SURVEY OF CHICKPEA WILT IN NORTH SHEWA ZONE, NORTH EASTERN ETHIOPIA AND EVALUATION OF CHICKPEA VARIETIES AGAINST FUSARIUM WILT (*Fusarium oxysporum* f. sp. *ciceris*) ATSIYA DEBRNA WAYU WOREDA

MSc THESIS

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HAWASSA UNIVERSITY

COLLEGE OF AGRICULTURE

HAWASSA, ETHIOPIA

AUGUST, 2020

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A THESIS SUBMITTED TO THE SCHOOL OF PLANT AND HORTICULTURAL SCIENCES, COLLEGE OF AGRICULTURE, SCHOOL OF GRADUATE STUDIES, HAWASSA UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PLANT 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 **"Survey of Chickpea Wilt in North Shewa Zone, North Eastern Ethiopia and Evaluation of Chickpea Varieties against Fusarium Wilt (*Fusarium oxysporum* f. sp. *ciceris)* at Siya Debrna Wayu Woredas" in partial fulfillment** for the degree of Master of Science with specialization in **Crop Protection** to graduate program of School of Plant and Horticultural Sciences, College of Agriculture, and is a record of original research carried out by **Basazin Demiss Taffese**, under our 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/We recommend that it be accepted as fulfilling the thesis requirements.

Name of Major Advisor	Signature	Date
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We, the undersigned, members of the Board of examiners of the final open defense by **BasazinDemissTaffese**have read and evaluated his thesis entitled "**Survey of Chickpea Wilt in North Shewa Zone, North Eastern Ethiopia and Evaluation of Chickpea Varieties against Fusarium Wilt (*Fusarium oxysporum* f. sp. *ciceris)* at Siya Debrna Wayu Woredas"** and examined the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree **Masters of Science** in plant sciences with Specialization in **Crop Protection (Plant pathology)**.

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FINAL THESIS APROVEL FORM

(Submission sheet-3)

As members of the Examining Board of the Final MSc Open Defense, we certify that we have read and evaluated the thesis prepared by BasazinDemiss entitled Survey of Chickpea Wilt in North Shewa Zone, North Eastern Ethiopia and Evaluation of Chickpea Varieties against Fusarium Wilt (*Fusarium oxysporum* f. sp. *ciceris)* at Siya Debrna Wayu Woredasand recommend that it be accepted as fulfilling the thesis requirement for the degree of the Master of Science inCrop Protection (Specialization: Plant Pathology).

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Final approval and acceptance of the thesis is contingent upon the submission of the final copy of the thesis to the Department of Graduate Council (DGC) of the candidate's major department.

I hereby certify that I have read this thesis prepared under my direction and recommend that it accepted as fulfilling the thesis requirement.

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

DEDICATION

I dedicate this thesis manuscript to my father Demiss Taffese, and my mother Mezengia Wondimkun for nursing me with affection and love and for their dedicated partnership in the success of my life. I dedicate this work to my husband Mr. Wasihun Kitaw for his irreplaceable love and support during my study.

STATEMENT OF AUTHOR (DECLARATION)

I declare that this thesis is my bonafide 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.

Name: Basazin Demiss Signature _____

Place: College of Agriculture, Hawassa University, Hawassa

Date of Submission _____

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LIST OF ABBEREVIATIONS

ANOVA	Analysis of variance
BCAs	Biological control agents
CFW	Chickpea Fusarium wilts
DZAR	Debre Zeit Agricultural Research Center
DARC	Debre Birhan Agricultural Research Center
FAO	Food and Agricultural Organization
FOC	*Fusarium oxysporium*f.sp.*ciceris*
IDM	Integrated Disease Management
LSD	Least Significant Difference
M.a.s.l	Meter above sea level
SAS	Statistical Analysis System
SPSS	Statistical Packaging of Social Science

TABLE OF CONTENTS

Content **Page**

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF TABLES IN THE APPENDIX

Table **Page**

LIST OF FIGURES IN THE APPENDIX

Figures **Page**

Survey of Chickpea Wilt in North Shewa Zone, North Eastern Ethiopia and Evaluation of Chickpea Varieties against Fusarium Wilt (*Fusarium oxysporum* f. sp. *ciceris*) at Siya Debrna Wayu Woreda

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ABSTRACT

Chickpea is an important source of human food and animal feed and is grown in many parts of the world including Ethiopia. Chickpea production is constrained by several biotic and abiotic factors. Chickpea wilt disease is among the major constrains of chickpea in Ethiopia. Presently the information on the status of Chickpea wilts disease and reaction of chickpea varieties to the disease in the study area is lacking. Therefore the present study was conducted with the main objective of determining the status of Chickpea wilt disease at selected woredas of North Shewa Zone, North Eastern Ethiopia and to evaluate chickpea varieties for their reaction against the disease. The survey was conducted at chickpea potential production woredas (Moretna Jiru, Ensaro, Mojana Wedera and Siyadebrna Wayu) during 2018/2019 cropping season. The field data (disease incidence and prevalence)were collected by using simple random sampling technique from farmers' and research fields. The pathogen was isolated from chickpea plants showing typical wilting symptoms. Pathogenicityof Fusarium oxysporum f.sp.ciceris was confirmed by root dipping inoculation technique in pots under plastic greenhouse condition by using susceptible variety kassech. Seven chickpea varieties were evaluated against Fusarium wilt reaction at Siyadebrna Wayu woredas naturally infested field. The design used for this experiment was randomized complete block design (RCBD) under field condition with three replications. Both incidence and severity data was taken from the field experiment. Chickpea Fusarium wilt incidence was recorded three weeks after sowing while severity data was taken for four consecutive weeks starting from three weeks after sowing. Disease prevalence of 100, 95, 90 and 85% were recorded in Siyadebrna Wayu, Ensaro, Mojana Wedera and Moretna Jiru woredas, respectively. While the percent disease incidence of four selected Woredaswere Ensaro (30.4%), Moretna Jiru (32.25%), Siyadebrna Wayu (36.55%) and Mojana Wedera (27.8%). The fungal mycelia of the isolates were smooth, white in color and, abundant on PDA. The macro conidia were falcate shaped i.e. straight to slightly curved with 3 septation, While the micro conidia was oval shaped. Based on these the pathogen was identified as F. oxysporum f. sp. ciceris. The re-isolated fungus was confirmed to be Fusarium oxysporium f.sp. ciceris in order to full filling the requirement for Koch's postulates by pathogenicity test. From the tested varieties Minijar, Yelbe and Kassech were relatively susceptible, the check variety Kutaye was relatively moderately resistant (tolerant) and Natoli, Mastewal and Areriti were relatively resistant. Based on the findings of the present study, the chickpea varieties, Natoli, Mastewal and Areriti are relatively resistance and can be recommended for the farmers in the study area since they perform better than the control.it is essential to do further research on molecular identification of the fungus associated with chickpea wilt.

Key words: Chickpea, Fusarium Wilt, Incidence, Prevalence, Severity, Varieties

1. INTRODUCTION

1.1. Background and Justification

Chickpea (*Cicer arietinum* L.) is the third largest produced food legume globally, after common bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.) (Gaur *et al*., 2010).The plant is a member of Fabaceae family and Faboideae subfamily of legumes originated from its wild ancestor, *C. reticulatus* in a relatively small area in Turkish Kurdistan of the Fertile Crescent some 8000-9000 years ago (Ladizinsky and Adler, 1976).It is cultivated mostly in the Mediterranean basin, the Near East, Central and South Asia, East Africa, South America, North America and, more recently, in Australia (Rubio *et al.,* 2004). It accounts for 12% of the world's pulse crop production. The Asian region contributes 83%,6% Oceania, 5% Africa,4% Americas and 2% Europe to the total world's chickpea production (FAO, 2019). Asia is the leading chickpea producing country with 83% of world chickpea production (FAOSTAT, 2019). Chickpea is grown throughout the world with different names, Chickpea (UK), Garbanzo (Latin America), Bengalgram (India), Hommes, Hamaz (Arab world), Shimbra/Shumbura (Ethiopia) and Nohut and Leblebi (Turkey) (Gul*et al.,* 2013).It is one of the most important pulses grown widely over an area of 242,703.73 ha across the highlands and semi-arid regions of Ethiopia (CSA, 2018). Ethiopia is secondary center of genetic diversity for chickpea and the wild relative of cultivated chickpea, *Cicer cuneatum* is found in Tigray region of Ethiopia (Yadeta and Geletu, 2002).

