



**EFFECTS OF FORAGE LEGUME INTERCROPPING AND
FERTILIZER APPLICATION ON FUNGAL CONTAMINATION OF
MAIZE (*Zea mays*) GRAIN IN DORA BAFANO WOREDA SIDAMA
REGION**

M.Sc. THESIS

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HAWASSA UNIVERSITY

HAWASSA, ETHIOPIA

DECEMBER, 2022

**EFFECTS OF FORAGE LEGUME INTERCROPPING AND
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MAIZE (*Zea mays*) GRAIN IN DORE BAFANA WOREDA SIDAMA
REGION**

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**A THESIS SUBMITTED TO
THE SCHOOL OF PLANT AND HORTICULTURAL SCIENCES,
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**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
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**HAWASSA UNIVERSITY
COLLEGE OF AGRICULTURE
SCHOOL OF PLANT AND HORTICULTURAL SCIENCES
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This is to certify that the thesis entitled “Effects of forage legume intercropping and fertilizer application of fungal contamination of maize (*Zea mays L.*) grain in Dore Bafano Sidama region of Ethiopia.” Submitted in partial fulfillment of the requirements for the MSc degree with specialization in **Plant Protection** Graduate program of the School of **Plant and Horticultural Sciences**, College of Agriculture, and is a record of original research carried out by **Asmamaw Sisay Aschenaki** under my supervision, and no part of the 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.

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DEDICATION

I dedicate this thesis to my Father Sisay Aschenaki and my mother Kasech Chebisa who nursed me with care and affection, tirelessly labored and sacrificed whatever they had for my education to reach me on this stage of education, the opportunity of which they themselves have never had.

STATEMENT OF THE AUTHOR

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

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ABBREVIATIONS AND ACRONYMS

CFU/g ⁻¹	Colony Forming Units/gram
CIMMYT	International Maize and Wheat Improvement Center
FAO	Food and Agriculture Organization
FAW	Fall Arm Worm
FHB	<i>Fusarium</i> Head Blight
GLM	General Linear Model
NPS	Nitrogen Phosphorus and Sulfur blended fertilizer
PDA	Potato Dextrose Agar
SNA	Spezieller Nährstoffarmer Agar

TABLE OF CONTENT

Contents	pages
DEDICATION.....	ii
STATEMENT OF THE AUTHOR.....	v
ACKNOWLEDGEMENT.....	vi
ABBREVIATIONS AND ACRONYMS.....	vii
TABLE OF CONTENT.....	viii
LIST OF TABLE.....	xi
LIST OF FIGURE.....	xii
LIST OF TABLE IN THE APPENDIX.....	xiii
ABSTRACT.....	xiv
1. INTRODUCTION.....	1
1.1. Background.....	1
1.2. Statement of the Problem.....	3
1.3. Objectives.....	5
1.3.1. General objective.....	5
1.3.2. Specific objectives.....	5
2. LITERATURE REVIEW.....	6
2.1. Production of Maize.....	6
2.2. Mycotoxins.....	6
2.3. Maize Ear Rots.....	7
2.3.1. <i>Fusarium</i> and <i>Gibberella</i> ear rot.....	8
2.3.2. <i>Aspergillus</i> ear rot.....	9
2.3.3. <i>Penicillium</i> spp.	10
2.4. Factors Affecting Grain Contaminations by Fungi.....	10

2.4.1. Insect damage	10
2.4.2. Temperature and moisture availability	11
2.5. Management of Fungi Contaminating Maize Grains	12
2.5.1. Resistant variety.....	12
2.5.2. Fungicides.....	14
2.5.3. Biological control	14
2.5.4. Cultural pre-harvest management.....	15
2.5.4.1. Intercropping with forage legume.....	15
2.5.4.2. Application of fertilizer	17
2.6. Population Density of Fungi in Soil Causative for Maize Ear Rot.....	18
3. MATERIALS AND METHODS.....	20
3.1. Description of the Study Area	20
3.2. Treatments, Experimental Design and Procedures	20
3.4. Soil and Grain Sampling.....	21
3.5. Isolation of Fungi from Maize Grain Samples	22
3.6. Isolation of Fungi from Soil Samples	23
3.7. Identification of Fungi	23
3.8. Data Analysis.....	24
3.9 Economic Analysis	24
4. RESULT AND DISCUSSION	25
4.1. Effects of Forage Legume Intercropping on Fungal Contamination of Maize Grain ..	25
4.2. Effects Fertilizer Application on Fungi Contamination of Maize Grain.....	27
4.3. Interaction between Forage Legume Intercropping and Fertilizer Application on Fungi Contamination of Maize Grain	29
4.4. Effect of maize intercropping with forage legume on fungal population of the soil....	31

4.5. Morphological identification of Fungi Species from Maize Grain and Soil	33
4.5.1. Fusarium spp.....	34
4.5.2. Aspergillus spp.	37
4.5.3. Other fungal spp.....	38
4.6 Economic Analysis	39
5. CONCLUSION AND RECOMMENDATION.....	43
5.1 Conclusion	43
5.2. Recommendation	43
6. REFERENCES	44
7. APPENDICES	64

LIST OF TABLE

Table	Page
Table 1. Effects of forage legumes intercropping on fungi contamination of maize grain in Dore Bafano woreda, during, 2021 cropping season.....	27
Table 2. Effects of fertilizer application on fungi contamination of maize grain in Dore Bafano woreda during, 2021 cropping season.....	29
Table 3. Interaction between forage legume intercropping and fertilizer application on frequency of <i>Aspergillus niger</i> , <i>Fusarium oxysporum</i> and <i>Rhizopus stolonifer</i> in maize grain in Dore Bafano woreda during, 2021 cropping season.	31
Table 4. Effect of forage legume intercropping on fungal population (CFU/g ⁻¹) in soil meant for maize production during, 2021cropping season.....	33
Table 5. Cost benefit analysis of forage legume intercropping and fertilizer application on maize grain in Dore Bafano, 2021	41

LIST OF FIGURE

Figure	Page
Figure 1. Colony and microscopic characteristics of <i>Fusarium</i> species	36
Figure 2. Colony and microscopic characteristics of <i>Aspergillus flavus</i>	37
Figure 3. Colony and microscopic characteristics of <i>Aspergillus niger</i>	38
Figure 4. Colony and microscopic characteristics of <i>Penicillium citrinum</i>	38
Figure 5. Colony and microscopic characteristics of <i>Penicillium chrysogenum</i>	39
Figure 6. Colony and microscopic characteristics of <i>Rhizopus stolonifer</i>	39

LIST OF TABLE IN THE APPENDIX

Appendix table 1: Mean squares of ANOVA for maize grain contaminating fungal frequency affected by forage legume intercropping and fertilizer application.....	64
Appendix table 2: Mean squares of ANOVA for maize grain contaminating fungal frequency affected by interaction of forage legume intercropping and fertilizer application	64
Appendix table 3: Mean squares of Selected Forage Legume Intercropping on Fungal Population in Soil Meant for Maize Production.	65
Appendix table 4. Major soil characteristics of experimental site.....	65

**EFFECTS OF FORAGE LEGUME INTERCROPPING AND FERTILIZER
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ABSTRACT

*Maize (*Zea mays*) is one of the most important cereal crops grown throughout the world. It serves as staple food for millions of people and a source of feed for animals. The crop gives 365 Kcal of energy per 100 g of grain. However, maize is reported as the most susceptible crop to fungi contamination. Hence, management of grain contaminating fungi is vital to ensure sustainable and safe food and feed production. In the present study, a field experiment was conducted to evaluate the effects of forage legume intercropping and fertilizer application on fungi contamination of maize grains. The experiment was done using a randomized complete block Design with 10 treatments replicate trice. The experiment employed factorial combinations of five cropping systems and two fertilizer (with ad without) application. Isolation and identification of fungi from the maize grain and soil was done. A total of 6 fungal genera consisting of 12 species were isolated and identified by their morphological characteristics. Fusarium species were the most frequently isolate, followed by Aspergillus and Pencilium spp. Both forage legume intercropping and fertilizer application significantly reduced the frequency of grain contaminating fungi. The frequency of fungi contamination observed to reduce from 17.66 to 8.66 % in case of Fusarium verticillioides when intercropped with cowpea in double rows. Similarly, when fertilizer is applied, the frequency of Fusarium verticillioides was reduced from 14.6 to 9.0 %. The population density ($CFUg^{-1}$) of Aspergillus, Fusarium, Penicilliu, Mucor and Trichoderama species in the soil were significantly reduced when maize was intercropped with forage legume. The finding revealed that intercropping of maize with forage legume and application of fertilizer highly reduce maize grain contaminating fungi.*

Key words: Maize grain, forage legumes intercropping, fertilizer and fungal frequency

1. INTRODUCTION

1.1. Background

Maize (*Zea mays* L.) belongs to family poaceae (Graminae) (Park, 2001; Wani *et al.*, 2014). Maize is one of the most important cereal crops grown throughout the world over a wide range of environmental conditions. The crop has the potential to produce a great amount of dry matter per hectare (ha) and it is widely grown because of its easiness of cultivation, adaptability to different agro-ecological zones, versatile food uses and storage characteristics (Shiferaw *et al.*, 2011). Maize plays an important role in the diet of millions of people; which supplies an energy density of 365 Kcal per 100 g, contains about 72 % starch, 10 % protein and 4 % fat (Ranum *et al.*, 2014). Maize is also extensively used for animal feed, and it can be processed into a number of industrial products including starch, sweeteners, oil, beverages, glue, industrial alcohol and fuel ethanol (Shiferaw *et al.*, 2011; Ranum *et al.*, 2014).

Maize has a success story to become a strategic food security crop, widely produced and consumed by smallholder farmers of Ethiopia (Abate *et al.*, 2015). Maize is the leading cereal crops in terms of production in Ethiopia, with 9.6 million tons produced in 2019/2020 on 2.27 million hectares of land. The national average yield of maize is estimated at 4.23 t/ha and approximately 88% of maize produced in Ethiopia is consumed as food, both as green and dry grain (CSA, 2020). According to Abate *et al.* (2015) more than eight million farmers were producing maize in Ethiopia in 2015/2016 and more than 95 percent of the total maize area and production in the country is covered by smallholder farmers. For smallholder farmers in maize-based systems, maize is directly associated with their food security.

Data on crop and livestock product utilization collected in 2017/2018 as part of the Agricultural Sample Survey (CSA, 2017), show that up to 76 percent of maize grain

produced was consumed at home on average, with sales representing only 12 percent of production.

Despite the impressive development of maize production in the last two decades, average yield in Ethiopia for the period 2015-2018 (3.6 tons/ha) was still lower than the world's average for the same period (5.8 tons/ha) according to (FAO, 2020). A significant portion of this yield gap is attributable to biotic and abiotic stresses (Worku *et al.*, 2012). Keno *et al.* (2018) point out that some of the main abiotic factors affecting maize production and productivity are drought, heat, soil acidity, frost and poor soil fertility mainly in N and P. Biotic stresses hindering maize production in Ethiopia include several diseases (e.g., Grey Leaf Spot, Common Leaf Rust, Maize Streak Virus), parasitic weeds (mainly *Striga hermonthica*) and insect pests such as the maize stem-borer, maize weevils and the newly emerged fall armyworm (Keno *et al.*, 2018).

