92	1.70	1.49	1.97	1.26	1.24	1.27	0.29	0.88

Means followed by the same letter (s) within a column are not significantly different from each other at 5% level of significance,LSD = least significance difference, CV=coefficient of variation; Fv : *Fusarium verticillioides,* Fg: *F. graminearum,* Fp *F. proliferatum* Af: *Aspergillus flavus*, Pc; *Penicillium citrianum*; An:*A. niger, Ai: Alternaria infectoria*, Rs: *Rhizopus stolonifer* and Th: *Trichoderma harzianum,* LSo(Limu sole), LHB (Limu with haricotbeen), LMB (limu with mung bean), LSP (limu with sweet potato), KSo (Kurtu sole), KHB (Kurtu with haricots bean), KMB (Kurtu with mung bean), KSP (Kurtu with sweet potato), BSo (BH540 sole), BHB (BH540 with haricot bean), BMB (BH540 with mung bean), BSP (BH540 with sweet potato).

4.4. Identification of fungi associated with maize grains

In present study total of nine species of fungi belonging to six fungal genera were isolated and identified using morphological characteristics. These are: *Fusarium verticillioides, Fusarium graminearum, Fusarium proliferatum, Aspegillus flavus, Aspegillus niger, Penicillium citrianum, Alternaria infectoria, Rhizopus stolonifer* and *Trichoderma harzianum*. Among these, *Fusarium*was the most dominant genera followed by *Aspergillus* and *Penicillium*.The finding of the present study is in agreement with the report of Getachew *et al.*(2018) ,which indicated *Fusarium, Aspergillus* and *Penicilium* species as the important fungal genera contaminating maize grain.

4.4.1. Morphological identification of fungi

***Fusarium* spp.**

Fusarium verticillioides

Fusarium verticillioides isolate grow slowly on PDA and with growth rates ranging from 32.5to 35mm after 3 days of incubation at25ºC (Table5). It formed cottony white mycelium on PDA(Fig 1 A). The isolates produced honey brown pigmentation on agar (Fig 1B). The fungus produced long chain of microconidia on SNA (Fig1D). Macroconidia were relatively long and slender and slightly straight with 3 to 5 septate (Fig1E).

Fusarium proliferatum

Fusarium proliferatum growth rates ranging from 31 to 34 mm after 3 days of incubation at 25Cº on PDA (Table 5).Initially cultures had white mycelia but developed pink to violet color with age(Fig 1F). It formed brown pigmentation on agar (Fig 1 G). Microconidia formed were with flattened base and0-septet. Macroconidia were relatively long, slander and slightly straight with 3 to 5 septet (Fig1I)

Fusarium graminearum

The isolate of *Fusarium graminearum* grew rapidly on PDA with growth rates of 44 to 47mm after 3 days of incubation at 25ºC (Table 5). It produced abundant aerial mycelium on PDA which was white to pale orange, to pink in color (Fig1J) and had pink to red pigmentation on agar (Fig 1K). Macro conidia formed were relatively slender, thick walled, moderately curved with 5 to 6 septa (Fig1L) Micro conidia were absent (it is the most important criteria to identify *Fusarium graminearum*