Chickpea is an important source of human food and animal feed and grown in many parts of the world (Millan *et al*., 2006).In addition, chickpea returns significant amount of nitrogen to soil and improves soil fertility and break the disease cycles of important cereal pathogens (Pande *et al*., 2011) and for this main reason, it is used in rotation with several cereals like teff on heavy

soils (Geletu and Yadeta, 1994). It can fix up to 140 kg N ha^{-1} from air and meet most of its nitrogen requirement.This saves the fertilizer input cost not only for chickpea but also for the subsequent crops (Anbessa and Bejiga, 2002).

The diverse agro-climatic conditions in Ethiopia make it very suitable for growing chickpea. Chickpea is widely grown across the highlands and semi-arid regions of Ethiopia and serves as a multi-purpose crop. It has a major role in the daily diet of the rural community and parts of urban population. The crop is being exported to Asian countries and is contributing positively to the country's foreign exchange earnings. Chickpea is cultivated in four regions of the country, Amhara, Oromia, Southern Nations, Nationalities and People's Region (SNNPR) and Tigray. Amhara and Oromia regions together produce 93% of total chickpea production in Ethiopiawhile SNNPR and Tigray produce 3.5% and 3%, respectively (CSA, 2015).

In Ethiopia the average chickpea yield is usually below 2ton/ha although its' potential yield is more than 5ton/ha (Melese, 2005). Chickpea production and productivity is affected by several biotic and abiotic factors. According to Schneider and Anderson (2010)among the biotic constraints, fungal and viral diseases are the important yield limiting factors in Ethiopia. The major fungal diseases of chickpea include Ascochyta blight (*Ascochyta rabiei*), Fusarium wilt (*Fusarium oxysporum* f.sp. *ciceris*) and dry root rot (*Rhizoctonia bataticola*). Besides the wet root rot (*R. solani*) and collar rot (*Sclerotium rolfsii*) are reported as less important disease in the country (Seid and Melkamu, 2006). Although, more than 70 pathogens have been reported so far on chickpea from different parts of the world, a few of them are recognized as significantly important to chickpea production (Pande *et al.,* 2010).

One of the most important disease reducing potential yields in chickpea is chickpea wilt caused by *Fusarium oxysporum* f.sp.*ciceris.* The disease causes a serious problem especially in the rain fed area. It is one of the major soil or seed borne diseases of chickpea worldwide (Jalali and Chand, 2012).It is a typical vascular disease causing xylem browning or blackening in chickpea.

Different cultivars of chickpea show wilting at different growth stages and this influence the degree of severity and yield loss due to the disease. The utilization of resistant varieties for the management of the disease and prevent the catastrophic losses caused by wilt disease can decrease the cost of cultivation and increase production. There are several reports indicating the importance of screening chickpea genotypes for FOC resistance as a means of genetic control in the integrated disease management program (Ayyub *et al*., 2013).

1.2. Statement of the Problem

Chickpea production and productivity in Ethiopia has recently reduced and there is a high potential yield gap because of several abiotic and biotic factors(Schneider and Anderson 2010). Despite of its importance for food security and soil fertility, an average chickpea yield in Ethiopia is usually below 2.8 ton ha^{-1} althoughits' potential yield is more than 5.6 ton ha^{-1} (Castro *et al.,* 2012). A number of factors contribute to the low productivity of chickpea.The major constraints leading to reduced yield are biotic stresses, low yield potential of local cultivars, susceptibility of chickpea landraces to heat stress, terminal drought, and water-logging and poor cultural practices(Merkuz and Getachew, 2012). One of the important biotic stresses reducing potential yield in chickpea is chickpea wilt caused by *Fusarium oxysporum* f.sp. *ciceris*which is serious problem especially in the rain fed chickpea producing areas(Schneider and Anderson, 2010). The disease was more prevalent in most of North Western and Central Ethiopia and high disease incidence was found on local than improved varieties (Asrat, 2017).

Chickpea *Fusarium* wilt/root rot is a soil borne fungal chickpea disease which has resulted in yield losses ranging between 10 and 15% worldwide (Warda *et al*., 2017). In extreme cases, the disease can lead up to 100% crop loss (Navas- Cortes *et al*. 2013). In central Ethiopia, it was reported to cause a yield loss of 30% (Mengistu andNegussie, 2014). According to Merkuz andGetachew (2012) the disease is prevalent in areas which are known for cultivation of susceptible varieties to the disease, limited awareness and access of farmers to disease resistant varieties.

Development of varieties resistant to *Fusarium* wilt is the most effective approach to the management of the disease. Breeding of resistant lines and identification of DNA markers for resistance to *Fusarium* wilt has been achieved in chickpea (Sharma *et al*., 2005). Chickpea varieties having resistance to wilt/root rot have been released by the national chickpea improvement program for cultivation in Ethiopia (Geletu *et al*., 1996). Arerti and Chefe which are wilt/root rot resistant varieties were evaluated at DZARC for their reaction to *Fusarium* wilt of chickpea on Fusarium infected field progressively and continually (DZARC, 2006). These varieties do not only excel the local varieties by their yield potential but also have larger seed size (Their seed size is three folds of the local cultivars). They possess desirable color, which makes them more marketable than local cultivar grains. For instance, Arerti variety is white seeded, which is the desirable color, and Chafe meets world market standard. In addition, these varieties have better abiotic stress tolerance; like water and soil, wider environment adaptability, and better food quality than local cultivars (DZARC, 2015).

The development of resistant varieties is the most effective method to manage *Fusarium* wilt and contribute to stabilizing chickpea yieldgap. Host resistance is the main component of integrated disease management and most efficient, cheapest, environmentally safe and economical way of

managing *Fusarium* wilt of chickpea (Bakhsh *et. al*., 2007). Identifying resistant chickpea varieties against *Fusarium* wilt is an important solution to minimize the yield gap in chickpea production. Presently the information on the status of *Fusarium* wilt disease and reaction of chickpea varieties to the disease in the study area is lacking. As a result research is needed to evaluate chickpea varieties for *Fusarium* wilt disease reaction and periodic survey of the disease is required to have up-to-date information on the distribution and importance of the disease in the study area. Therefore, this research was proposed with the following objectives.

1.3. Objectives

General Objective

To determine the status of *Fusarium* wilt of chickpea at selected Woredas of North Shewa Zone (most chickpea producer woredas) and to evaluatechickpea varieties for their reaction against *Fusarium* wilt (*Fusarium oxysporium* f.sp*ciceris*) under field condition.

Specific Objectives

- To determine the prevalence and incidence of *Fusarium* wilt of chickpea in North Shewa Zone of Amhara region.
- To evaluatechickpea varieties for their reaction against *Fusarium* wilt disease under hotspot field condition.

2. LITERATURE REVIEW

2.1. The Chickpea Crop

2.1.1. Origin and Distribution of Chickpea

Chickpea is believed to be originated in south-eastern Turkey and adjoining Syria and Iran. The earliest remains of chickpea seeds date back to around 7000 BC (Syria and Turkey) (Van der Maesen, 1987). According to Ladizinsky (1975), it is believed to have been domesticated in Turkey from *Cicer reticulatum,* a closely related wild species. After its domestication in the Middle East, the crop spread throughout the Middle East, the Mediterranean region, India and Ethiopia. Chickpea cultivation is expanded to countries including Australia, New Zealand, the United States and Canada. In tropical Africa, it is mainly cultivated in East Africa (Sudan, Eritrea, Ethiopia, Kenya, Tanzania) and in Malawi; where it is grown particularly in areas with a marked cool season (Geletu Bejiga andVan der Maesen, 2006).

According to Van der Maesen (1972), as cited in Redden and Berger (2007), the primary center of diversity of Chickpea is in the Fertile Crescent where the crop was originally domesticated, and with the geographic spread of chickpea secondary centers of diversity developed, some older than 2000 years in Mediterranean Europe, the Indian subcontinent and north-east Africa, and lately in Mexico and Chile with post-Colombus introduction. According to the same authors, the distribution of old landraces and wild relatives of chickpea occurs in three main regions from 8° to 52°N latitude and 8°W to 85°E longitude: (i) Western Mediterranean, Ethiopia, Crete and Greece; (ii) Asia-minor, Iran and Caucasus; and (iii) Central Asia, Afghanistan and the Himalayan region. The geographic distribution differs for these two types, with the Kabuli tending to be restricted to the western Mediterranean where the desi are mainly absent. The desi range more widely from the eastern Mediterranean to central Asia and the Indian sub-continent (Moreno and Cubero, 1978).