Fungi are ubiquitous and diverse, inhabiting various environments including agricultural soils and the crops grown on them (Stajich *et al.*, 2009). Fungi can contaminate, invade and colonize crops on the field during pre-harvest stages and can remain present during the post-harvest processing stages. Depending on the processing steps, these fungi may later spoil foods during storage or in households or markets when storage conditions are sub-optimal and climatic conditions are favorable for their growth (Prange *et al.*, 2005; Taniwaki *et al.*, 2018). Thus, fungal contamination and colonization of crops could directly lead to pre- and post-harvest food losses, mycotoxin contamination and indirectly to public health risks from consumption of mycotoxin-contaminated foods (Avery *et al.*, 2019). Additionally, soil could serve as a reservoir for pathogenic fungi, constituting public health hazards to farmers who spend much of their time on farms and have direct contact with agricultural soils. On the positive side, beneficial fungi, including biological control strains and species of industrial

relevance, are also present in agricultural soils. (Donner *et al.*, 2009; Bandyopadhyay *et al.*, 2016).

Maize-legume intercropping has enough potential in the form of more yields from limited resource, proper utilization of resources, and restoration of soil fertility, efficient pest management and creation of above and below ground diversity. In the moisture stress or resource poor conditions, intercropping provides natural insurance against crop failure caused by biotic and abiotic factors and thus ascertains economic stability of small holders (Sagar *et al.*, 2020)

1.2. Statement of the Problem

Maize grains are widely considered the most susceptible of major cereal crops to grain contaminating fungi than other cereal crops. It can be contaminated with various mycotoxin-producing fungi species that belong to the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, and *Calviceps* (Jones and Toal, 2003). In Ethiopia several fungal species have been isolated from the maize grain Such as *Aspergillus* spp, *Fusarium* spp. and *Penicillium* species (Girma *et al.*, 2009; Chauhan *et al.*, 2016).

Grains contaminated by toxigenic fungus can lead to reduced grain quality, crop yield, reduction of nutritional value, and mycotoxin production. Exposure to grain contaminating by mycotoxigenic fungal leads to the health risk that has been associated with liver cancer, growth retardation and stunting in children, suppression of the immune system, esophageal cancer, and neural tube defect leading to abortion (Strosnider *et al.*, 2006; Okoth *et al.*, 2012). The management of maize grain contaminating fungal is vital towards ensuring sustainable, safe food and feed production. Pre-harvest management of fungi are considered the most important mitigating strategies which include the resistant cultivars, use of micro-organisms or biological, fungicides control, management of moisture stress, intercropping system and the management of soil fertility

Intercropping systems and fertilizer application are an effective strategy for managing maize ear rots. Intercropping contributes to soil health improvement via nitrogen fixation, improved organic matter content, and conservation of soil moisture, resulting in improved grain yield (Khan and Pickett, 2004). Cropping system reduced the incidence of maize ear rots and associated mycotoxins through effective stem-borer and FAW management (Njeru *et al.*, 2020). Fertilizer is one of the most important sources of plant nutrition. Crops may be suffering from a shortage of some elements in soil, so providing nutrients such as fertilizer helps to provide adequate nutrition for crops. Many studies have shown that inorganic fertilizer; such as silicon, chloride, potassium and phosphates have the ability to fight diseases (Fauteux *et al.*, 2005; Deliopoulos *et al.*, 2010). Maize is the most common crop produced with legume intercropping in the study area.

In Ethiopia, resistant cultivars commonly grown for grain contaminating fungal in the country are not known, use of micro-organisms or biological and fungicidal control are not wide spread and need skill and resources. It is very important to develop more relevant management strategy to smallholders who lack skill and financial resources to purchase chemical pesticides and applied. Intercropping systems and fertilizer application are play a great role to reduce ear rot incidence and fungal contamination frequency in maize grain by controlling insect damage because insect damage is the most important factor that promotes fungal contamination in maize. However, potential contribution of the forage legumes intercropping and fertilizer application in management of fungal contamination of maize grain infections has not been studied in study area. Therefore this study is designed for the following objective.

1.3. Objectives

1.3.1. General objective

- To evaluate effects of forage legume intercropping and fertilizer application on fungi contamination of maize grain in Dore Bafano Woreda, Sidama Region.

1.3.2. Specific objectives

- To determine the effect of maize-forage legume intercropping and fertilizer application on grain contamination and fungal population in the soil.
- To determine the interaction effect of maize-forage legume intercropping and fertilizer application on grain contamination and fungal population in the soil.

2. LITERATURE REVIEW

2.1. Production of Maize

Maize is the critical staple food for millions of people across the African continent. It has a good nutritional value and is one of the main sources of calories (Abebe *et al.*, 2009). It is considered to be the cheapest source of calorie intake in the Ethiopia, providing 20.6% of per capita calorie intake nationally (IFPRI, 2010). Maize is grown virtually in all of Africa, predominantly by smallholder farmers (Guilpart *et al.*, 2017). In 2018, the highest maize producers in Africa were South Africa, Nigeria, Ethiopia, Egypt, and Tanzania, with 12.5, 10.2, 7.4, 7.3, 71 and 6.0 million tons, respectively (Logrieco *et al.*, 2021). It becomes an important livestock feed ingredient. For example, in Nigeria, about 2 million tons of maize are used by the poultry sector alone. However, maize production in the African continent faces serious challenges preventing it from reaching its maximum potential (Guilpart *et al.*, 2017).

Both the productivity and the safety of maize grown in Africa is threatened by climate change (Medina *et al.*, 2017) and diverse mycotoxins, most importantly aflatoxins and fumonisins (Ezekiel *et al.*, 2019). Frequently contaminate maize grain throughout the value chain and this is driven by both agronomic and climatic challenges. In addition, sociological and institutional challenges in the continent also contribute to mycotoxin contamination (Ezekiel *et al.*, 2019). Large numbers of scientific papers continuously report single or multiple mycotoxins contaminating maize produced across in Africa (Mahuku *et al.*, 2019; Misihairabgwi *et al.*, 2019).

2.2. Mycotoxins

Mycotoxins are secondary fungal metabolites that contaminate agricultural commodities and can cause sickness or death in humans and animals. Risk of mycotoxin contamination of food and feed in Africa is increased due to environmental, agronomic and socio-economic

factors. Environmental conditions especially high humidity and temperature favors fungal proliferation. Farming practices in Africa sustain fungal and toxin contamination of food and feed. The socio-economic and food security status of the majority of inhabitants of sub-Saharan Africa leaves them little option in choosing good quality products (Hell et al., 2010). There are four types of mycotoxins that consistently affect maize and the animals that consume it; these are aflatoxins, deoxynivalenol and its derivatives, fumonisins, and zearalenone (Munkvold et al. 2019). The fungi that produce them primarily, *Aspergillus flavus*, *Fusarium graminearum* and *Fusarium verticillioides*. Although there are dozens of other mycotoxins and toxigenic fungal species in maize.

Several reports indicated that mycotoxins are common contaminants of stored maize in Sub-Saharan Africa (Kimanya et al., 2008; Probst et al., 2014). Because of the traditional post-harvest practices and the prevailing environmental conditions in Ethiopia, the risk of maize grain contamination is expected to be high. Still, available information regarding occurrences of mycotoxins in maize produced in the country is fragmented: they vary in age of grain (duration from harvest to sampling), targeted stage of production and supply chain for sampling, coverage of high producing areas, or number and type of mycotoxins investigated (Chauhan et al., 2016; Getachew et al., 2017; Tsehaye et al., 2017).

2.3. Maize Ear Rots

Maize ear rots are fungal infections with distinct discolorations and infection route that affect several plant parts including: roots, stalks and ears in the field (White, 2000). Infected maize ears are deleterious due to mycotoxin contamination, but on appearance are mainly manifested as deterioration, on weight, taste and nutritional value. This invites stringency in phyto-sanitary regulations thus reducing trade in cereals (Dohlman, 2003). Different types of ear rots exist, but those of agricultural importance are mainly: *Gibberella*, *Fusarium*, *Aspergillus* and *Penicillium* spp. (Xiang et al., 2010) and *Diplodia* (Bigirwa et al., 2007). In

Ethiopia, also *Aspergillus*, *Cladosporium*, *Drechslera*, *Fusarium* and *Penicillium* spp. are identified from maize grain (Tsedaly and Adugna, 2016; Garbaba *et al.*, 2018)

2.3.1. *Fusarium* and *Gibberella* ear rot

Fusarium species may cause seedling blight, root rots, stalk rots, ear and kernel rot on maize (Leyva-Madriral *et al.*, 2015). Two types of ear rot in maize are caused by different *Fusarium* species. These are recognized as *Gibberella* ear rot, also referred to as red ear rot, and *Fusarium* ear rot (pink ear rot) (Mesterházy *et al.*, 2012; Das, 2014). *Fusarium* ear rot disease is recognized by white to light-pink cottony mycelium growth on kernels, and the ear rot occurs on ear tips or as random individual kernels or groups of kernels in scattered areas on the maize ear (Logrieco *et al.*, 2002; Munkvold, 2003). Infected kernels also exhibit white streaks, known as “starburst” symptom, radiating from top of kernels (Das, 2014). Reddish mold growth, starting from the ear tip and eventually cover large portion of the ear, is a typical symptom of *Gibberella* ear rot. Blue-black perithecia of the teleomorph, *G. zeae* can be observed on infected husks and ear shanks (Das, 2014).

Fusarium ear rot is predominantly caused by *F. verticillioides* (Sacc.) Nirenberg, *Fusarium proliferatum* (Matsush.) Nirenberg, and *F. subglutinans* (Wollenw. & Reinking) P.E. Nelson, Toussoun, & Marasas have also been associated with *Fusarium* ear rot (Logrieco *et al.*, 2002; Munkvold, 2003). *Gibberella* ear rot is mainly caused by *F. graminearum* (Schwabe), but it may also be caused by other *Fusarium* species including *F. culmorum* (Wm.G. Sm.) Sacc., *F. cerealis* (Cooke) Sacc. and *F. avenaceum* (Fr.) Sacc. (Munkvold, 2003; Mesterházy *et al.*, 2012).

Infection of maize by *Fusarium* species may result in premature death of plants, by interfering with the translocation of water and nutrients to upper plant parts, causing yield losses and reduce grain quality (Williams *et al.*, 2007; Presello *et al.*, 2008). The main concern associated with maize ear rot diseases is that some *Fusarium* species produce

secondary metabolites known as mycotoxins, which render the grain inedible or toxic to humans and domestic animals (Wańkiewicz *et al.*, 2012a; Marín *et al.*, 2013). Colonization of maize grains by *Fusarium* ear rot fungi may result in contamination of grains with high level of fumonisins, as well as lower levels of fusarins, fusaric acid, moniliformin and beauvericin, depending on the species involved (Brown *et al.*, 2012; Darnetty and Salleh, 2013). *Gibberella* ear rot disease may lead to contamination with deoxynivalenol, nivalenol and zearalenone (Logrieco *et al.*, 2002; Mesterházy *et al.*, 2012). Some data suggested that over 25 % of the world's food crops are affected by mycotoxin contamination each year, with *Fusarium* species playing the significant role for food contamination (FAO, 2013).