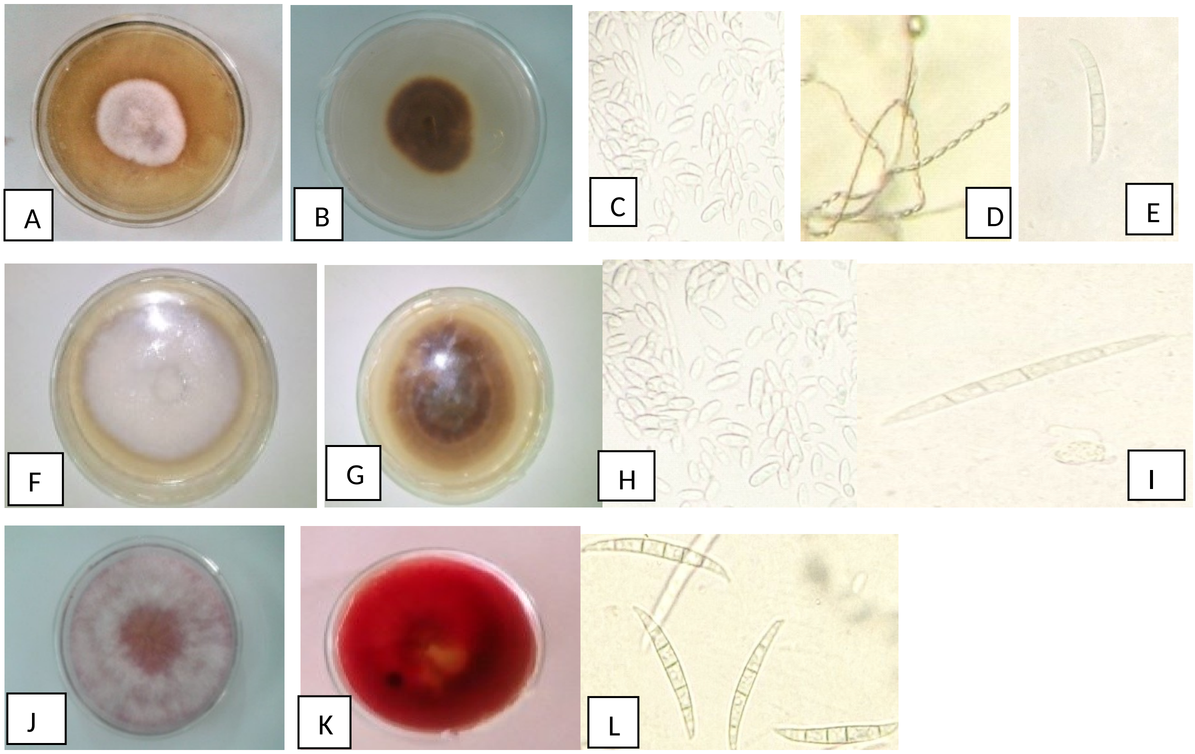

Figure 1. Morphological features of *Fusarium* spp. isolated from maize grain *F.verticilloides;* surface (A), reverse (B), microconidia (C), chain of microconidia (D). and macroconidia (E) *F. proliferatum;* surface (F), reverse (G), microconidia (H) and macroconidia (I). *F. graminearum;* surface (J) reverse (K), macroconidia (L).

Table 5. Growth rate of *Fusarium* species

Fungal spp	**Growth rate(mm)***
Fusarium verticilloides	32-35
Fusarium graminearum	43-47
Fusarium proliferatum	30-34

*Growth rate was measured after 72 hours of culturing isolates on PDA

Aspergillus species

Aspergillus flavus

Aspergillus flavus was moderately growing fungus with growth rates ranging from 67 mm to77mm after 7 days of incubation at 25ºCon PDA. It produced yellowish-green, colonies encircled by a white border produce exudates on PDA (Fig2A). They produced light brown pigment that was seen on reverse side of PDA(Fig2). The fruiting body Vesicle (chein of conidia attachment area), conidiophores (holding stick) characters, size and shape of conidial heads colonies (Fig2 C-D)..

Aspergillus niger

Aspergillus niger was moderately growing and had growth rates ranging from 60.3 mm to65.4mm after 7 days of incubation at 25 ºC on PDA (Table 6). The mycelium was velvety which was black in color and was radially furrowed which was clearly seen in the reverse side (Fig 2E, F). It produced abundant conidia in the conidium. Conidia heads were more than bi serieate and globose, the vesicle very big in size (Fig 2G).