In Ethiopia, archaeological evidence from Labella caves dated seed samples of Chickpea as over 2500 years of age (Mitiku, 2011). The country is also a secondary diversity for chickpea (Yadeta and Geletu,

2002). It is widely grown in different agro-ecological zones falling between 1400 to 2300m above sea level where the mean annual rainfall ranges from 700 to 2000mm (Geletu Bejiga andMillion Eshete, 1996). Although chickpea is widely grown in Ethiopia, the major producing areas are concentrated in the two regional states - Amhara and Oromia. These two regions cover more than 90% of the entire chickpea area and constitute about 92% of the total chickpea production. The top nine chickpea producing zones (North Gonder, South Gonder, North Shewa, East Gojam, South Wello, North Wello, West Gojam, (Gonder Zuria) belong to the Amhara region and account for about 80% of the country's chickpea production. In the Oromia region, the major chickpea producing zones are in West Shewa, East Shewa and North Shewa, which account for about 85% of the total area and production in this regional state. In line with this idea, Menale Kassie *et al*. (2009), noted that Tigray, Southern Nation Nationalities and Peoples Region of Ethiopia and other regions contribute 7.1%, 1.3% and 0.7 % average cultivated chickpea area and chickpea production share of 6 %, 1%and 1%, respectively during 1999-2008.

2.1.2. Biology and Ecology of Chickpea

Chickpea *(Cicer arietinum*L.) is a self-pollinated, diploid (2n = 16), annual grain legume crop (Bharadwaj*et al*., 2010), though 2n=14 has been reported for some landraces, the species *Cicer songaricum* and for some accessions of *Cicer anatolicum* (van der Maesen, 1972), and cross-pollination is not common event; only 0-1% is reported (Singh, 1987) with a small genome (Aggarwal *et al*., 2013). Pollination occurs in the flower bud stage, prior bees visit open flowers in the field (van der Maesen, 1972). Usually only one seed per pod is set (Van der Maesen *et al*., 2007). It is an herbaceous annual legume crop and the plant height ranges from 30 - 70 cm. It has a tap root system, which is deep and strong. The lateral roots develop symbiotic relationship with the *Rhizobium* bacteria, which are capable of fixing atmospheric nitrogen in plant-usable form. The nodules (slightly flattened fan-like lobes) are seen about one month after seedling emergence and are confined to the top 15 cm of the soil surface.

Based on seed morphology (size and color), cultivated chickpeas are of two types. These are; Microsperma (Desi chickpea) and Macrosperma (Kabuli chickpea).Desi type of chickpea have colored and thick seed coat. The seeds have a combination of brown, yellow, green and black colors. The seeds are generally small and angular with a rough surface. There are 2 - 3 ovules in each pod, but 1 - 2 seeds are produced per pod. The plants are short with small leaflets. The flowers are generally pink and the plants show various degrees of anthocyanin pigmentation, although some Desi types have white flowers, but there is no anthocyanin pigmentation on the stem. The Desi types account for 80 - 85% of the chickpea area. On the other hand the Kabuli type chickpeas are characterized by white or beige colored seed with a ram's head shape, thin seed coat, and smooth seed surface. It is medium to tall in height, with large leaflets and white flowers and contains no anthocyanin. As compared to the Desi types, the Kabuli types have higher levels of sucrose and lower levels of fiber.

Chickpea is planted under wide agro climatic conditions around the world. It is grown between 20^{o}N and 40^{o}N in the Northern hemisphere and is also produced on a small scale between 10^{o}N and 20^{o}N in India and Ethiopia at comparatively higher elevations (Berger *et al*., 2006). In the Southern hemisphere, where chickpea is relatively recent introduction, it is grown between 27^{o}S and 38^{o}S. Growing regions of chickpea can be broadly divided into two, non-tropical dry areas and semiarid tropics (SAT) (Imtiaz *et al*., 2011). Chickpea is grown usually as a rain-fed cool-weather crop or as a dry climate crop in semi-arid regions, with relative humidity of 21 to 41% as sufficient for seed setting. The time available for chickpea crops to produce adequate vegetative structures and then grain yield often depends on hot or cold temperatures, rainfall distribution, or competition for use of land by other crops in rotation (Roberts *et al.*, 1985; Smithson *et al*., 1985).

Chickpea is cultivated mainly in crop-livestock based farming systems of the Central, North and Northwest highlands of Ethiopia where Vertis soil are dominating. Chickpea is mainly produced in Amhara (52.5%), Oromia (40.5%), SNNP (3.5%) and Tigray (3%) regions (CSA, 2016). In Ethiopia, chickpea is cultivated at an altitude ranging from 1400 to 2300 meters above sea level (m.a.s.l.) with annual rainfall ranging from 700 to 2000 mm on Vertis soil having a pH range of 6.4-7.9. It is mainly grows under residual moisture at the end of the main rainy season in water-logging areas. Chickpea is a less labor-intensive crop and its production demands low external inputs compared to cereals (Bekele *et al*., 2007).

2.1.3. Importance of Chickpea

2.1.3.1. Nutritional Value of Chickpea

Pulses are primary sources of nourishment and, when combined with cereals, provide a nutritionally balanced amino acid composition with a ratio nearing the ideal for humans. Chickpeas are an ancient crop usually grown for their seed which is nutritionally of a very high quality (Saxena, 1990). The main use of chickpea is for human consumption and the seed provides an excellent source of protein, especially for vegetarians or vegans. The seeds may be eaten as whole; split into halves after removing the seed coat processed into flour or the young shoots may be eaten as a vegetable (Muehlbauer andTullu, 1997). Due to their good balance of amino acid, high protein bioavailability and relatively low levels of anti-nutritional factors, chickpea seeds have been considered a suitable source of dietary proteins. Ranging among varieties, the seeds contain approximately 12.4-31.5 % crude protein, 3.8-10.2 % fat, 52.4-70.9 % total carbohydrate and 1.7-10.1 % crude fiber. True digestibility, biological value and net protein utilization of chickpea seed ranges from 85-89 %, 83-85 % and 92-97 %, respectively (Williams andSingh, 1987). Nutritionally, Kabuli chickpeas are very slightly higher in protein

content and fat, however, desi chickpeas provide more than three times the dietary fiber (Petter son, 1997).

2.1.3.2. Medicinal value of chickpea

Legume seeds contain large number of compounds that are qualified as phytochemicals with significant potential benefits to human health (asanticarcinogenic, hypocholesterolemic or hypoglycemic agents) (Muzquiz and Wood, 2007). On the medicinal side, chickpea is known to be a nutraceutical (or health benefiting food) because of its high nutritional value and near absence of anti-nutritive components (Williams and Singh, 1987). Desi chickpea have a very low 'glycemic index' making them a healthy food source for people with diabetes (Walker and Walker, 1984). As described in Muzquiz and Wood (2007), chickpea does not contain any specific major anti nutritional factors; the only negative factor ascribed to its consumption is more flatulence due to a higher concentration of raffinose family oligosaccharides (RFOs) than other dry edible legumes. Seeds are mainly used for the treatment of bronchitis, leprosy, skin diseases, blood disorders and biliousness (Muehlbauer and Tullu, 1997).

2.1.3.2. Nodulation and nitrogen fixation

Legume crops are economically important in cropping systems because of their ability to assimilate atmospheric nitrogen. Biological nitrogen fixation occurs inside the root nodules of legume species as a result of a symbiosis between the host plant and bacteria (Thavarajah *et al.,* 2005). The fertility benefits are derived from the N-rich legume residues remaining after grain harvest and from the higher levels of nitrate that are often found in the root-zone of legume crops at the end of growth. The origin of this nitrate is contentious (Unkovich, 1997). Unkovich andPate (2000) suggested that the nitrate most likely originates from **mineralized** rhizobium deposits, legume roots, and nodules. Soil in which nodulated legumes are growing often contains

more nitrate nitrogen (N) than soil in which unnodulated legumes or non-legumes are growing (Turpin *et al.,* 2002). N_2-fixing legumes use less soil nitrate than an adjacent non-N_2-fixing crop, resulting in nitrate conservation.

2.1.4. Chickpea Productionin Ethiopia

Ethiopia is the largest producer of chickpea in Africa accounting for about 46% of the continent's production (Joshi *et al*., 2001).Above 1.86 million farmers are involved in producing chickpea and lentils. The total area covered by chickpea in Ethiopia is estimated at 258,486.29ha and from which annual production of 472,611.39 tons of chickpea grain is produced (CSA, 2016). Ethiopian chickpea production is predominated by Desi type chickpea (about 95%). However, in recent years there has been an increase in the interest of farmers in growing large seeded Kabuli varieties due to their higher price in the market (Guar *et al*., 2005). However, there has been a substantial export of chickpea by Ethiopia during the past five years, with the highest of 48,549 tons (valued at US$14.7 million).