2.3.2. *Aspergillus* ear rot

Colonization of maize by *Aspergillus* spp. is a major challenge in maize production due to aflatoxin contamination (Wagacha & Muthomi, 2008). *Aspergillus* species are ubiquitous in the environment such as soil, air and debris and are widely distributed in tropical and subtropical environments (Klich, 2002). They grow as saprophytes in the soil which serves as the main reservoir of their propagules and as a source of primary inocula (Scheidegger and Payne, 2003). Black or greenish-yellow or tan growth on and between kernels characterize the infection with *Aspergillus niger* and *A. flavus*. The rot begins to appear at the tip of the ear and many follow along the tracks by ear worms. The kernels become light in weight, shrunken and uneconomic (Jacobsen, 2007 and Palencia *et al.*, 2010).

An important challenge for maize production is contamination with aflatoxin, which poses negative health effect to humans and animals and causes huge economic losses (Okoth *et al.*, 2012). Aflatoxins are toxic secondary metabolites produced by several *Aspergillus* spp. Aflatoxins have been associated with stunted growth in children, immune-system suppression, cancer and even death in humans (Strosnider *et al.*, 2006). In animals, aflatoxin contaminated feeds have been associated with aflatoxicosis, impaired growth,

immunosuppression, liver and kidney tumors in rodents and reduced quality of milk and milk products because of the presence of aflatoxin M1, a derivative of aflatoxin B1 (Lizárraga-Paulín *et al.*, 2011).

2.3.3. *Penicillium* spp.

The toxins produced by *Penicillium* spp. can affect the liver and kidney function in humans or animals (Peterson *et al.*, 1999; Scott, 2004). Mycotoxins such as: Ochratoxin and Citrinin commonly produced by *Penicillium* (Peterson *et al.*, 1999; Lund and Frisvad, 2003). Toxins were reported in freshly harvested maize samples as well as in stored maize (Mansfield *et al.*, 2008). *Penicillium* spp. invade particularly on ears injured mechanically or by corn earworms, European corn borers and blue-eye mold occurs in stored corn with high moisture content and blue eye damage is characterized by a blue-green discoloration in the germ area. The discoloration results when *Penicillium* fungi invade the germ area through the tip of the kernel and the damage usually occurs at the tip of the ear (Wagacha and Muthomi, 2008). *Penicillium* were reported from stored maize (Njobeh *et al.*, 2009)

2.4. Factors Affecting Grain Contaminations by Fungi

Factors such as high temperatures and moisture, insect damage, drought stress and delayed harvesting predispose maize to contaminating by *Fusarium*, *Aspergillus* and *Penicillium* spp. (Miller, 2001; Atanda *et al.*, 2011).

2.4.1. Insect damage

Kernels wounded by insect feeding are susceptible to contamination of *Fusarium*, *Aspergillus* and *Penicillium* spp (Cao *et al.*, 2014; Njeru *et al.*, 2020). Insects serve as vectors, transferring inoculum between plants or causing wounds and enabling entry of the fungus in to the plant. Experiments have shown positive correlation between insect damage and cob rots with reporting an increased *F. verticillioides* incidence in maize from *Buseola fusca*

infestation (Bakan, *et al.*, 2002; Ncube *et al.*, 2017). Because insects feeding on maize ears either act as vectors of ear rot fungi or open the ear to fungal inoculum dispersed by raindrops and wind (Mays, 2015).

A variety of insect species has been reported as agents in the dispersal of grain contamination fungi. Some of the most frequently reported insect pests in this regard are the maize stem borers (*Ostrinia nubilalis* and *Sesamia nonagrioides*) (Fandohan *et al.*, 2003; Folcher *et al.*, 2009), Angoumois grain moth (*Sitotroga cerealella*) (Cao *et al.*, 2014), thrips (*Frankliniella occidentalis*) (Parsons and Munkvold 2010) and fall arm worm (*Spodoptera frugiperda*) ((Montezano *et al.*, 2018).

2.4.2. Temperature and moisture availability

Moisture and temperature conditions during the growing season, as well as during storage are key environmental factors for growth of *Fusarium*, *Aspergillus* and *Penicillium* spp. as grain contamination fungi (Waśkiewicz *et al.*, 2012b; Pitt *et al.*, 2013). *Aspragillus* ear rot is promoted by drought conditions, occurrence of cracks or silk-cuts on kernels, high temperature range between 27 and 38°C and relative humidity (85%) during grain filling (DuPioneer, 2010).). Kernel integrity can also be compromised by water stress resulting into silk-cut at silking stage. For instance, hot and dry weather which increase soil and air temperature (>28°C) was found to be favorable for occurrence of *Fusarium* ear rot (Jurado *et al.*, 2008).

Maize genotypes, several morphological and genetic are other factors have been reported as important factory to ear rot and fumonisin contamination. Maize genotypes with good husk cover exhibit less fumonisins (Cao *et al.*, 2014); husk leaves that extend beyond the ear tip and adhere tightly to the developing ear excludes insects that facilitate fungal infection (Butrón *et al.*, 2006). An exposed ear may be more vulnerable to ear rot than one ear enclosed

in the husk. The open-tip husked maize variety is also a source of exposure to *Aspergillus* fungal spores (Manozo *et al.*, 2017).

2.5. Management of Fungi Contaminating Maize Grains

A number of pre- and post-harvest measures have been advanced to manage ear rots and mycotoxin contamination in grain crops (Hell *et al.*, 2008), since ear rot attacks begin in the field, pre-harvest measures can ensure achievement of good yields and grain quality in terms of mycotoxin reduction (Munkvold, 2003). Generally, factors including fungal taxon, humidity, rainfall, insect damage, drought, irrigation and maize germplasm influence incidence of maize ear rots in the field (Parsons, 2008). Pre-harvest management is considered the most important in limiting the overall contamination of crops. Therefore, the use of tolerant varieties, optimal plant production, cultural practices, chemical control, fertilizer application, intercropping and the management of myco-toxigenic fungi by non-toxigenic strains or/and bacteria could further reduce fungal incidence and subsequent mycotoxin contamination (Hell *et al.*, 2010).

2.5.1. Resistant variety

Diallel analyzed to determine the general and specific combinability of resistant genotypes has been reported for *Aspergillus* and *Fusarium*, mostly performed on maize (Henery, *et al.*, 2009; Hung and Holland, 2012) and wheat (Mardi, *et.al.*, 2004; Malla, *et al.*, 2010). The response of an inbred line to *F. verticillioides* and fumonisin and the corresponding General combine in hybrids, was significantly correlated. This indicates that an efficient way to improve resistance to *F. verticillioides* and fumonisin in maize hybrids, specifically, is to first evaluate and select resistant inbred lines that can be used to develop resistant hybrids (Hung and Holland, 2012). This was also demonstrated for breeding resistance to *Fusarium* head blight of wheat (Mardi, *et al.*, 2004).

Inbred lines with resistance to aflatoxin contamination were evaluated for General Combining Ability and specific Combining Ability for resistance to fumonisin accumulation, and two lines with resistance to fumonisin and aflatoxin were registered (Henery, *et al.*, 2009). That research demonstrated the ability to breed resistance to multiple mycotoxigenic fungi and/or their mycotoxins. Furthermore, improved resistance to *F. verticillioides* and fumonisin in inbred lines derived from cross-pollination of resistant and elite maize lines has been demonstrated (Eller, *et al.* 2010). The subsequent hybrids produced from the crossing of improved lines with elite lines, however, did not demonstrate an improved activity against *Fusarium* ear rot and fumonisin accumulation, and although some improved lines performed well as an inbred line and as a component of a hybrid (Mardi, *et al.*, 2004).

Genetically modified crops are plants of which the DNA has been altered through the introduction of a foreign gene to express a trait not inherent to the modified plant. Three transgene-mediated strategies have been proposed for the management of mycotoxigenic fungi and mycotoxins in maize (Duvick, 2001). These include (1) the reduction of fungal infection, (2) the degradation of mycotoxins and (3) interfering with the mycotoxin biosynthetic pathway. To reduce infection by the fungus, the incorporation of antifungal and/or resistance genes, as well as the overexpression of defense-related genes, is required. The best-known example of using genetically modified maize for reducing *Fusarium* ear rot and fumonisin contamination of grain is *Bacillus thuringiensis* maize (Abbes, *et al.*, 2013). This is due to the close association between kernel damage by insects and infection by *F. verticillioides* (Munkvold, 2003a). *Bacillus thuringiensis* maize plants that prevent insect damage, therefore, also reduce fumonisin contamination of maize grain (Abbes, *et al.*, 2013).

2.5.2. Fungicides

Although some progress has been observed in the management of the ear rot causing fungi by use of fungicides. Application of fungicides has been evaluated in several previous studies. For example, Haidukowski *et al.* (2005) reported up to 77% disease severity reduction of FHB caused by *Fusarium graminearum* and *Fusarium culmorum* in wheat and up to 89% subsequent reduction in Deoxynivalenol accumulation in the grains by treatment with different fungicides, cyproconazole, prochloraz, tebuconazole and azoxystrobin under field conditions. However, Simpson *et al.* (2001) reported differential effects of different fungicide applications on the causal agents of FHB and Deoxynivalenol production in field trials. In that study, treatment with tebuconazole reduced the populations of *F. culmorum* and *Fusarium avenaceum*.

2.5.3. Biological control

Biological control is a sustainable solution for plant disease control, since its effect is long-term with few undesirable side effects compared to other pest control options (Vinale *et al.*, 2008). Several fungal and bacterial strains have been identified as biological control agents of plant pathogens. Bacterial strains belonging to *Bacillus*, *Agrobacterium*, *Pseudomonas* and *Streptomyces* and fungi in the genera *Trichoderma* and *Gliocladium* are widely used bio control agents for the control of plant pathogens (Vinale *et al.*, 2008; Pereira *et al.*, 2010). The antagonistic effects of fungal and bacterial strains against plant pathogenic fungi are through several mechanisms, including production of volatile and non-volatile antibiotics to suppress target pathogens, competition for space and nutrients, hyper parasitism and production of lytic enzymes that result in killing of the pathogen (Howell, 2003; Vinale *et al.*, 2008). *Trichoderma* species, bacterial strains such as *Bacillus amyloliquefaciens* and *Enterobacter hormaechei* have been observed to provide good control of *F. verticilloides* (Pereira *et al.*, 2010).

Another study on biological control has been reported by (Cotty, 2006) when the spore number of non-toxigenic strains in the soil is high, they will compete with other strains, both toxigenic and non-toxigenic, for the infection sites and essential nutrients needed for growth. Moreover, soil inoculation with non-toxigenic strains has a carryover effect, which protects crops from contamination during storage (Dorner and Cole, 2002; Atehnkeng, *et al.*, 2014)

2.5.4. Cultural pre-harvest management

Field management before planting is very critical for the control of fungal grain contamination. This includes deep plowing to remove debris of the previous crop plants that could harbor inoculum and crop rotation with non-host plants for fungi to prevent inoculum build-up in the field (Aldred and Magan, 2004). After planting, good field management practices and stress-free conditions will help in reducing the possibility of the establishment of grain contaminating fungal populations by facilitating the growth of healthy plants. Proper irrigation timing can avoid drought stress, thereby reducing susceptibility of plants to fungal contamination. Further, proper fertilization is important for improving plant health and vigor, and sustaining its resistance (Park *et al.*, 1999; Aldred and Magan, 2004). Moreover, weed and insect controls are critical factors for grain contamination fungal in the field. Insects are known to enhance fungal infection by dissemination of fungal inocula and rendering grains more susceptible to fungal infection by inflicting physical damage (Dowd, 2003).