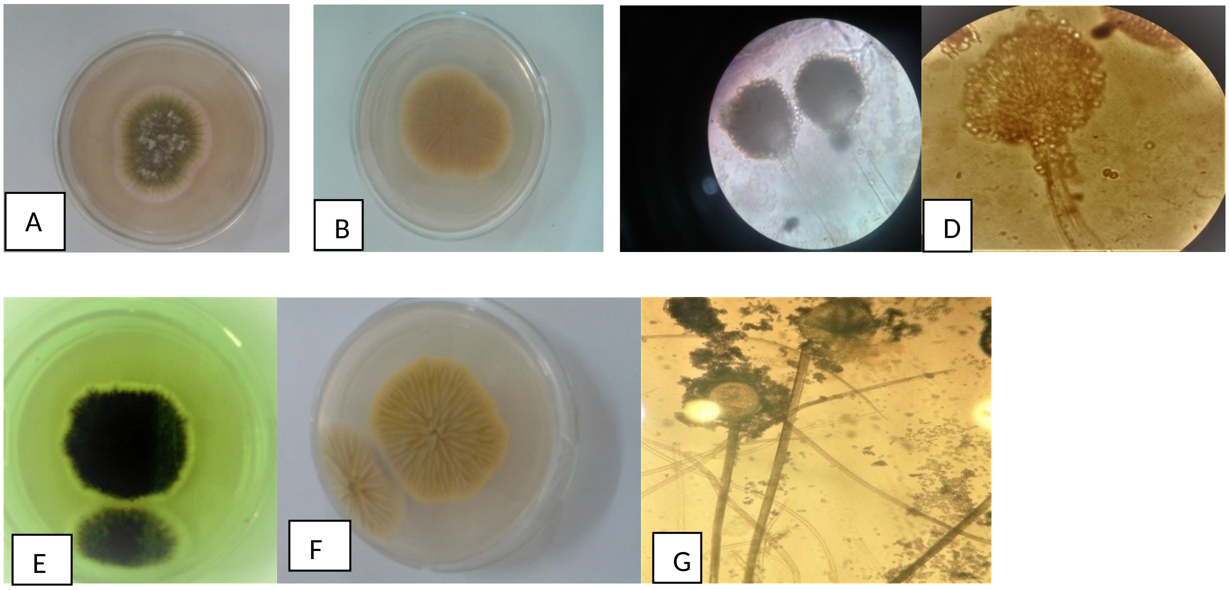

Figure 2. Morphological features of *Aspergillus* spp. isolated from maize grains:

Aspergillus flavus; surface (A) and reverse (B) of culture on PDA; (7days old).Vesicle, sterigmata, conidiophore characters, size and shape of conidial heads (40x), (C–D). *Aspergillus* *niger;* surface (E) and reverse (F) of culture on PDA; (7days old).Vesicle, sterigmata, conidiophore characters, size and shape of Conidial heads (40x), (G).

Table 6. Growth rate of *Aspergillus* species

Fungal spp	**Growth rate(mm)**
Aspergillus flavus	24-35
Aspergillus niger	21-24

Alternaria infectoria

Alternaria infectoria was fast growing with growing rates ranging from 80.5 mm to81.3mm after 7 days of incubation at 25 ºC on PDA (Table7). The colony appeared flat, downy to wooly and was covered by grayish, short aerial mycelium (Fig 3A). It formed reddish brown pigmentation on the reverse side (Fig3B). Typical *Alternaria* spores were evident during mycropscopic observation of fungal cultures (Fig 3C).

Trichoderma harzianum

Trichoderma harzianum fast growing with growth rates ranging from 80.5 mm to83.3mm after 7 days of incubation at 25ºCon PDA (Table7).It produced cottony myceliumon PDA which was blue grayish green colored (Fig 3D). The fungus produced grayish green pigmentation on agar (Fig 3E). Conidiophores produced were branched in pyramidal structure which is typical for *Trichoderma* and conidia were sub globose to short ovoid in shape (Fig 3F).

Rhizopus stolonifer

*Rhizopus stolonifer*was fast growing with growing rates ranging from 83.5 mm to84.6 mm after 7 days of incubation at 25 ºc on PDA (Table 7). The colony was initially white in color and becoming grayish brown with time due to brownish sporangiophore and brown black sporangia(Fig 3G,H). Sporangiophore were irregular in shape often polygonal and non-septet hyphal with upright sporangioshere connected by stolon (Fig 3I).