2.2. Major Production Constraints of Chickpea

Chickpea production is exposed to different biotic and abiotic constrains which reduces seed yields. The major biotic stresses which lead to yield reduction and instability are those caused by fungal, bacterial and viral diseases, insect pests, parasitic nematodes (Ranalli andCubero, 1997) and parasitic weeds of chickpea (Cubero *et al*., 1986). Some of the diseases caused by biotic stresses are described below. *Ascochyta* blight, caused by *Ascochyta rabiei*, is a highly devastating foliar disease of chickpea. It occurs mainly in areas where cool, cloudy and humid weather prevails during the crop season (Singh *et al*., 2008). *Fusarium* wilt, caused by *Fusarium oxysporum*f.sp. *ciceris*, is the most important root disease of chickpea, particularly in the semiarid tropics where the chickpea growing season is dry and warm. Viral diseases have been

reported to cause sporadic but significant yield loss in some areas. Major symptoms include discoloring (yellow, orange or brown) of foliage, browning of phloem and stunting of growth. Many viruses have been identified that can cause stunt disease. Insects especially the gram caterpillar or gram pod borer (*Helicoverpa armigera* Hubner) can cause problems (Winch, 2006). According to the same author, the insect is highly polyphagous and sources with high levels of resistance are not available in chickpea germplasm. Furthermore, seed beetle or bruchid (*Callosobruchus* spp.) is the most important storage pest of chickpea. Cyst nematode (*Heterodera cicer*) is another major biotic stress to chickpea (Singh *et al.,* 2008). As with most weeds in a particular crop, weeds affecting chickpea have a similar ecology and biology. Generally, cool-season broadleaf weeds are the most difficult to control in chickpea (Yenish, 2007).The most common abiotic stresses affecting chickpea production are drought (particularly terminal drought), salinity, heat, frost and cold. Resistance or tolerance to these stresses is more complex. Chickpea, an important food legume grown in the arid and semi-arid tropical regions, suffers substantial yields loss due to water deficit at the end of the growing season (Khamssi, 2011).

2.2.1. Chickpea Fusarium Wilt (CFW)

2.2.1.1. The Biology and Ecology of Causal Organism

Fusarium wilt of chickpea is caused by *Fusarium oxysporum* f. sp. *ciceris* (Jimenez-Femandez *et al*., 2011, Landa *et al*., 2006 and Haware, 1990). *Fusarium* wilt of chickpea can produce microconidia, macroconidia and chlamydospore. The microconidias (2.5–4.5 μm × 5–11 μm) are oval or cylindrical, straight or curved. Macroconidias (3.5–4.5 μm × 25–65 μm) are produced more distantly than microconidias and usually they are three to five septate or fusoid. Hyphae are septate and profusely branched. Chlamydospores are formed after 15-days on culture media and

infected chickpea tissues, formed singly, in pairs or in chains, and are smooth or rough-walled (Castro *et al*., 2012 and Jimenez Diaz *et al*., 2015). The color of the fungus on potato sucrose agar and potato dextrose agar and under near-UV light is different, i.e. the aerial mycelium is at first white and cottony, but later it may become cream or salmon in color or remain white (Jimenez Diaz *et al*., 2015).

The fungus can survive in soil and chickpea residues by means of chlamydospores for at least 6 years (Haware *et al*., 1996) but infection of symptomless dicotyledonous weeds can initiate survival of the pathogen in fallow soils. Thus, infested soil is a core source of primary inoculums for the development of Fusarium wilt disease epidemics in chickpea. Abundant chlamydospores produced in infected tissues as severe symptoms develop and the plant senesces. Gradually, these chlamydospores are released into the soil as infested debris decomposes. Chlamydospores may undergo cycles of renewal by limited saprophytic growth of the fungus by the help of organic debris and root exudates (Haware *et al*., 1982). The fungus can grow at temperatures of 7-35 °C and pH 4-9.4 (Jimenez Diaz *et al*., 2015). Temperature and pH ranges for mycelia growth of the pathogen are 7.5 to 35 ^{0}C and 4 to 9.4, respectively; the optimal conditions being 25 to 27.5 ^{0}C and 5.1 to 5.9, depending upon the strain. Optimum pH for sporulation is 7.1-7.9.

2.2.1.2. Disease Symptoms

Disease can be observed about 3 weeks after sowing. Entire seedlings collapse without discoloration in 3 to 6 weeks after sowing. When uprooted, the affected seedlings usually show uneven shrinking of the stem above and below the collar region. Wilt symptoms in adult plants are very common at flowering and padding stages. The affected plants show characteristic wilting i.e. dropping of the petioles, rachis and leaflets. The lower leaves become chlorotic but most of the other leaves droop while still green.

Gradually, all the leaves turn yellow and then light brown or straw colored (Figure 1A). Dried leaflets then shed at maturity. No external rotting, drying of root is seen when the wilted plants are uprooted. When the stem is split vertically, internal discoloration is seen. The xylem in the central inner portion (pith part of wood) is discolored in the form of dark brown or black around the collar region (Figure 1B). Sometimes only a few branches are affected, resulting in partial wilt (Anon, 2010).

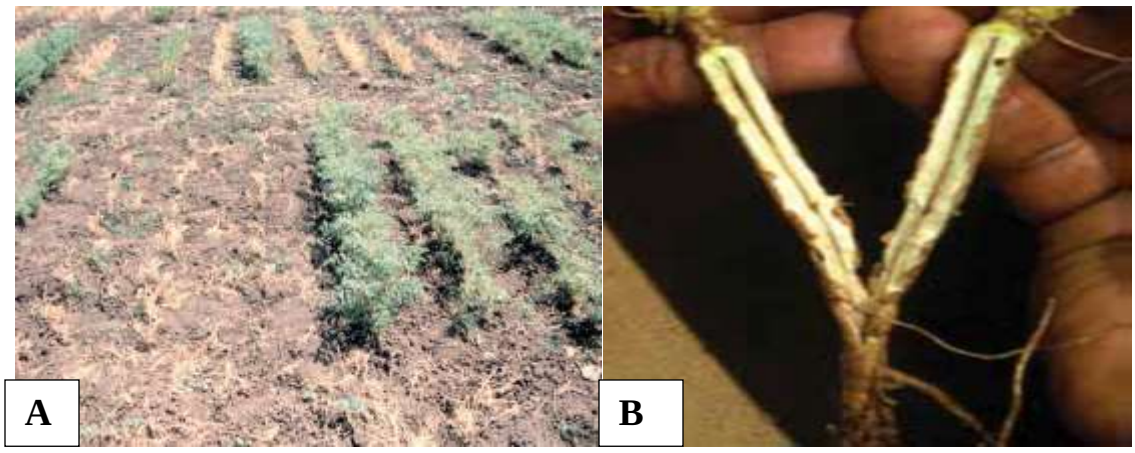


Figure 1.Chickpeas affected by Fusarium wilt (A)Internal blackening of chickpea stem caused by Fusarium wilt (B)

2.2.1.3. Host Ranges of the Pathogen

Pathogenic strains of FOC have been studied for more than 100 years. The host range of these fungi is broad and includes animals, and ranging from arthropods (Teetor-Barsch and Roberts, 1983) to humans (Nelson *et.al.*, 1984) as well as plants including a range of both gymnosperms and angiosperms. While collectively, plant pathogenic FO. Strains have abroad host range; individual isolates usually cause disease only in narrow range of plant species. This observation has led to the idea of special form or forma specialis in *F. oxysporium*. Formae specialis have been defined as an informal rank in classification. Used for parasitic fungi characterized from a

physiological stand point (e.g. by the ability to cause disease in particular hosts) but scarcely or not at all from a morphological stand point. Exhaustive host range studies have been conducted for relatively few formae specialis of *F. oxysporium* (Kistler, 2001). Like *Fusarium oxysporium* f.sp.*ciceris, albedinis, asparagi, batatas, betae, cattleyae, cannabis, cepae, citri, coffea, cubense, cyclaminis, herbemontis, lentis, lini, lycopersici, pisi, radices-lycopersici, strigae, niveum,*and *nicotianae.*

2.2.1.4. Disease Cycle and Epidemiology

The *Fusarium oxysporium* f.sp *ciceris*can be transmitted by seed and may survive in plant debris in soil. It is reported that the fungus chlamydospore was found free in soil(Haware *et al*., 1996), in the hilum of the seed (Haware *et al*., 1978), in cotyledons and axis (Shakir and Mirza, 1994). The primary infection can be through chlamydospores or mycelia. The conidia of the fungus are short lived; whereas, the chlamydospores are long lived and it can remain viable up to the next cropping season (Chand and Khirbat, 2009). Chlamydospore formation relies on the nutrient level of the inoculums. The pathogen may be found in roots and stems, even in apparently healthy looking plants growing among diseased ones harboring sufficient fungus (Haware and Nene, 1982, and Trapero-Casas andJimenez Diaz, 1985).