2.5.4.1. Intercropping with forage legume

In intercropping system, two or more crop species are cultivated which creates complexity in food and habitat of pests. Further, intercropping of maize with legumes is known to increase population of beneficial insects and decrease the population of bud worm, corn borer, leaf hopper and maize stalk borer (Seron and Brintha, 2010; Kinama and Pierre, 2010). The population of beneficial insects such as parasites and predators are enhanced in poly-

culture due to diversity of crops (Maitra *et al.*, 2019) and presence of harmful pests may remain below the economic threshold level.

Inter-crops can reduce pest damage by (i) improving soil health and promoting vigorous plant growth, especially in the case of N-fixing intercrops or those that serve to ameliorate the field microclimate (Sida *et al.*, 2018b), (ii) inhibiting movement of larvae among plants (Van Huis, 1981), (iii) preventing female moths from laying eggs, through visual or chemical disruption (Khan *et al.*, 2010), and (iv) providing habitat for natural enemies (Midega *et al.*, 2006)

The incidence of *F. verticillioides*, *F. subglutinans* and *Fusarium girardinum* reduced in maize grown under the push-pull cropping system compared to maize grown as a mono-cropping system (Owuor *et al.*, 2018; Njeru *et al.*, 2020) and a lower incidence was revealed under the Push-pull cropping system than mono-cropping system. The reduction of *Fusarium*, *Aspergillus*, *Penicillium* and other species under the forage legume intercropping system could be caused by the reduction of inocula entry as a result of reduced insect damage (Njeru *et al.*, 2020). Ear rot fungi mainly gain entry into maize kernels through wounds caused by insect infestation or systemically from the soil through the stalk (Mesterházy *et al.*, 2012; Cao *et al.*, 2014).

A variety of insect species has been reported as agents in the dispersal of fungal and increase of mycotoxin contamination. Some of the most frequently reported insect pests in this regard are the maize stem borers (*Ostrinia nubilalis* and *Sesamia nonagrioides*) (Fandohan *et al.* 2004; Folcher *et al.* 2009). The incidence of damage by the FAW and maize stalk borer pests reduced under the push–pull cropping system than mono cropping (Midega *et al.*, 2018; Njeru *et al.*, 2020), that contribute to reductions in fungi grain contamination in maize. The management of insect pest by intercropping maize with non-host plants such as legumes or other crop is common in Africa (Schulthess *et al.*, 2004), it reduced stem borer infestations

by up to 80% (Schulthess *et al.*, 2004; Chabi-Olaye *et al.*, 2005b). Maize + lablab intercrops reduce stem borer populations compared with sole cultures (Maluleke *et al.*, 2005; Jiang *et al.*, 2006). Similarly, maize with beans at ratios of 1:1 to 2:1 significantly decreased stem borer population densities compared to pure maize stands (Difabachew and John, 2010).

In addition, legumes use as a catch crop can reduce nitrate and K leaching (Askegaard and Eriksen, 2008), and act not only as a N₂ fixing crop but also as a catch crop by taking up additional soil minerals N,P, and K (Flores-sanchez *et al.*, 2013). These findings make legumes an important tool in the cropping systems where N and K are the major yield limiting factors (Flores-sanchez *et al.*, 2013). Rusinamhodzi *et al.* (2012) reports that deficiencies of micro nutrients such as Zinc, molybdenum and boron in the field may bound legume growth as well as limit nitrogen fixation. In a study, Vesterager *et al.* (2008) found maize and cowpea intercropping as beneficial on nitrogen poor soil. Maize /cowpea intercropping increases the amount of nitrogen, phosphorus, and potassium contents associated to mono crop of maize (Dahmardeh *et al.*, 2010).

2.5.4.2. Application of fertilizer

Nutrient supply plays an important role in plant growth and development. Fertilizer is one of the most important sources of plant nutrition. Crops may be suffering from a shortage of some elements in soil, so providing nutrients such as fertilizer helps to provide adequate nutrition for crops. Many studies have shown that inorganic fertilizer; such as silicon, sulfur, chloride, potassium and phosphates have the ability to fight diseases (Fauteux *et al.*, 2005; Deliopoulos *et al.*, 2010).

Adequate supply of plant nutrition is one of the potential good agricultural practices against *Aspergillus flavus*. Deficiency or excess of plant-available nitrogen and phosphorus can lead to stress, increasing their susceptibility to being attacked by pests and pathogens (McMahon, 2012; Dolezal *et al.*, 2014). Souza *et al.* (2016) revealed that non-application of nitrogen

resulted in higher contamination of kernels by *Fusarium* sp., *Penicillium* sp. and yeast. Similarly, the application of a low rate of nitrogen level increases the colonization of *Aspergillus* species on maize kernel reported by (Mutiga *et al.*, 2017). Blandino *et al.* (2008) observed a significant increase in the incidence and severity of diseases with non-application of nitrogen fertilizer. Inadequate soil nutrition can affect maize ear and kernel development, which may in turn influence susceptibility to fungal colonization (Seebauer *et al.*, 2004). The application of sufficient N and P often speeds up the growth of maize seedlings and roots, making the plant healthier and stronger against damage from weak pathogens. (Hubber and Haneklaus, 2007; Zhao *et al.*, 2009).

2.6. Population Density of Fungi in Soil Causative for Maize Ear Rot

Fungi are part of diverse living components of soil, with several of them living as saprophytes and symbionts contributing to various soil services including structure formation, organic decomposition, recycling of major elements (for example carbon, nitrogen and phosphorus) and toxic removal (Aislabie and Deslippe, 2013). Pathogenic fungi also exist as major causal agents of soil borne diseases affecting roots, stalks, leaves and ears of various crops including maize (Shurtleff, 1980). Nevertheless, the presence of certain non-pathogenic (mainly saprophytes) or pathogenic fungi on grains, soils and other reservoirs are potential for grain contamination and mycotoxin production, especially species in the *Aspergillus* *Penicillium* and *Fusarium* genera (Pereira *et al.*, 2011).

In soil fungal ecology, cultural practices greatly encourage or discourage fungal distribution. For instance, rotation of susceptible crops like wheat with maize together (Schaafsma *et al.*, 2005) increase contamination incidence. Addition of organic matter either by cultural practice through minimum tillage, or application of organic amendments increases *Aspergilli* propagules (Zablotowicz *et al.*, 2007) while decreasing those of *Fusarium* in soil (Alakonya *et al.*, 2008).

Reduced tillage and no tillage practices are increase the propagules of soil borne pathogens by protecting the pathogen's refuge in the residue from negative microbial interactions or maintaining soil temperature appropriate to the spread of structures of pathogen population. (Nesci *et al.*, 2006). Intercropping maize with forage legume contributes to soil health improvement which is potentially impactful on soil fungal community. The technology improves organic matter content of the soil, nitrogen fixation, overall improvement in soil macro- and micro arthropods and conservation of soil moisture (Khan *et al.*, 2011)

3. MATERIALS AND METHODS

3.1. Description of the Study Area

This study was conducted during 2021 rainfall season in Dore Bafano district Sidama region of Ethiopia. The district is located at 7° 03'11" N latitude and 38° 29' 59.99" E longitude, at an altitude ranging from 1709–2300 meters above level. The area is characterized by a 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 and the experiment was conducted during April-October, 2021G.C.

3.2. Treatments, Experimental Design and Procedures

The experiment consisted of factorial combinations of five cropping system and two fertilizer (with and without fertilizer) application; which made 10 treatments,

No	Treatments
T1	Maize alone without fertilizer
T2	Maize+ cowpea single row without fertilizer
T3	Maize + cowpea double rows without fertilizer
T4	Maize+ lablab single row without fertilizer
T5	Maize + lablab double rows without fertilizer
T6	Maize alone with fertilizer
T7	Maize+ cowpea single row with fertilizer
T8	Maize + cowpea double rows wit fertilizer
T9	Maize + lablab single row with fertilizer
T10	Maize + lablab double rows with fertilizer

The experiment was arranged in a randomized complete block Design (RCBD) with three replications. In all treatments maize was planted at inter and intra-row spacing of 75 cm and 25 cm, respectively. Cowpea double rows were planted in the space available between maize rows at an inter-row spacing of 30 cm. While Lablab double rows were planted at an inter-row spacing of 25cm between maize rows. In a single row intercropping plots both Lablab and Cowpea were planted in equal middle line of two maize rows.

Each treatment was assigned to plots that were 4m long and 4m wide with 5 rows per plot. The commonly used maize variety (limmu variety) in the study area was obtained from the Dore Bafano Agriculture office. At the time of planting, two seeds were placed per planting hole and two weeks after emergence, plants were thinned to one plant per hole. The treatment with fertilizer were fertilized with 150kg/ha NPS Blended fertilizer (N 19%; P₂O₅ 38%; S 7.5%) at planting and 100kg of urea (N 46%) was applied as top dressing one time, at 4 weeks after crop emergence. The time of planting, weeding and harvest were as per farmers' practice.

3.4. Soil and Grain Sampling

When the maize plant reaches the physiological maturity stage. Soil samples were collected from the top of the 4 cm layer of each plot. The soil was taken from five points, one from the middle area and four from corners of each plot of treatments. Then the samples from the plot were mixed in a paper bag to make a composite mixture (Owuor *et al* 2018). The composite soil sample (0.25kg) was drawn and placed in a polyethylene bag, labeled, and transported to Hawassa University plant protection laboratory. The samples were air-dried under a cool area for 1 week. Finally, the samples were sieved through a standard sieve (0.50mm opening) (Horn and Dorner, 1998) and stored under a cool area for further studies.

At the time of harvest, the three middle rows of ears were harvested together per plot and replica. These samples were further dried in an open air to moisture level of 13% measured

using moisture meter. Samples were then hand shelled and stored for fungal frequency analysis.