Penicillium citrianum

*Penicillium citrianum*was fast growing with growth rates ranging from 68.6.3 mm to70.3 mm after 7 days of incubation at 25 ºC on PDA (Table 7). It produced leathery colonies on PDA which were blue, greyish green colored (Fig 3J). The fungus produced yellowish pigmentation on Agar (Fig 3K). It produced typical *Penicillium* conidia formed on phialides on conidiophores (Fig 3L).

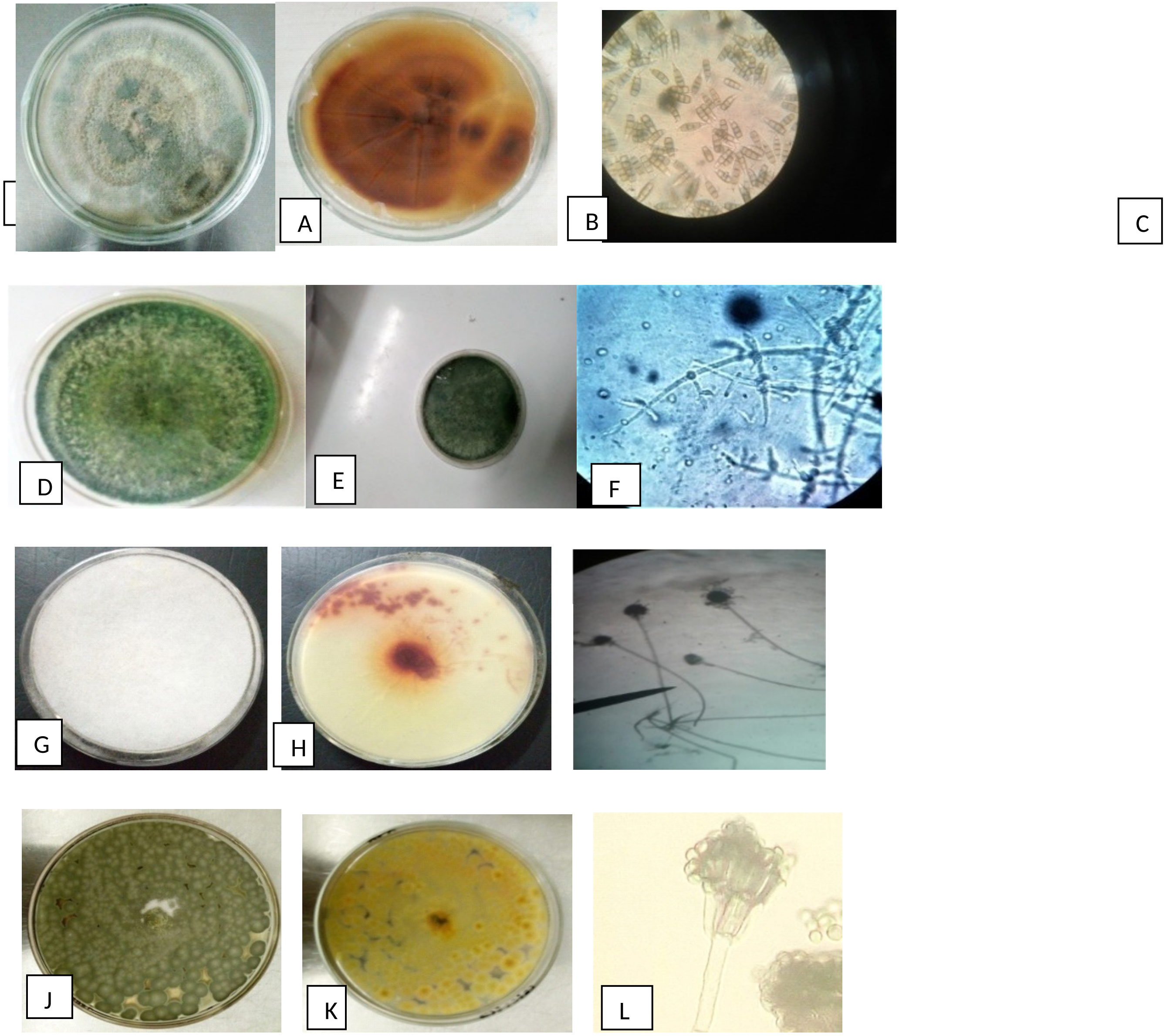

Figure 3. Morphological features of other fungal spp. isolated from maize grains:

Alternaria infectoria; surface (A) and reverse (B) of culture on PDA; (7days old). Conidiophore characters, size, septa and shape of conidial heads (40x), (C).*Trichoderma harzianum;* surface (D) and reverse (E) of culture on PDA; (7days old). Mycelium, conidiophore characters, size and shape of conidial heads (40x), (F**).** *Rhizopus stolonifer;* surface (G) and reverse (H) of culture on PDA; (7days old).mycelia, sporangiophore, columela, rhizoids40x), (I).*Penicillium citrianum;* surface (J) and reverse (K) of culture on PDA; (7days old). Phialides, conidiophore characters, size and shape of conidial heads (40x), (L).

Table 7. Growth rate of *Penicillium* and other fungal species.

Fungal spp	**Growth rate(mm)**
Penicillium citrianum	*15-17*
Alternaria infectoria	*20-23*
Trichoderma harzianum	*22-24*
Rhizopus stolonifer	29-32

5. Summary, Conclusion and Recommendations

5.1. Summary and Conclusion

Maize is a key cereal crop that plays a tremendous role in increasing food security and household nutrition in Ethiopia. Maize production is constrained by biotic and abiotic factors. Among the biotic factors fungi are the major ones. During pre-harvest, several kinds of fungi can remain associated to maize seeds either causing their deterioration or simply remain viable to infect germinating seedling.

From the present study a total of nine fungal species belonging to six fungal genera were identified from maize grain. The fungal genera identified were, *Fusarium, Aspergillus*, *Penicillium*, *Alternaria*, *Rhizopus* and *Trichoderma.* From these *Fusariu*m spp. was the most dominant genera followed by *Aspergillus*. The fungal species were, *Fusarium verticilliodes*, *F. graminearum*, *F. proliferatum*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium citrianum*, *Alternaria infectoria*, *Trichoderma harzianum* and *Rhizopus stolonifer*. From these, *Fusarium verticillioides* was the most predominant one followed by *Fusarium graminearum*.

Agronomic practices such as intercropping and variety selection can be used to reduce fungal contamination in maize (Borg *et al.,* 2017). .Similarly, the present study has shown thatfungal contamination was reduced by intercropping than the sole cropping treatment. This research also showed that not only intercropping but also variety selection can play a role in reducing fungal contamination in maize grain.

The result of the present study indicated that maize varieties tested vary in the level of susceptibility for fungal contamination. The variety Kurtu was the most susceptible maize variety for fungal contamination among the varieties tested and had the highest percentage of fungal contamination. It is also clearly seen that maize cropping system affects the level of fungal contamination. Intercropping maize with haricot bean has resulted in the lowest fungal contamination followed by intercropping maize with mung bean.

The finding of this study revealed that, intercropping and variety selection played important role and influenced fungal contamination in maize. When we compare maize sole planting with intercropping, fungal contamination level was low in case of intercropping. It was also clearly seen that some varieties of maize were free from fungal contamination.

Most of the fungi genera identified in the present study are very important in causing postharvest yield losses and production of mycotoxins. Therefore, due attention must be given to reduction of maize contamination with these important fungi. Intercropping and variety selection can play a vital role in reduction of contamination of maize with this fungi and minimize the risk of mycotoxin exposure to human and animals.

5.2. Recommendations

1. To avoid contamination of maize by mold fungi, use of tolerant maize variety and intercropping should be used as management option. .
2. There is a need for increased awareness creation about the importance of intercropping and variety selection that play role in reduction of fungal contamination.
3. There is need for more research about intercropping and variety selection role on maize grain fungal contamination in different agroecologies to come up with conclusive research.

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