The fungus stays dormant as chlamydospores in plant debris until it initiate to germinate, once carbohydrates are released from decaying plant tissue or from roots (Schippers and Van Eck, 1981). The stimulus for germination may be host or non-host plant roots, or contact with pieces of fresh (not colonized) plant debris (Nelson, 2012). After the germination of chlamydospores, conidia and new chlamydospores may be formed as well as Hyphae. Following germination, a thallus is produced from which conidia form in 6–8 hour, and chlamydospores in 2–3 days if conditions are suitable. Invasion of the roots is followed by the penetration of the epidermal cells

of the host or the non-host (Beckman andRoberts, 1995) and the development of a systemic vascular disease in host plants (Stover, 1970). Penetration can occur either through wounds or directly (Nelson, 2012).The most common sites of direct penetration are found at or near the root tip of both taproots and lateral roots (Lucas, 1998). Penetration depends on a combination of different factors that include fungal compounds, plant surface structures, activators or inhibitors of fungal spore germination, and germ tube formation (Mendgen *et al*., 1996). During colonization, the mycelium advances within the cell through the root cortex until it arrives at the xylem vessels and enters them through the pits. The fungus then remains exclusively within the xylem vessels using them to colonize the host (Bishopt and Cooper, 1983).The pathogen is primarily confined to the xylem vessels in which the mycelium branches and forms micro conidia (Haware and Nene, 1982). The micro conidia detach and are carried upward in the vascular system until movement is stopped, at which point they germinate and the mycelium penetrates the wall of the adjacent vessel (Beckman and Turner, 1989). Lateral movement between vessels is through the pits. Infected plants water economy is eventually severely compromised by blockage of vessels, resulting in stomatal closure, wilting and death of leaves, often followed by death of the whole plant (Gupta, 1991; Singh *et al.*, 2006).

The development of *Fusarium* wilt of chickpea is affected by many factors in fields. These include: the presence of virulent strains in the soil, susceptibility of the crop varieties, soil type, soil fertility, climatic conditions, irrigated or non-irrigated crops and interactions with other soil borne micro-organisms. The amounts of inoculums in the soil are also important as disease symptoms may not be apparent when there are low levels of the pathogenic strains in the soil (Davis *et. al*., 2006). The disease can occur at any stage of plant growth, particularly when crops are stressed. Factors such as, high plant populations, improper cultivation, other soil-borne

pathogens and various herbicides are all known to induce injury of young roots, causing plant stress. These can aggravate *Fusarium* wilt damage. Early infections can kill seedlings soon after emergence, leaving bare areas in the crop. Later infection can cause wilting, yellowing and death of adult plants. The effect of *Fusarium* wilt is most apparent during blossoming and early pod set when the plant and its productivity are more sensitive to stress.

Fungal spores can be splash dispersed, rain splash and moving water can carry chlamydospores and conidia short distances to surrounding plants and adjoining paddocks. The pathogen can be transported over large distances by infested seed and harvesting equipment into new areas. Seed infected by *Fusarium oxysporum* f. sp. *ciceris*may not show external symptoms of infection. Windblown plant debris could spread the pathogen over moderate distances following harvest into adjacent paddocks (Pande *et al*., 2007).

2.2.1.4. Isolation and Ecology of *Fusarium* species

Fusarium species can be found in soil, water and on seeds, roots and leaves of most plants. Several selective media have been developed for the isolation, growth and sporulation of *Fusarium* species, including Selective Fusarium Agar (SFA), Dichloran Chloramphenicol Peptone Agar (DCPA), Spezieller Nahrstoffarmer Agar (SNA) and Modified Potato Dextrose Agar (MPDA). The isolation of *Fusarium s*pecies from plants is affected by the nature of the source material, method of surface sterilization, plating procedures, medium and incubation conditions (Burgess *et al.,* 1994). The choice of medium depends largely on the nature of the tissue involved in the isolation exercise. Selective media are normally used for the isolation of *Fusarium* species from diseased crown or root samples. There are several other techniques for recovering *Fusarium* species, directly or indirectly, from plant samples, which do not involve plating tissue segments on agar media. Some species produce sporodochia on the surface of the

diseased tissue. Macro conidia can be taken from these sites and used to prepare a conidial suspension, which is plated on Water Agar containing antibiotics. Germinated single conidia are later taken to initiate pure cultures for identification of *Fusarium* species (Burgess *et al.,* 1994).

2.2.1.5. Morphological Identification of Fusarium Oxysporum

Morphological identification of*Fusarium oxysporum*is based on the micro conidia produced on short monophialides as a false head, mostly unicellular, varying from oval–ellipsoid to cylindrical and from straight to curve. Macro conidia are also formed in abundance, with an attenuated apical cell and a pedicellate basal cell, generally with 3–5 septate, produced in short branched or unbranched monophialides or sporodochia. Another striking characteristic used for identification is the constant presence of chlamydospores, with a smooth wall, the most formed singly, with intercalated or terminal location. Colony on PDA initially with white aerial mycelium becoming salmon with a tendency towards violet and a purple back.

Macro conidia are produced from phialides on unbranched or branched conidiophores (Burgess *et al.,* 1994). They are two or more celled, thick-walled, smooth, and cylindrical or sickle shaped with pointed distal ends. Micro conidia and chlamydospores, which are resting structures produced by hyphae and conidia, may be present or absent. The perithecial states (teleomorph or sexual state) are known for some species and belong to the Hypocreales in the Ascomycotina (Burgess *et al.,* 1994). Micro conidia (2-4 x4-8 μm), are formed on long or short simple conidiophores. They are 1- celled (occasionally 2- or 3-celled), smooth, hyaline, ovoid to cylindrical, and are arranged in balls. Chlamydospores, when present, are sparse and grow in pairs, clumps or chains.They can be thick-walled, hyaline, intercalary or terminal. Phialides are cylindrical structures, with a small collarate and maybe solitary or produced as a component of a complex branching system. Monophialides and polyphialides (in heads or in chains) may also be

observed. Macroscopic and microscopic features such as length and shape of the macro conidia, the number, shape and arrangement of micro conidia, and presence or absence of chlamydospores are key features for the differentiation of *Fusarium* species.

2.2.1.6. Disease Management

Fusarium wilt of chickpea can be managed using resistant cultivars, adjusting sowing dates, and fungicidal seed treatment (Navas-Cortes *et al*. 1998). The use of wilt-resistant chickpea varieties and adjustment of sowing dates are potentially cheap and easily adoptable methods in managing chickpea wilt. In addition to the use of fungicides, follow good agronomic practices to keep crop healthy and do not grow chickpea outside of the area of best adaptation.

2.2.1.6.1. Resistant Variety

The use of resistant cultivars becomes the most practical and economically efficient control measure for management of chickpea *Fusarium* wilt. Host plant resistance provides the cheapest and most sustainable disease control (Malik *et al*., 2006). Resistant chickpea cultivars represent a key component in integrated disease management (IDM) programs that involve the use of additive or synergistic combinations of biotic, cultural, and chemical control measures (Jimenez Diaz *et al*., 2015, Landa *et al*., 2004, Jimenez Diaz and Jimenez Gasco, 2011andConway, 1996). Resistance to FOC races had been identified mainly in Desi germplasm and to a lesser extent in Kabuli chickpeas, as well as in wild *cicer* spp. (Jimenez Diaz *et al*., 2015). The development of resistant cultivars has not been extensive because of undesirable agronomic characteristics in some developed materials. Furthermore the high pathogenic variability in *Fusarium oxysporium* f.sp.*ciceris*populations may reduce the effectiveness and extensive use of available resistance varieties (Bayraktar and Dolar, 2012). Developing and releasing wilt/root rot-resistant cultivars is the major objective of the national chickpea improvement program and chickpea varieties

having resistance to wilt/root rot have been released for cultivation in Ethiopia (Geletu *et al*., 1996).Seventeen chickpea varieties (DZ-10-4, DZ-10-11, Dubie, Mariye, Worku, Akaki, Arartie, Shasho, Habiru, Chefe, Ejere, Tjie, Natoli, AcosDubie, Minjar, Teketay and Dalota) from DebreZeite Agricultural Research Center (DZARC) , four from Sirinka (Fetenech, Yelebe, Kassech and Akuri), One from Debre Birhan (Mastewal) were released at different years. Generally twenty one improved chickpea varieties released by the national and regional programs in Ethiopia (Million and Asnake, 2015).