3.5. Isolation of Fungi from Maize Grain Samples

Four hundred seeds of maize grain were randomly drawn from each plot and taken to Hawassa University plant protection laboratory for mycological analysis. Fungal frequency were assessed by using the blotter technique method (ISTA, 2003). One hundred maize seed from each plot were surface sterilized by soaking in 2.5% sodium-hypochlorite solution for one minute and rinsed three times with sterile distilled water and dried in a laminar flow cabinet and then plated directly on top of three layers of well-soaked sterile blotter paper. Then the seeds were incubated at a temperature of (25°C) for 7-10 days. Infected and healthy seeds were counted to calculate the proportion of seeds contaminated by fungal agents. The fungi growing on seeds were observed under the stereomicroscope

Potato Dextrose Agar (PDA) was used as growth media for isolation of fungal pathogen. Media were prepared by dissolving 39g of commercially formulated PDA powder in to one liter of distilled water. The mixtures were boiled while stirring with a magnetic stirrer for 10 minutes to completely dissolve the powdered agar and then autoclaved at 121°C for 15 minutes to sterilize the media. The liquid media were maintained under aseptic condition and allowed to cool to about 50°C, then 50 mg streptomycin was added to suppress bacterial growth and poured into sterilized Petri-dishes inside laminar air flow. Then allowed to cool and solidify. Fungal colonies were observed from maize grain transferred to PDA and incubated at a temperature (25°C) for 5 - 7 days. Colonies of *Fusarium* spp. were also transferred to synthetic nutrient agar and incubated at 25°C in alternating cycle of 12 hours light and 12 hours darkness for 14 - 21 days to allow sporulation and the frequency of fungi species was calculated based on the following formula.

$$\text{Fungi frequency (\%)} = \frac{\text{no.of isolates of fungi species with in a sample}}{\text{total no.of grain/ kerner of a samle}} \times 100$$

3.6. Isolation of Fungi from Soil Samples

One gram of the fine sieved soil was mixed with 9 ml of sterile distilled water, mixed by mechanical shaker for 3 minutes. The mixture of soil solution was serially diluted by mixing 1 ml aliquot in 9 ml sterile distilled water up to 10^{-3} . One milliliter (1 ml) of 10^{-2} and 10^{-3} dilutions were pipetted into 20ml PDA media amended with antibiotics 50 mg streptomycin/L was dispensed, swirled and allowed to set. Each plot soil dilution was pipetted and the plates incubated for 3 – 5 days at a temperature (25°C). The number of each fungi type growing on the media were counted immediately as colony-forming units per plate.

Fungal colonies were sub-cultured on PDA incubated at a temperature of (25°C) for 3 - 7 days. Colonies of *Fusarium* spp. were also transferred to synthetic nutrient agar (SNA) media and incubated at 25°C in alternating cycle of 12 hours light and 12 hours darkness for 14 - 21 days to allow sporulation and calculate colony forming units/gram of soil as follows (Bollman *et al.*, 2010):

$$\text{CFU/ g}^{-1} = \frac{\text{Average count}}{\text{dilution factory}} \times \text{volume planted}$$

3.7. Identification of Fungi

Identification was done based on microscopic morphological characteristics using the manual (Dugan, 2006; Klich, 2007; Leslie and Summerell, 2006). Some of the morphological features used for the identification of fungus included growth pattern and diameter, the color of aerial mycelia, reverse colony color, macro-conidia presence and micro-conidia presence or absence were observed. The mycelia were spread well on the slide using the sterile needle and a coverslip gently placed with little pressure to eliminate air bubbles. The slide was mounted on the microscope and observed with $10\times$ and $40\times$ objective lenses.

3.8. Data Analysis

Analyses of variances (ANOVA) for fungi frequency on maize grain and population density in soil were analyzed by using the SAS (2002) version 9.0 procedure with GLM model. Treatment means were separated at the 5% level of significant using of fishers protected least significant difference (LSD) test.

3.9 Economic Analysis

Economic analysis was performed following the CIMMYT partial budget methodology (CIMMYT, 1988). The mean grain and straw yield data was adjusted down by 10%. Total costs that varied (seed and planting cost) for each treatment was calculated and treatments were ranked in order of ascending total variable cost (TVC). A net return per hectare was calculated by deducting cost of production per hectare from gross income per hectare. Dominance analysis was executed to eliminate those treatments costing more but producing a lower net benefit than the next lowest cost treatment. Marginal Rate of return (MRR) was calculated for the non-dominated treatments. A treatment which is having an acceptable MRR level (>100 %) and having the highest net benefit is said to be economically profitable (CIMMYT, 1988)

4. RESULT AND DISCUSSION

4.1. Effects of Forage Legume Intercropping on Fungal Contamination of Maize Grain

The present study revealed that cropping system had a significant effect on frequency of fungi species contaminating maize grain as shown in Table 1. The result showed that the frequency of maize grain contaminating fungi species were significantly ($P < 0.05$) affected by forage legumes intercropping system and fertilizer application. The interaction between forage legumes intercropping system and fertilizer application did not significantly affect frequency of fungi species shown in (Appendix Table 1).

The highest frequency of *Fusarium verticilloides* (17.66%), *Fusarium giraminreaium* (8.16%) and *Fusarium subglutinans* (5.50%) were observed in maize alone cropping plots whereas, the lowest frequency *F. verticillioides* (8.66%), *Fusarium giraminreaiumn* (4.0%) and *F. subglutinans* (3.50%) were observed from maize intercropping with cowpea double rows plot (Table 1). For *Aspergillus flavus* (5.50%), *Penicillium citrinum* (2.50%) and *Penicillium chrysogenum* species (4.16%) were observed in maize alone cropping plots while the lowest frequency of *Aspergillus flavus* (3.50%), *Penicillium citrinum* (0.50%) and *Penicillium chrysogenum* species (2.66%) were observed in maize intercropping with cowpea double rows plot (Table 1).

The frequency of *Fusarium*, *Aspergillus* and *Penicillium* fungi species under the forage legume intercropping system was reduced. This might be forage legume intercropping reduced insect damage on maize crop, which cause wounds and enabling entry of the fungus into the plant or serve as vectors, transferring inoculum among plants. Insects feeding on maize ears either act as vectors of ear rot fungi or open the ear to fungal inocula dispersed by raindrops and wind (Mays, 2015). Therefore, maize grown under the forage legume intercropping system had significantly lower ear rot infections. Similarly, less contamination on maize intercrop systems has been reported (Mutiga *et al.*, 2015). Intercropping helps to

create a suitable growing condition or avoid stress factors for the plants that helps to reduce fungal infection.

Fusarium and *Aspergillus* species causes little damage on kernels under good growing conditions for the maize plant (Pitt *et al.*, 2013), whereas stress conditions usually enhance fungal infection contamination (Picot *et al.*, 2010). These results support findings of a preliminary study that reported a reduced incidence of *Fusarium verticillioides* and *Fusarium subglutinans* on maize grain which grown under the push pull cropping system as compared to maize grown in mono-cropping system (Njeru *et al.*, 2020). Owuor *et al* (2018) also reported a lower incidence of *Fusarium*, *Aspergillus* and *Penicillium* species was observed under the push-pull cropping system than mono-cropping system.

In addition, the current findings from maize mono-crops are consistent with observations from previous studies that reported *Fusarium* ear rot as the most common type of maize ear rot in Ethiopia (Ayalew, 2010). Similarly, Tsehaye *et al.* (2017) also reported that *Fusarium* species were recovered throughout all maize growing areas and agro-ecological zones of Ethiopia. This indicates its intimate association with the plant and its adaptation to the tropical climate. Ayalew (2010) has reported that 99 % of the *Fusarium* spp. on the internal and external surface of maize kernels was *Fusarium verticillioides*. Among the *Fusarium* spp. reported in this study, the *F. verticillioides* species was the most frequent grain contaminating fungi and the *F. graminearum* species complex was the second major contaminant of maize kernels.

Table 1. Effects of forage legumes intercropping on fungi contamination of maize grain in Dore Bafano woreda during, 2021 cropping season

Intercropping type	Frequency (%)					
	AF	FV	FG	FS	PC	PCH
Maize sole	5.50 ^a	17.66 ^a	8.16 ^a	5.50 ^a	2.50 ^a	4.16 ^a
Maize +Cowpea single row	4.16 ^{bc}	9.66 ^{cd}	4.50 ^{cd}	4.33 ^{bc}	1.0 ^{bc}	2.83 ^b
Maize + cowpea double row	3.50 ^c	8.66 ^d	4.0 ^d	3.50 ^c	0.50 ^c	2.66 ^b
Maize + lablab single row	4.33 ^{bc}	11.0 ^{bc}	5.33 ^{bc}	4.66 ^{ab}	1.16 ^b	3.16 ^b
Maize + lablab double row	4.66 ^{ab}	12.0 ^b	5.83 ^b	5.16 ^{ab}	1.33 ^b	3.33 ^{ab}
Means	4.43	11.79	5.56	4.63	1.29	3.22
LSD	0.91	1.98	1.32	1.07	0.57	0.88
CV	15.5	12.61	17.81	17.17	32.9	20.55

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; AF *Aspergillus flavus*, FV=*Fusarium verticilloides*, FS= *Fusarium subglutinan*, FG = *Fusarium graminreaum*, PC= *Penicillium citrinum*, PCH = *Penicillium chrysogenum*,

4.2. Effects Fertilizer Application on Fungi Contamination of Maize Grain

Maize grain contaminating fungi frequency varied significantly with fertilizer application ($p < 0.05$) (Table 2). Plots with without fertilizer had the highest fungal frequency as compared to maize grown with fertilizer. The highest frequency of *Fusarium verticilloides* (14.6%), *Fusarium graminreaum* (7.53%), *Fusarium subglutinan* (6.40%), *Aspergillus flavus* (5.20%), *Penicillium critinum* (1.53%) and *Penicillium chrysogenum* (4.33%) were recorded from maize grow without fertilizer application. While the lowest frequency of *Fusarium verticilloide* (9.0%), *Fusarium graminreaum* (3.60%), *Fusarium subglutinan* (2.86%), *Aspergillus flavus* (3.66%), *Penicillium citrinum* (1.06%), and *Penicillium chrysogenum* (2.13%) were recorded from maize grown with fertilizer application (Table 2).

Application of fertilizer gives strength to maize crop against infection of maize grain contaminating fungal pathogen. Because, nutrients can affect the physiology and biochemistry and especially the integrity of the cell walls membrane leakage and the chemical composition of the plant. The finding from this study in line with those of Souza *et al.* (2016) non-application of nitrogen (0 kg/ha^{-1}) resulted in higher contamination of kernels by *Fusarium* sp., *Penicillium* sp. and yeast ($p < 0.05$). Similarly, the application of a low rate of nitrogen level increases the colonization of *Aspergillus* species on maize kernel reported by Mutiga *et al.* (2017). Blandino *et al.* (2008) observed a significant increase in the incidence ($p < 0.05$) and severity ($p < 0.01$) of diseases with non-application of nitrogen fertilizer in maize crop. Inadequate soil nutrition can affect maize ear and kernel development, which may in turn influence susceptibility to fungal colonization (Seebauer *et al.*, 2004).

Deficiency or excess of plant-available nitrogen can lead to stress, increasing their susceptibility to being attacked by pests and pathogens (McMahon, 2012). A high level of phosphorus can harm plants by decreasing the amount of oxygen available for the plant while deficiency of phosphorus makes the plants look weak characterized by thin stem. The effects make the crop susceptible to infection by opportunistic fungi (*Aspergillus flavus*) which contaminates maize grain (Dolezal *et al.*, 2014).

Table 2. Effects of fertilizer application on fungi contamination of maize grain in Dore Bafano woreda during, 2021 cropping season.

Treatments	Frequency (%)					
	AF	FV	FG	FS	PC	PCH
Without fertilizer	5.20 ^a	14.6 ^a	7.53 ^a	6.40 ^a	1.53 ^a	4.33 ^a
With fertilizer	3.66 ^b	9.0 ^b	3.60 ^b	2.86 ^b	1.06 ^b	2.13 ^b
Means	4.43	11.8	5.56	4.63	1.29	3.23
LSD	0.58	1.25	0.83	0.67	0.36	0.55
CV	15.5	12.61	17.81	17.17	32.9	20.55

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; AF *Aspergillus flavus*, FV=*Fusarium verticilloides*, FS= *Fusarium subglutinan*, FG = *Fusarium graminreaum*, PC= *Penicillium citrinum*, PCH = *Penicillium chrysogenum*.

4.3. Interaction between Forage Legume Intercropping and Fertilizer Application on Fungi Contamination of Maize Grain

The frequency of maize kernels contaminated with *Aspergillus niger*, *Fusarium oxysporum* and *Rhizopous stolonifer* fungi species were significantly different in forage legume intercropping and fertilizer application. The interaction between forage legumes intercropping system and fertilizer application significantly affected the frequency of fungi species shown in Table 3 (Appendix Table 2).

The highest frequency of *Aspergillus niger* (6.0%) was observed in maize alone without fertilizer cropping plots while the lowest (2.0%) was observed in maize + cowpea double rows with fertilizer. In *Fusarium oxysporum* and *Rhizopous stolonifer* species a significance difference was observed between maize alone without fertilizer and other remaining treatments however there was no significant difference among those remaining treatments (Table 3). This study revealed that the frequency of grain contaminating fungi *Aspergillus niger*, *Fusarium oxysporum* and *Rhizopous stolonifer* species were reduced under forage

legume intercropping with maize along with fertilizer application. This might be due to the fact that forage legume intercropping with maize, reduced stem and ear borer insect damage on maize crop. Whereas, fertilizer application gives strength to the plant against disease and insect pest. Similarly, less contamination on maize from intercropping systems has been reported (Mutiga *et al.*, 2015). *Fusarium* and *Aspergillus* species ear rot development is aggravated under drought and fertilizer stress conditions (Miller, 2001; Hell *et.al.* , 2008; Parsons and Munkvold, 2010). Legumes can minimize water stress in a farm by covering the soil (Tédihou *et al.*, 2012), and serve as moisture conservation in a farm. It improves soil health and promotes vigorous plant growth (Sida *et al.*, 2018b), by increasing the amount of nitrogen, phosphorus, and potassium contents to maize (Dahmardeh *et al.*, 2010).

Table 3. Interaction between forage legume intercropping and fertilizer application on frequency of *Aspergillus niger*, *Fusarium oxysporum* and *Rhizopous stolonifer* in maize grain in Dore Bafano woreda during, 2021 cropping season.

Treatments	<i>Aspergillus</i>		<i>Fusarium</i>		<i>Rhizopous</i>	
	<i>niger</i>		<i>oxysporum</i>		<i>stolonifer</i>	
	NFt	Ft	NFt	Ft	NFt	Ft
Maize alone	6.0 ^a	4.3 ^b	2.3 ^a	1.0 ^b	2.3 ^a	1.0 ^b
Maize + Cowpea single row	3.3 ^{cd}	2.6 ^{de}	1.0 ^b	1.0 ^b	1.0 ^b	1.0 ^b
Maize +Cowpea double row	3.0 ^d	2.0 ^e	1.0 ^b	1.0 ^b	1.0 ^b	1.0 ^b
Maize + Lablab single row	4.0 ^{bc}	4.0 ^{bc}	1.0 ^b	1.0 ^b	1.0 ^b	1.0 ^b
Maize +Lablab double row	4.3 ^b	4.3 ^b	1.33 ^b	1.0 ^b	1.0 ^b	1.0 ^b
Means	3.8		1.16		1.3	
LSD	0.87		0.43		0.31	
CV	11.0		23.47		16.1	

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; NFt = non fertilized, Ft = Fertilized

4.4. Effect of maize intercropping with forage legume on fungal population of the soil

The study showed that cropping system had a significant effect on fungal population in soil meant for maize production as shown in Table 4. The result showed that the population of fungi species in soil was significantly ($P < 0.05$) affected by forage legumes intercropping system. A fertilizer application and the interaction between forage legumes intercropping system and fertilizer application were not significantly affected on the population of fungi species in soil shown in (Appendix Table 3).

The lowest population density of *Fusarium* species (916.6 CFU/g⁻¹) in maize alone grown plot and the highest population density (1050 CFU/g⁻¹) Maize + Lablab double rows intercropping system plots were observed in the study area. For *Aspergillus niger*,

Aspergillus flavus, *Penicillium chrysogenum* and *Mocar* species a significant difference was observed only between maize alone grown plots and forage legume intercropping plots but no significant difference between forage legume intercropping system (Table 4).

Intercropping forage legumes with maize crop increase fungi population in soil which meant for maize grain contamination because intercrop legume maintaining soil temperature appropriate to the spread of structures of the pathogen population. As Nesci *et al.* (2006) and Dubova *et al.* (2016) stated the population of *Aspergillus* spp. and *Fusarium* spp. significantly increases under minimum tillage and organic matter amendments. This result has contradicted the findings which showed insignificant difference in the population of *Aspergillus* in between push-pull and maize mono-crop systems (Owuor *et al.*, 2018). This is due to most farms being maize intercropped with food legumes such as common bean (*Phaseolus vulgaris* L.) and peanuts (*Arachis hypogaea* L.) before doing the experiments (Owuor *et al.*, 2018).

Table 4. Effect of maize - forage legume intercropping on fungal population (CFU/g⁻¹) in soil during, 2021cropping season.

Treatments	Fungal population (CFU/g ⁻¹) ¹⁾							
	AN	AF	FSS	PC	PCH	MU	RH	TRI
1	100.00 ^b	129.17 ^b	916.6 ^c	100.0	108.3 ^b	95.8 ^b	133.3 ^{ns}	116.6 ^{ns}
2	170.83 ^a	270.83 ^a	1008.3 ^b	108.3	187.5 ^a	100 ^{ab}	141.6 ^{ns}	137.5 ^{ns}
3	179.17 ^a	237.50 ^a	1008.3 ^b	112.5	187.5 ^a	112 ^{ab}	125 ^{ns}	133.3 ^{ns}
4	170.83 ^a	279.17 ^a	1016.6 ^b	116.6	187.5 ^a	125 ^a	141.6 ^{ns}	141.6 ^{ns}
5	154.17 ^a	241.67 ^a	1050.0 ^a	120.8	204.1 ^a	125 ^a	133.3 ^{ns}	125 ^{ns}
LSD	25.0	43.6	33.1	-	19	25	-	-
CV	13.4	15.7	19.0	9.0	2.7	18.8	23.6	22.0

Means followed by the same letter (s) within a column are not significantly different from each other at 5% level of significance **1**=Maize alone, **2** =Maize+ cowpea single row, **3**= Maize + Lablab single rows, **4**= Maize+ cowpea double row and **5** = Maize + Lablab double rows; LSD least significance difference, CV=coefficient of variation; AN = *Aspergillus niger*, AF= *Aspergillus flavus*, FSS= *Fusarium* species, PC= *penicillium citrinum*, PCH *Penicillium chrysogenum*, MU= *mucor* sp, RH= *Rhizopous stolonifer* and TRI= *trichoderma* sp

4.5. Morphological identification of Fungi Species from Maize Grain and Soil

Fungi species that contaminating maize grain in the study area was identified. Four genera and nine species of fungi were identified from maize grain. Those species are *Fusarium verticillioides*, *Fusarium giraminreaiumn*, *Fusarium subglutinans*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium citrinum*, *Penicillium chrysogenum*, and *Rhizopous stolonifer*. Whereas six genera and twelve species of fungi were identified from soil sample. Those species are *Fusarium verticillioides*, *Fusarium giraminreaiumn*, *Fusarium subglutinans*, *Fusarium oxysporum*, *Fusarium crookwellense*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium citrinum*, *Penicillium chrysogenum*, *Rhizopous stolonifer*, *Trichoderma* sp., and *Mocur* species.

4.5.1. *Fusarium* spp.

Fusarium graminearum

Fusarium graminearum isolate grew rapidly on PDA media and had growth rate of 48 to 60 mm after 3 days of incubation at 25 °C. *Fusarium graminearum* were produced abundant cottony aerial mycelium with white to pale orange to pink on colony color and pigmentations from pink to red pigmentation on PDA media (Figure 1 2A and 2B). Macroconidia were more common with straight and relatively slender in shape. Macro conidial septation was ranged from 5 to 6 septation (Figure 1. 2C). There was an absence of microconidia.

Fusarium subglutinan

Fusarium subglutinan isolate grew rapidly on PDA media and had growth rate of 25 to 34mm after 3 days of incubation at 25 °C. *Fusarium subglutinan* was produced abundant mycelium with white colony initially but becomes violet as the culture aged. Pigmentation was colorless to a dark purpled that is nearly black on PDA (Figure 1. 3A-3B). A number of macroconidia septation was usually 3-septate (Figure 1. 3C). Microconidia was Oval and 0-septate. The polyphialides were proliferated extensively.

Fusarium crookwellense

Fusarium crookwellense were produced white dense aerial mycelium on PDA media that grew at rate of 53 to 64mm after 3 days of incubation at 25 °C (Table 5). A red pigmentation was produced on agar. Macroconidia septation was usually 5-septate (Figure 1. 5C) with an absent of microconidia.

Fusarium verticilliodes

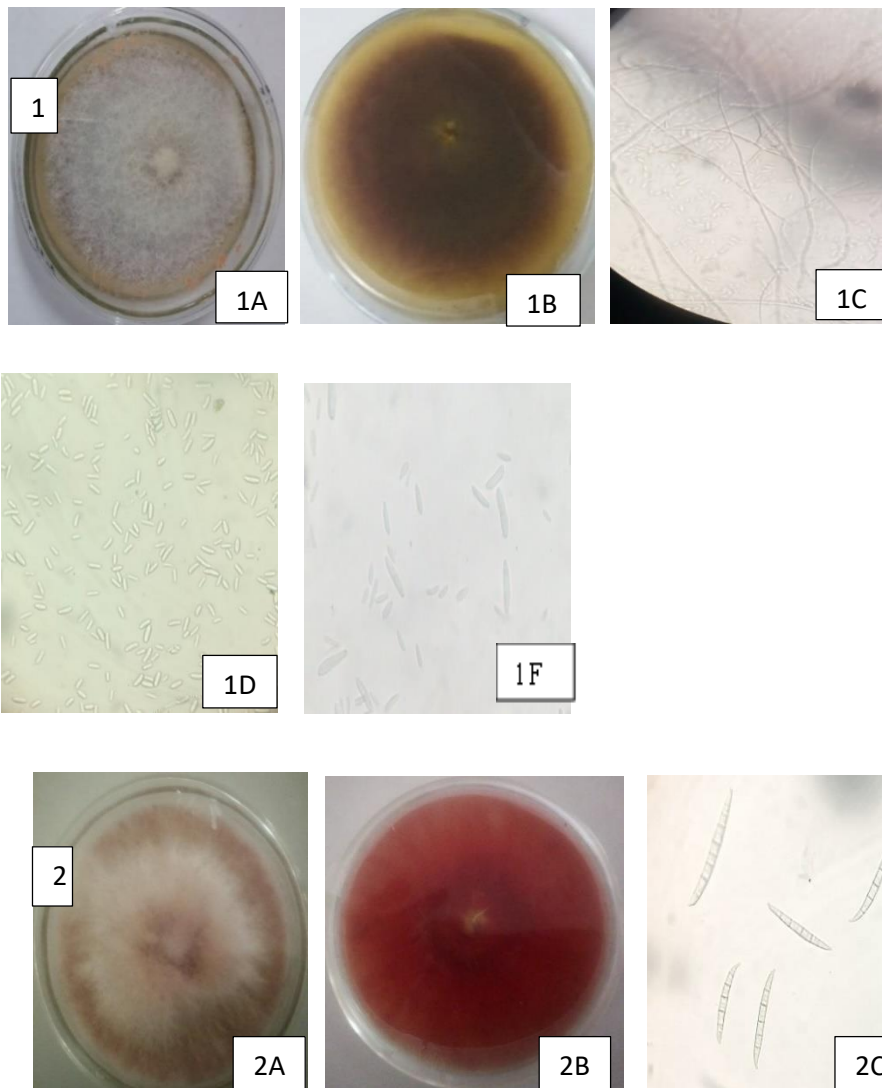
Fusarium verticilliodes isolate grow rapidly on PDA and growth rates ranging from 22 to 29 mm after 3 days of incubation at 25°C. It produced initially white mycelia but later develop into violet with age on PDA (Figure 1. 1A). Pigmentation was violet grey on agar (Figure 1.1B). Abundant microconidia were produced in long chains from monophialides in false

heads (Figure 1.1C). The conidia were oval to club-shaped with a flattened base, and zero-septate (Figure 1. 1D). Macroconidia was vary from slightly falcate or sickle-shaped to slender straight with 3 to 5 septate (Figure 1. 1F).