2.2.1.6.2. Chemical Control

In view of the economic importance of chickpea, as well as the seriousness of the disease and associated yield loss, farmers apply fungicides to control the disease. Research has indicated that foliar fungicide applications are not cost effective when *Fusarium* wilt severity is very low. On the other hand, some fungicides reduce losses and their use is not economical if disease pressure is high. One or more applications of a foliar fungicide during flowering, or even at early podding, can increase seed yield and quality. Timely application of fungicide is especially important if the forecast calls for rain. Different fungicides and soil fumigants are currently used to control FOC. However, many of these compounds proved to be quite toxic to the environment and to the ground water. Methyl bromide is a good example for a very efficient soil fumigant that has a great impact on the environment and has been recently phased out due to the public concern and international agreements. Yet pesticide application does not always prove economic (Bayraktar and Dolar, 2012). In addition, chemicals have various limitations and pose risk of health hazard and environmental contamination. Use of FOC-free seed and fungicide-treated seed are some of the measures usually employed to control Fusarium wilt in chickpea, but with

limited success (Haware *et al*., 1996; Navas-Cortes *et al*., 1998). Apron and Mancozeb are the two most adopted chemicals in the study areas.

2.2.1.6.3. Cultural Control

Successful disease management requires planning well in advance. This disease is most effectively managed with the integration of several different strategies. Several cultural practices such as rotation with non-host crops, not growing chickpeas more frequently than every 3–4 years, and not planting new crops near previous blighted fields, the use of disease free seeds and destruction of plant diseased debris, will all help to reduce inoculums level and inhibit severe epidemics (Gan *et al*., 2006). In addition to these there are agronomic practices which reduce primary inoculum, like draining wet soils, soil preparation by tillage, fertilization, and weed control pre- planting have been reported as tools to manage root rot diseases. Planting within the recommended seed rate to avoid overcrowding can also decrease disease pressure. Crop rotation can break the disease cycle and affect soil chemistry. Tillage practices like burial of infected residue and controlling volunteer chickpeas will also be important (Navas- Cortes *et al*., 1995). Burning of chickpea residue in certain environment can also reduce the inoculum survival but may not be favored because of negative effects on soil health due to loss of organic matter and essential nutrients. Solarization of soil and proper planting date are some of the measures usually used to control Fusarium wilt in chickpea, but with limited success (Haware *et al*., 1996; Navas-Cortes *et al*., 1998). It has been demonstrated that some cultural practices, such as planting date proved to be very effective in reducing fungal attack to plants, but they are insufficient under high disease pressure, especially when weather conditions are particularly conductive to disease development (Abdel-Monaim, 2011). According to Merkuze A and Getachew A in 2012 reported that management of FOC by integrating sowing date with resistant variety and seedbed

preparation are among the cultural practice mostly apply for the management of FOC in Ethiopia.

2.2.1.6.4. Biological Control

Now a day, biological control of this soil and seed-borne plant pathogenic fungi has been addressed using bacterial and fungal antagonists. Both *Trichoderma* spp. and *Bacillus* spp. are wide spread throughout the world and have been recognized as the most successful bio control agents for soil borne pathogens. Several modes of action have been described, including competition for nutrients, antibiosis, induced resistance, mycoparasitism, plant growth promotion and rhizosphere colonization capability (Hassanein *et al.*, 2006; Siddiqui and Akhtar, 2007 and Bailey *et al.,* 2008). The species of *Trichoderma* have been evaluated against the wilt pathogen and have exhibited greater potential in managing chickpea wilt under glasshouse and field conditions, but its effectiveness is not similar in all areas (Kaur and Mukhopadhayay, 1992).

3. MATERIALS AND METHODS

3.1. Description of study area

The survey was conducted on **November** 2018 growing season, since chickpea plant could produce wilt disease symptoms at this period of time.During the survey four selected Woredas of North Shewa Zone in Amhara region were covered (Figure 2). These are Moretna Jiru, Ensaro, Mojana Wedera and Siyadebrna Wayu. According to zonal agricultural office report the Woredas are listed under **potential chickpea producing Woredas**. The Woredas were selected based on the production status and agro ecology of chickpea. The study areas have bimodal rainfall distribution with short and long rainy seasons covering from March to April and June to September, respectively. About 52% of the study Woredasfall under the Highland (*Dega*) agro-ecological zone which is characterized by severe frost attack every year from October to December and the remaining 48% are located at low land kola. Dega and kola agro ecology were representing total woredas found in North Shewa Zone. But I have sown only in one woreda, where chickpea wilt was more sever. Detail descriptions of study areas are presented under Table 1.

Table 1:Description of the study areas

No.	Lists of Woredas	Distance from Addiss Ababa (Km)	Annual rainfall (mm)	Annual Temp. (^oC)	Soil type	Altitude (m.a.s.l)	Latitude	Longitude
1	Moretna Jiru	195	900-1000	16-23	clay	1350-2860	$9^0$90' N	$39^0$15'E
2	Mojana Wedera	200	800-1060	0-18	clay loam	1500-3500	9^o92'N	39^o62'E
3	Ensaro	130	900-1500	18-30	Clay loam	1345-2650	9^o80'N	39^o 90'E
4	Siyadebrna Wayu	176	900-1000	16-22	Verti	2033-2600	9^o85'N	39^o14'E

Source: Woredas agricultural office record file

3.2. Field Survey and Sample Collections

The survey covered the most important chickpea producing Woredas (i.e. Moretna Jiru, Ensaro, Mojana Wedera and Siyadebrna Wayu) of the North Shewa Zone of Amhara region (Figure 2). Fifteen chickpea fields in each Woredas were randomly selected and the survey route was following major roads to towns and Villages in the Woredas with sampling sites separated by 5km from each other. Disease prevalence and incidence were assessed from the surveyed fields. Chickpea fields were assessed for disease incidence and prevalence and percent disease levels were calculated by the formula below. In each field, a total of five quadrant (1m*1m) was established in X fashion. In each quadrate the total number of plants and the number of plants show chickpea wilt disease symptoms (yellowing and wilting)were counted in situ from one month old seedlings.Percent disease incidence was then calculated based on the following formula (Formula 2). From each quadratefive plants (onemonth old) showing typical symptom of Fusarium wilt were collected in separate paper bag and brought to the laboratory for further

investigation. Whenever possible, whole plants including roots were collected. In case of samples which included the root system with soil attached, the sample was put into a plastic bag and tied at the base of the stem (Plant Health Australia,2008).

Disease prevalence (%) $= \frac{\text{Number of Infected fields}}{\text{Total number of fields assessed}} \times 100$.....Formula 1

Disease Incidence (%) $= \frac{\text{Number of Infected plants}}{\text{Total number of plants assessed}} \times 100$…….....Formula 2

During the survey both primary[for disease prevalence and incidence by direct observationandquestionnaire (Appendix II)] and secondary data(from agricultural office reports of the selected Woredas) were collected.Data on plant parts affected, symptoms observed, distribution, types of varieties grown and cropping history and management practices was recorded.

Figure 2. Map Showing Survey Areas in North Eastern Ethiopia

(Where EN=Ensaro, M.J=Moretna Jiru, SW=Siyadebrna Wayu and MW=Mojana Wedera in North Shewa Zone of Amhara region, North Eastern Ethiopia)

3.3. Isolation and Identification of Fungi

Fungal isolation was carried out from seventy five plant samples collected during survey; whichshowing typical symptoms of Fusarium wilt. Isolation was made from rootsof affected plants due to several other saprophyte fungi associated with root. The tissues were washed under running tap water and cut into pieces. Samples were then surface disinfested with 70% ethanol alcohol for 1 minute and rinsed in water three times then blotted dry on filter paper. Surface disinfected samples were aseptically plated in 9 cm diameter Petri-dishes containing Potato Dextrose Agar (PDA supplemented with chloramphenicol as an antibiotics)and kept at 25^{o}C temperature in an incubator to allow fungal growth in the laboratory of Debre Birhan Agricultural Research Center and Debre Birhan University. Once fungal growth was evident, subsequent sub culturing to new PDA was carried out to get fungal pure cultures. The pure cultures obtained were stored at 4^{o}C for further studies.

The colony diameter of thepure cultures of the fungal isolatesgrown on PDA for 72 hr. under dark condition was measured by taking two radial measurementsat perpendicular angle and averaged. The growth rate of the isolatesgrowing on PDA, in the dark condition was calculated by taking the radial growth of the isolates every 24 hrs.forthree days. Colony morphology and pigmentation was recorded after 7 days of culturing on PDA by observing the surface and reverse side of cultured plates, respectively. The macro and micro conidia were obtained by growing the fungal pure cultures on special media i.e. **Spezieller Nährstoffarmer Agar (SNA)** under 12 hours of Fluorescent light and 12 hours dark condition for 10-14 days at Hawassa University crop protection laboratory. For this purpose, SNA was prepared by autoclaving, in 1 L of distilled H_2O: 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.2 g Glucose, 0.2 g Sucrose and 20 g Agar. After getting the conidia, fungal identification was carried out by

observing the macro and micro conidia under compound microscope at40 x magnifications. Thenconidial structure, fungal cultures showing typical cultural characteristics of *Fusarium oxysporuim* f.sp. *ciceris were* confirmed using morphological identification keys(Nelson *et al*., 1983; Leslie&Summer ell, 2000 and Burgess *et al*., 1994).

3.4. Pathogenicity test

Pathogenicityof*Fusarium oxysporum* f.sp.*ciceris*isolated from chickpea was confirmed by root dipping inoculation technique in pots under plastic greenhouse condition by using susceptible variety kassech based on the field experiment result. For the preparation of spore suspension of theFOC isolatesto inoculate chickpea seedlings, FOC culture was inoculated on PDB media in 1000ml volume flask (Figure 3), and incubated for 10 to 14 days under florescent light condition to initiate conidia formation. Conidia wereharvested by adding 5 ml of sterilized distilled water in each flaskand surface of medium was scraped with the help of sterilized spatula. Spore suspension from each tube was passed through two layers of muslin cloth to remove mycelial mass. Spores were countedusing haemocytometer and the suspension was adjusted to $1x10^6$ conidia/ml spore concentration.

Figure 3.Mass production of *Fusarium oxysporium* f.sp.*ciceris* on PDB media

For the plastic greenhouse assay, first chickpea seeds of susceptible variety (kassech) were surface sterilized with 70% ethanol for 10 minute and rinsedthree times in sterile distilled water. The disinfected seeds were planted in 15 cm sized pots filled with loam soil, compost and sand at a ratio of 2:1:1. Roots of two weeks old seedlings were uprooted and washed under running tap water. Prior to FOC inoculation, root tips of the seedlings were cut (1 cm) using sterile scissors. Each of the injured plant roots was dipped in 1ml of spore suspension ($1x10^6$ conidia/ml) of FOC for 30 minutes. Then the inoculated plants were removed and the roots system wasallowed to dry out in order to avoid moisture and rotting of seedlings.

Root-inoculated seedlings were transplanted in pre-irrigated 15 cm diameter plastic pot filled with sterile sand, compost and clay loam soil mixed at ratio of 1:1:2 and kept in a greenhouse at 25 ± 3^oC using Randomized Complete Design (CRD) with three replications. Water was applied regularly to maintain the substrate at field capacity level (enough water but not large amount of

water that causes water logging).Inoculated seedlings were assessed for disease initiation period, incidence and severity from 7 up to 30 days after inoculation.

Re isolation of the pathogen was made from stem base of artificially inoculated and diseased plants showing the typical symptoms of wilting. The obtained fungus were transferred to PDA and SNA for comparison of morphological features of the re isolated fungi with the original culture of FOC using the same *Fusarium* identification manuals used in the first step identification under section 3.3.

3.5. Evaluation of Chickpea varieties against *Fusarium oxysporum* f.sp. *ciceris*in Siyadebrna Wayu Woreda

3.5.1. Description of Experimental Design and Treatment

The field experiment was carried out using randomized complete block design (RCBD) with 7 treatment varieties i.e. (Natoli (V 1), Mastewal (V 2), Arerti (V 3), Kutaye (V 4-check), Minjar (V 5), Yelbe (V 6) and Kassech (V7) with 3 replications at naturally infested field or hot spot condition in Siya Debrena Wayu Woreda. Seeds were obtained from DBARC (Debre Birhan Agricultural research center) and these chickpea varieties were not tested in the study area.Treatment four (Kutaye) was the check variety because it was recommended by agricultural expertise as a wilt resistant and dominantly used by the farmers in the study area. The description of the chickpea varieties' tested is presented under Table 2. The field experiment had a total of 21plots with size of 2.2×2 m, plant to plant spacing of10cm, row to row spacing of 30cm and 0.5 m spacing between plots. Seeds were sown at planting depth of 5cm. The chickpea was cultivated under rain fed condition and weeding was carried out one month after sowing. It did notneed that much fertilizer since it can fix nitrogen by its roots.

Table 2.Description of chickpea varieties evaluated in the field experiment.

No.	Varieties	Chickpea type	Seed color	Altitude (m.a.s.l)	Year of release	Genetic background	Productivity (Q/ha	
							Research station	Farmers field
1	Natoli	Desi	Light Golden	1800-2700	2007	ICCX-910112-6	22-26	20-25
2	Mastewal	Desi	Light Golden	1800-2600	2006	ICCV-92006	25-33	15-19
3	Arerti	Kabuli	White	1800-2600	1999	FLIP 89-84c	26-46	20-32
4	Kutaye	Desi	Red	750-1900	2005	ICCV-92033	24–50	20-46
5	Minjar	Desi	Golden	1800-2600	2010	ICC97103	22–50	20-40
6	Yelbe	Kabuli	White	750-1900	2006	ICCV-14808	18.2	14
7	Kassech	Kabuli	White	750-1900	2011	FLIP 95-31C	20-25	16-20

Source: Asnake *et al.,* 2018 and Ayana *et al.,* 2019.

3.5.2.Disease assessment

From the experimental fielddata on disease incidence, severity and yield was collected.The seed emergence was recorded 18 days after sowing. Observations on number of plants wilted(disease incidence) from each variety were recorded 30days after ofsowing.

The chickpea wilt was assessed using the following parameters.

Disease incidence

The percent wilt incidence was calculated on the basis of initial plant count and total number of wilted plants in each treatment (variety).Percent disease incidence was calculated using Formula 2 described under section 3.2.

The level of resistance and susceptibility of each variety was determined by using 0-9 rating scale (Table 3)given by Iqbal *et al*., (2005).

Table 3: Grade for accounting per cent mortality of *Fusarium oxysporum* f. sp. *ciceris*

Grade	Percent mortality	Disease reactions
1	0-10	Highly Resistant (HR)
3	11-20	Resistant (R)
5	21-30	Moderately Resistance (MR)
7	31-50	Susceptible (S)
9	50 above	Highly susceptible (HS)

Source: Iqbal *et al*., 2005.

Disease Severity

The disease severity was also recorded for four consecutive weeks by tagging ten to fifteen infected plants per plot and followed the tagged plants to take the severity data. A total of 105 plants were tagged from 21 plots. The reaction of chickpea varieties against FOC was evaluated using a 1-9 disease severity scale according to Pastor Corrales and Abaw (1987). The severity grades were converted into percentage of severity index for analysis (Cooke, 2006) as written in the formula 3 below.

Based on shoot wilting intensity the 1-9 scale represents:

- 1=no visible symptoms
- 3=1-10% of symptomatic leaves (leaves with mild chlorosis & wilting)
- 5=11-25% of symptomatic leaves (leaves with moderate chlorosis & wilting)
- 7=26-50% of symptomatic leaves (leaves with sever wilting & chlorosis)
- 9= Plants dead (severely infected plants)

Based on this scale varieties reaction is classified as follows:

- 1-3 = Resistant
- 3.1-6= Intermediate
- 6.1-9= Susceptible

$$\mathbf{PSI} = \left(\frac{\text{SNR}}{\text{NPR}\times\text{MSS}}\right) \text{X}100 \text{.....Formula 3}$$

Where, PSI is percent severity index; SNR is the sum the numerical rating; NPR is number of plants rated; MSS is the maximum score of the scale. The area under disease progress curve (AUDPC) for each treatment was computed using the formula 4 by Jerger and Vinjanen-Rollinson (2001) as follows:

$$\mathbf{AUDPC} = \sum_{\text{i}=1}^{\text{n}-1} 1/2(\text{Xi} + \text{Xi} + 1)(\text{ti} + 1 - \text{ti}) \text{.....Formula 4}$$

Where, n is total number of assessments, t_i is the time of the i^{th} assessment in days,$X_i$is the percentage of the disease severity or disease incidence at i^{th} assessment and AUDPC the area under disease progress curve was expressed in percent-days.