Fusarium oxysporum

Fusarium oxysporum isolate grow rapidly on PDA and growth rates ranging from 28 to 40 mm after 3 days of incubation at 25°C. It produced abundant woolly mycelium with white colony color and produced white - yellowish pigment on PDA media (Figure 1 4A-4B).

Macroconidia was straight to slightly curve with usually 3-septate (Figure 1 4C).



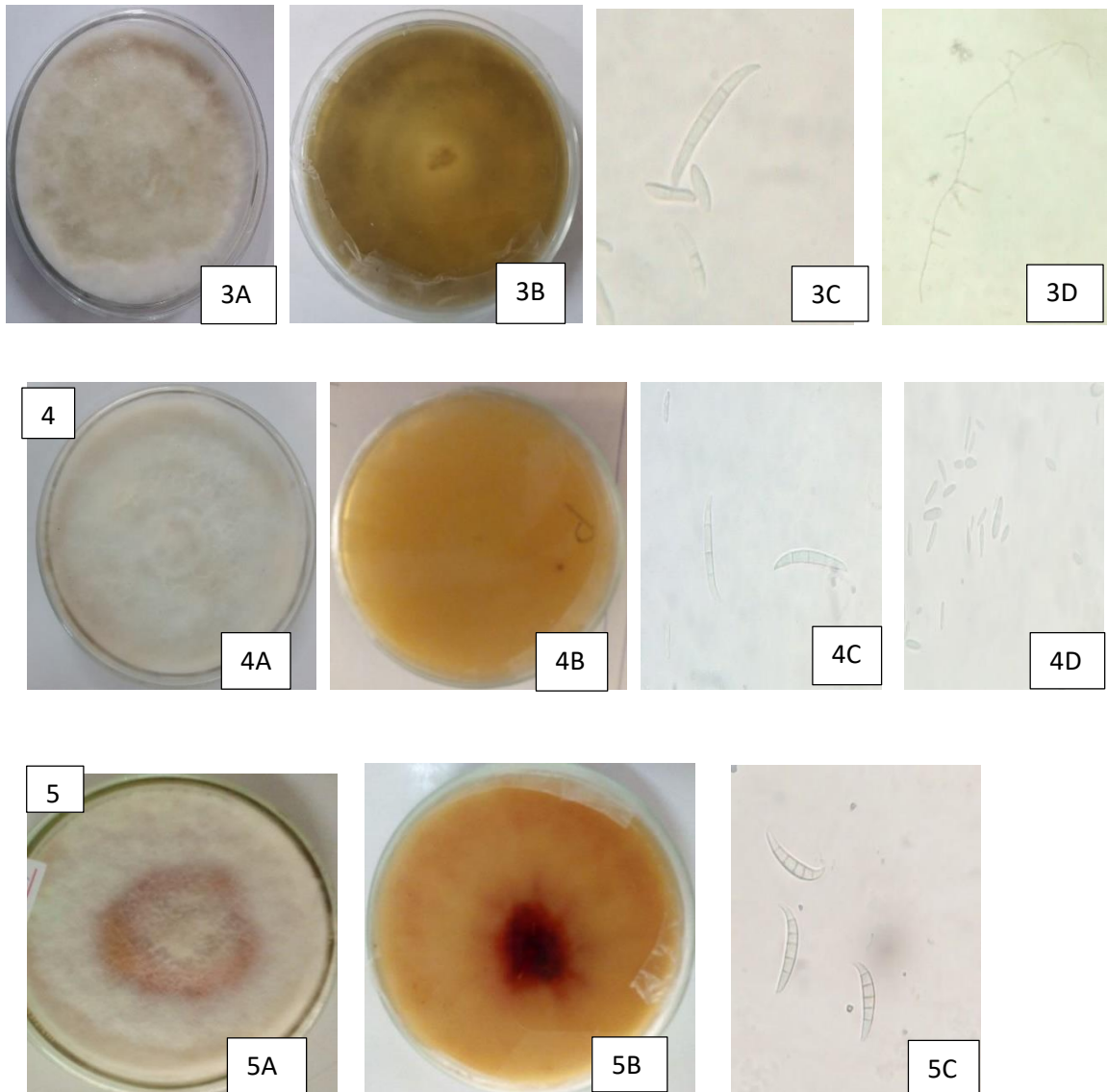


Figure 1. Colony and microscopic characteristics of *Fusarium* species

1. *Fusarium verticilloides*: **1A** obverse, **1B** reverse colony, **1C and D** Microconidia and **1F** Macroconidia
2. *Fusarium graminearum*: **2A** obverse, **2B** reverse colony and **2C** Macroconidia.
3. *Fusarium subglutinans*; **3A** obverse, **3B** reverse colony, **3C** Macroconidia, and **D** microconidia
4. *Fusarium oxysporum*; **4A** obverse, **4B** reverse colony, **4C** Macroconidia and **4D** Microconidia
5. *Fusarium crookwellense*; **5A** obverse, **5B** reverse colony and **5C** Macroconidia

4.5.2. *Aspergillus* spp.

Aspergillus flavus

Aspergillus flavus was fast growing with growing rates ranging from 40 mm to 45 mm after 7 days of incubation at 25 °c on PDA. It produced yellowish-green, colonies encircled by a white border produce exudates on PDA (Figure 2 A1). They produced soluble pigments with brown color that was seen on reverse of colonies. The conidiophores were bearing vesicles. Vesicle shape was sub globose to globose. The conidia was thin wall and yellow green color as shown (Figure 2 A3).

Aspergillus niger

Aspergillus nigri was fast growing with growing rates ranging from 41 mm to 43mm after 7 days of incubation at 25 °c on PDA. *Aspergillus nigri* was produced white mycelia in initially on PDA which quickly become black with conidial production (Figure 3 A1). The reverse was pale yellow with radial fissures in the agar (Figure 3.A2). Conidiophores were beard spherical vesicles and hyaline or lightly pigmented hyphae near the apex (Figure 3. A3).

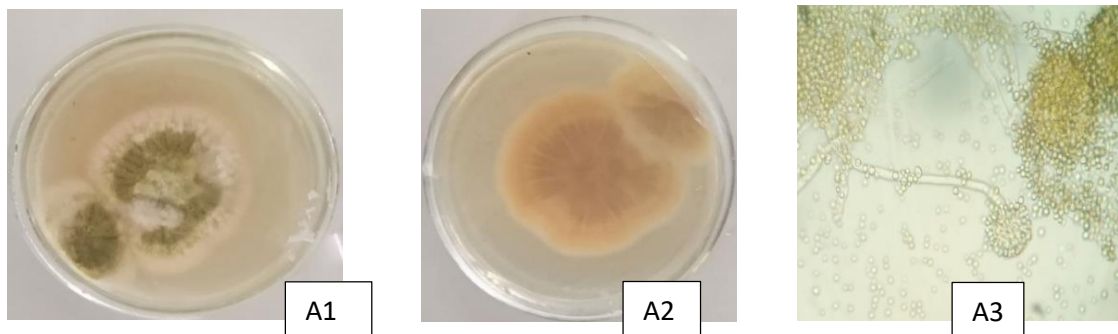


Figure 2. Colony and microscopic characteristics of *Aspergillus flavus*.

A obverse, **A2** reverse colony and **A3** conidiophores

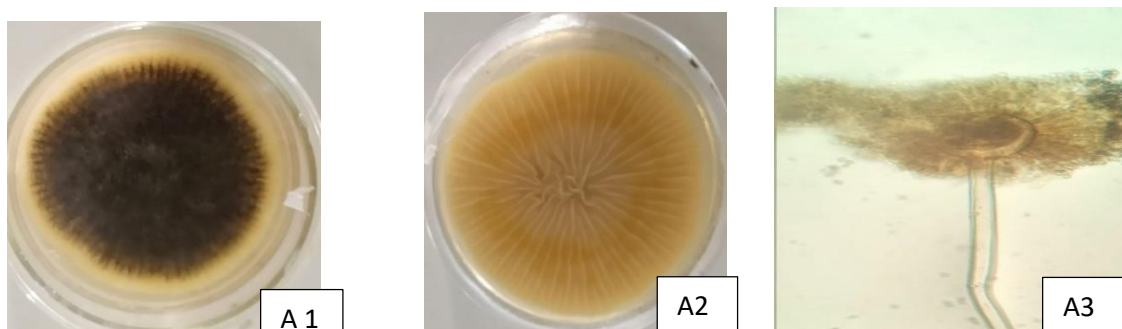


Figure 3. Colony and microscopic characteristics of *Aspergillus niger*.

A obverse, **A2** reverse colony and **A3** conidiophores

4.5.3. Other fungal spp.

Penicillium citrinum

Penicillium citrinum was rapid growing with growing rates ranging from 22 to 24 mm after 7 days of incubation at 25 °c on PDA. It produced dark green color, granular powdery colony and the back side of colony was pale yellow in color on PDA media (Figure 4 A1-A2).

Penicillium chrysogenum

Penicillium chrysogenum was fast growing with growing rates ranging from 30 mm to 35 mm after 7 days of incubation at 25 °c on PDA. It was produced a whitish color in beginning and over time, produced blue green color with a yellowish pigment (Figure 5 A1-A2). It was reproduced a dry chains of spores from brush-shaped conidiophores (Figure 5 A3).

Rhizopus stolonifer

Rhizopus stolonifer was fast growing with growing rates ranging from 87 mm to 90 mm after 3 days of incubation at 25 °c on PDA. It was produced white cottony mycelia it become gray brown (Figure 6 A1). The pigmentation was colorless. Hyphae was broad and not septate with rhizoids and stolon's present (Figure 6 A2). Sporangiohophores were solitary or in tufts on the stolons (Figure 6 A3).

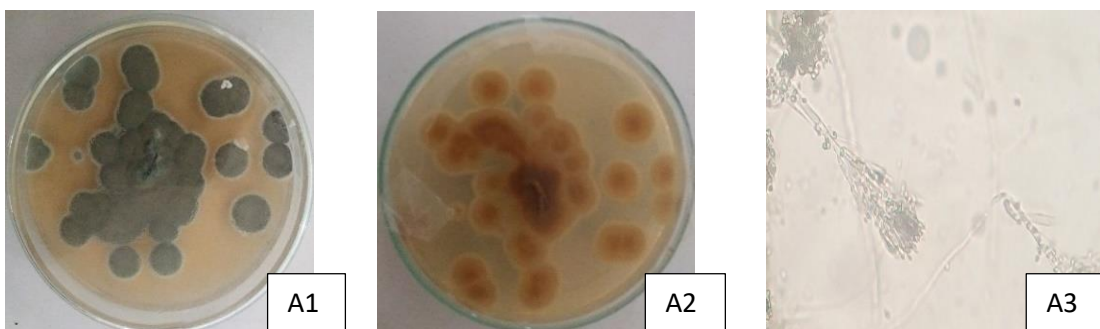


Figure 4. Colony and microscopic characteristics of *Penicillium citrinum*

A obverse, **A2** reverse colony and **A3** conidiophore

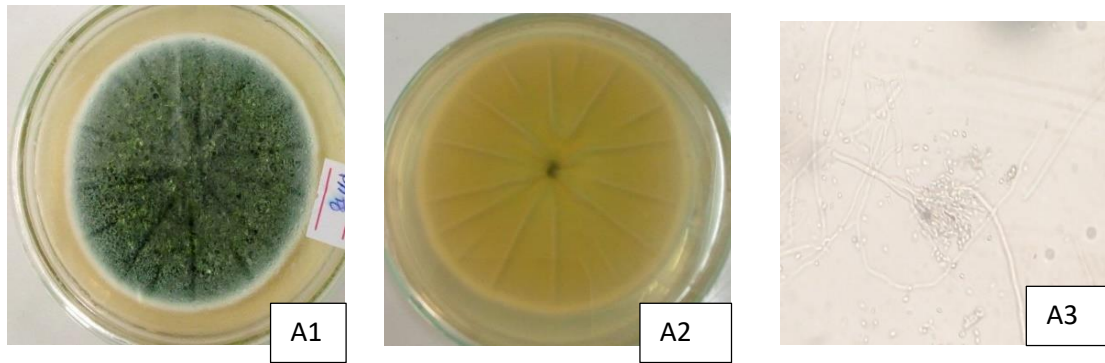


Figure 5. Colony and microscopic characteristics of *Penicillium chrysogenum*
A obverse, **A2** reverse colony and **A3** conidiophore



Figure 6. Colony and microscopic characteristics of *Rhizopus stolonifer*

A obverse, **A2** reverse colony and **A3** Sporangioophore

4.6 Economic Analysis

Cost benefit analysis was done to determine the relative economic returns on the applied treatments using the prevailing market prices. The yields were adjusted by 10% downwards due to management level variability between a researcher and a farmer (CIMMYT, 1988). The price of maize, lablab and cowpea was obtained from personal communication with maize, lablab and cowpea producers and retailers around Hawasa city, which was the nearest market to the study area. Gross benefit was estimated as the product of the adjusted gain of maize and straw yield of lablab and cowpea (kg ha^{-1}) multiplying by market price. A sale price for maize was (27 Birr kg ha^{-1}) and for lablab and cowpea (5 Birr kg^{-1}) respectively. Net benefit was calculated by subtracting the total cost of production from the gross benefit.

The cost of production includes: current fertilizer cost; Urea and NPS 13.8 and 13 Birr kg ha⁻¹ respectively, seed cost of maize 60 Birr kg /ha⁻¹ and lablab and cowpea were 115 Birr kg/ha⁻¹. Cost of labor per day in the area is 100 Birr. Marginal rate of returns were calculated (MRR), where the percentage change in benefit over change in total variable cost (Table 7).

$$\text{MRR (\%)} = \frac{\Delta \text{net benefit}}{\Delta \text{total variable cost}} \times 100$$

The partial budget analysis indicated that Intercropping maize + cowpea single row with fertilizer application had acceptable MRR and the highest net benefit (178,264.1 Ethiopian birr/ ha) (Table 7). Due to having maximum benefit than other, this treatment is recommended for farmers in the study area and other similar agro ecologies.

Table 5. Cost benefit analysis of forage legume intercropping and fertilizer application on maize grain in Dore Bafano, 2021

Treatment	Variable					Income per			
	Seed cost	Labor cost	NPS	Urea	TVC	ha/ Birr from adjusted	TVC in Ethio. Birr	Net benefit (GB- TVC)	MRR%
1	1500	800	0	0	2300	92,735.1	2300	90,453.1	D
2	3225	1500	0	0	4725	141,013	4725	136,288	1890
3	4950	2000	0	0	6950	147,811.2	6950	140,861.2	205.5
4	3225	1500	0	0	4725	123,817	4725	119,092	D
5	4950	2000	0	0	6950	117,764.2	6950	110,814.2	D
6	1500	1300	2850	1500	7150	130,491	7150	123,341	6263.4
7	3225	2000	2850	1500	9575	187,839.1	9575	178,264.1	2264.9
8	4950	2500	2850	1500	11800	190,554.9	11800	178,754.9	22.06
9	3225	2000	2850	1500	9575	158,395.9	9575	148,820.9	D
10	4950	2500	2850	1500	11800	150,812.2	11800	139,012.2	D

1 = maize alone, 2 = maize+ cowpea single row, 3 = maize + lablab single row, 4 = maize+ cowpea double rows, 5 = maize + lablab double rows, 6 = Maize alone with fertilizer, 7 = maize+ cowpea single row with fertilizer, 8 = maize + lablab single row with fertilizer, 9 =maize+ cowpea double row with fertilizer, 10 = maize + Lablab double row with fertilizer, MRR = Marginal rate of returns, TVC = Total variable cost and GB = Gross benefit

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The results of the present study revealed that intercropping forage legume and fertilizer application has effects on maize grain contaminating fungi. Intercropping legume crop with maize reduced the frequency of grain contaminating fungi through reduction of insect damages, because insect make wounds and enabling entry of the fungus into the plant or serve as vectors, transferring inoculum among plants. Intercropping maize with cowpea has low frequency than lablab forage legume this was probably cowpea inhibiting movement of larvae among plants, preventing insects from laying eggs, through visual or chemical disruption and providing habitat for natural enemies than lablab forage legumes

The fungi genera typically found in grain harvested grains in the present study were *Fusarium*, *Aspergillus*, *Penicillium* and *Rhizopus* spp. All identified grain contaminating fungi has low frequency under fertilizer applied plots this is due to the fact that fertilizer gives strength to the crop against the pathogens and insect pests. The population density of maize grain contaminating fungi were lower in soil under maize grown alone than intercropping with forage legume; this was due to intercropping amended soil organic matter and conserve soil moisture contents

5.2. Recommendation

In the present study, intercropping of maize + cowpea single row with fertilizer application was the most appropriate combination for reducing maize grain contaminating fungi in the study area. However, it is difficult to conclude and give reliable recommendation using a one year's experiment at one site, hence further investigation over locations and years should be carried out for developing concrete recommendation. Moreover, there is a need for increased awareness creation about the impact of fungal contamination in maize grains.

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7. APPENDICES

Appendix table 1: Mean squares of ANOVA for maize grain contaminating fungal frequency affected by forage legume intercropping and fertilizer application.

Source variation	df	AF	FV	FG	FS	PC	PCH
block	2	0.23 ^{ns}	6.30 ^{ns}	0.23 ^{ns}	0.93 ^{ns}	0.10 ^{ns}	0.13 ^{ns}
fertilizer	1	17.63*	235.20*	116.03*	93.63*	1.63*	36.30*
intercrop	4	3.21*	74.20*	15.71*	3.61*	3.28*	2.05*
Ferti* inter	4	0.55 ^{ns}	6.86 ^{ns}	1.78 ^{ns}	1.38 ^{ns}	0.38 ^{ns}	0.71 ^{ns}
Error	18	0.47	2.21	0.99	0.63	0.18	0.44
CV		15.54	12.61	17.81	17.17	32.9	20.55

**=Significant: ns= non-significant at (P < 0.05); DF=degree of freedom; CV=coefficient of variation; AF *Aspergillus fluvs*, FV= *Fusarium verticilloides*, FP= *Fusarium proliferatus*, G = *Fusarium graminreaum*, PC= *penicillium citricum*, PCH *Penicillium chysogenum*,

Appendix table 2: Mean squares of ANOVA for maize grain contaminating fungal frequency affected by interaction of forage legume intercropping and fertilizer application

Source variation	df	AN	FO	RH
block	2	0.4 ^{ns}	0.03 ^{ns}	0.03 ^{ns}
fertilizer	1	3.3*	0.8*	0.53*
intercrop	4	6.7*	0.5*	0.53*
Ferti* inter	4	0.75*	0.5*	0.53*
Error	18	0.17	0.075	0.03
CV		11.0	23.47	16.1

**=Significant: ns= non-significant at (P < 0.05); DF=degree of freedom; CV=coefficient of variation; AN = *Aspergillus niger*, FO= *Fusarium oxysporum*, RH= *Rhizopous stolonifer*

Appendix table 3: Mean squares of Selected Forage Legume Intercropping on Fungal Population in Soil Meant for Maize Production.

Source	DF	AN	AF	PC	PCH	FS	MU	RH	TRI
block	2	437.5 ^{ns}	770.8 ^{ns}	270.8 ^{ns}	187.5 ^{ns}	62.5 ^{ns}	83.3 ^{ns}	3000 ^{ns}	20.8 ^{ns}
Fertilizer	1	333.33 ^{ns}	83.3 ^{ns}	83.3 ^{ns}	750 ^{ns}	83.3 ^{ns}	333.3 ^{ns}	0 ^{ns}	20.8 ^{ns}
intercrop	4	6166.6*	21645.8*	385.4*	8645.8*	14791.6*	1114.5*	291.6 ^{ns}	604.1 ^{ns}
Fer*inte	4	20.8 ^{ns}	500 ^{ns}	239.5 ^{ns}	20.8 ^{ns}	83.3 ^{ns}	177.0 ^{ns}	0.0 ^{ns}	20.8
Error	18	520.8	1327.5	479.16	291.6	833.3	458.2	1416.6	916.6
CV		13.4	16.36	19.6	9.75	2.8	19.17	27.88	23.14

**=Significant: ns= non-significant at (P < 0.05); DF=degree of freedom; CV=coefficient of variation; AN = *Aspergillus niger*, AF *Aspergillus fluvs*, FSS= *Fusarium species* PC= *penicillium citrinum*, PCH *Penicillium chysogenum*, MU *mucor spp* RH *Rhizopous stolonifer* and TRI= *trichoderma spp*

Appendix table 4. Major soil characteristics of experimental site

Soil parameters	Value	Rating	References
Physical properties			
Sand (%)	59.17		
Silt (%)	34		
Clay (%)	6.83		
Textural class	Sandy loam		
Chemical properties			
pH	6.28	Slightly acidic)
EC (dS/m)	0.013		
TN (%)	0.155	Low	Landon (1991)
OC (%)	1.859	Low	Karltun (2013)
P (mg/kg)	9.164	Medium	Landon (1991)

TN = Total nitrogen, P = phosphorus, OC= organic carbon, EC= exchanging capacity