Grain Yield

The time of harvesting is crucial in maintaining the quality of seeds. The crop was harvested when leaves start to senesce and start shedding. The pods on the plant were turn yellow and seeds in the pods feel hard and rattle within the pod.It was harvested at physiological maturitywhen about 90-95% of the crop matures and with 60% moisture contents. Chickpea harvesting was done by hand. After harvest, the plants were dried in the sun for a few days to ensure that seeds get dry enough (Ellis *et al*., 1987). The grain yield data was recorded after harvesting by measuring the weight of the grain from each plot with their respective treatment

varieties and replications. It was measured first in unit of gram per meter square (g/m^2) then the unit was converted to standard unit kilo grams per hectare (Kg/ha) base. It was important for comparisons of treatment varieties and to know which variety gives better yield than others.

3.6. Data Analysis

Data obtained was subjected to Analysis of variance (ANOVA) using statistical packaging of social science (SPSS) and Statistical Analysis System version 9.2 (SAS 2002). Means were separated according to LSD at 5% probability level. The result of data analysis is organized and presented in the form of graphs, charts and tables.

4. RESULTS AND DISCUSSION

4.1. Distribution and Significance ofChickpea Fusarium Wilt in NorthShewa Zone

The survey results showed that chickpea wilt was widely distributed and very serious problem in the study areas. The farmers were asked if the disease occurred in their field previously and 87.0% of the respondents said that the disease existed in their fields previously. According to farmers' response the disease is dispersed by seed and planting material. The disease occurs three weeks after sowing and it is favoured by moisture. Farmers locally called the disease "**leblibe**". According to their response, farmers in the study areas use different management options to minimize the effects of the disease including adjusting sowing date (since it related with soil moisture as soil moisture decrease disease incidence also decrease at mid-August), soil solarisation (since it kills soil microbes), and crop rotation (Teff and Wheat). Crop rotation can control the disease due to host specificity of the pathogen.

According to the respondents and Woredas agricultural office reports different varieties of chickpea are adopted by the farmers in the study areas; because the woredas had different agro ecology.The most commonly used chickpea varieties in Moretna Jiru were Natoli, Local and Kutaye; in Mojana Wedera wereKutaye, Natoli and Local; in Siyadebrna Wayu kutaye, Arerti, Habru and Natoli; where as in Ensaro Woreda farmers adopted Kutaye, Arerti and Natoli varieties. Farmers in the study area mostly use Kutaye and Natoli. In the study areas farmers rotate teff and wheat with chickpea.According to the woredas agricultural office record file; the types of soil in the surveyed field were verti soil in Siya Deberna Wayu, clayin Moretna Jiru, and clay loamin Ensaro and Mojana Wedera (Table 1). In the study areas, chickpea is produced in the main raining season.

4.1.1. Prevalence and Incidence of Chickpea Wilt in North Shewa Zone

Disease prevalence and incidence varied in assessed four Woredas of the North Shewa Zone (Table 4). Disease prevalence ranged from 85 to 100 %. The highest (100%) and the lowest (85%) disease prevalence were recorded from Siyadebrna Wayu and Moretna Jiru weredas, respectively. Chickpea *Fusarium* wilt was highly prevalent in Siyadebrna Wayu as compared to other Woredas. The disease incidence on the other hand ranged from 27.8 to 36.55%. The highest (36.55%) and the lowest (27.8%) incidence were observed in Siyadebrna Wayu and Mojana Wedera, respectively. The result was similar with previous studies conducted in central Ethiopia, depending on agro ecologies, locations, crop stages and seasons when the surveys were carried out, wilt/root rot incidences ranging from 1-68% have been reported (Beniwal *et al*., 1992; Negussie, 1996; Merkuz *et al*., 2011).

Table 4: Incidence and prevalence of chickpea wilt disease in the study areas.

Site	**Prevalence (%)**	**Incidence (%)**
Moretna Jiru	85	27.8
Ensaro	95	30.4
Mojana Wedera	90	32.25
Siyadebrna Wayu	100	36.55

4.2. Identity of Fungi Associated With Chickpea Wilt in North Shewa Zone

A total of twelve FOC isolates were identified (which associated with chickpea wilt disease) morphologically by analyzing colony morphology and conidia structure in the laboratory using *Fusarium* identification manuals (Nelson *et al*., 1983;Leslie andSummer ell, 2000 and Burgess *et*

al., 1994). The wilt affected chickpea plants were identified in the field based on key symptoms like wilting, yellowing of leaves, leaf senescence and drying of plants (Appendix figure 2B). The symptoms observed were yellowing and wilting of leaves which result in the drooping of entire plant or its branches. When the roots of infected plant splited in to two brown to black discoloration of the xylem vessels were observed.

Similar results were reported by Magar (2012) and Sonkar *et al.*, (2014) i.e. in both seedling and adult infection, inspection of roots longitudinally shows a brown to black discoloration of internal vascular tissue (pith and xylem). Nikam *et al*., (2011) observed wilting symptoms on chickpea variety JG-62 after 25 days of inoculation with FOC the initial symptoms were light yellow and drooping of leaves and finally wilting of the host plant. Whereas, Patil *et al*., (2017) did not observed rotting of outer surface of the wilted chickpea in seedling stage, however in adult stage, they observed brown discoloration of internal tissues upon splitting vertically the discolored region downward as well as typical wilting. In another report, it is indicated that sometimes only a few branches of the plant were affected which results in partial wilting (Jalali *et al*., 1980).

Table-5: Morphological Characteristics of *F. oxysporum*

No.	Sample code	Average colony diameter (mm)	Mycelium		Macro conidia
			Side	Reverse	Septation
1	EN1	80	White	no pigment	3
2	SW1	74	“	“	3
3	EN2	73	“	“	3
4	SW3	45	“	“	3
5	SW2	45	“	“	“
6	MJ3	38	“	“	“
7	MJ1	37	“	“	“
8	EN3	53	“	“	“
9	MW2	49	“	“	“
10	MJ2	47	“	“	“
11	MW3	44	“	“	“
12	MW1	39	“	“	“

Where MJ-Moretna Jiru, SW-Siyadebrna Wayu, EN-Ensaro and MW-Mojana Wedera
(The suffix 1, 2 and 3 represent the sample variety taken 1=Natoli, 2=Areriti and 3=Local)

The colony characteristics of the FOC isolates isolated fromchickpea were similaras shown in the above table. The fungal mycelia of the isolates were smooth, white in color and, abundant on PDA at 7 days (Figure 4A). The result of the present study is also confirmed by Jimenez Diaz *et al*., (2015) who found that the aerial mycelium of FOC was at first white and cottony, butlater it

became cream or salmon in color or remain white. The FOC isolates did not form any pigmentation on agar (Figure 4B).

Table 6: Colony diameter and growth rate of the FOC isolates isolated from Chickpea

No.	Isolate	Colony Diameter* (mm)	Growth Rate (mm/day)
1	SW	80[a]	11.6[a]
2	MJ	73[a]	10.2[ab]
3	EN	71.7[a]	9.7[ab]
4	MW	68.7[a]	9.3[b]
LSD(0.05)		15.7	1.5
CV(%)		11.40	8.01

*Colony diameter of FOC isolates grown on PDA for 7 days .in the dark
Where MJ-Moretna Jiru, SW-Siyadebrna Wayu, EN-Ensaro and MW-Mojana Wedera

There was no significant difference (P<.0.05) in the colony diameter of the FOC isolates. The colony diameter ranged from 68.7– 80mmas indicated in the above table. On the other hand the growth rate varied significantly (P<0.05)among the FOC isolates. It ranged from 9.3-11.6 mm/day. The isolate obtained from Siyadebrna Wayu was the fastest growing colony compared to other isolates obtained from the other woredas. Whereas the isolate from Mojana Wedera was slow growing one at the same temperature 25^{o}C.

The macro conidia was falcate shaped i.e. straight to slightly curved with 3 septation(Figure 4D), while the micro conidia was oval shaped as illustrated in (Figure 4C). The fungi were identified as *Fusarium oxysporum* f. sp. *ciceris*based on colony characteristics and macro and micro conidia structure observed under microscope using *Fusarium* identification manuals (Nelson *et al*., 1983; Leslie & Summer ell, 2000 and Burgess *et al*., 1994).

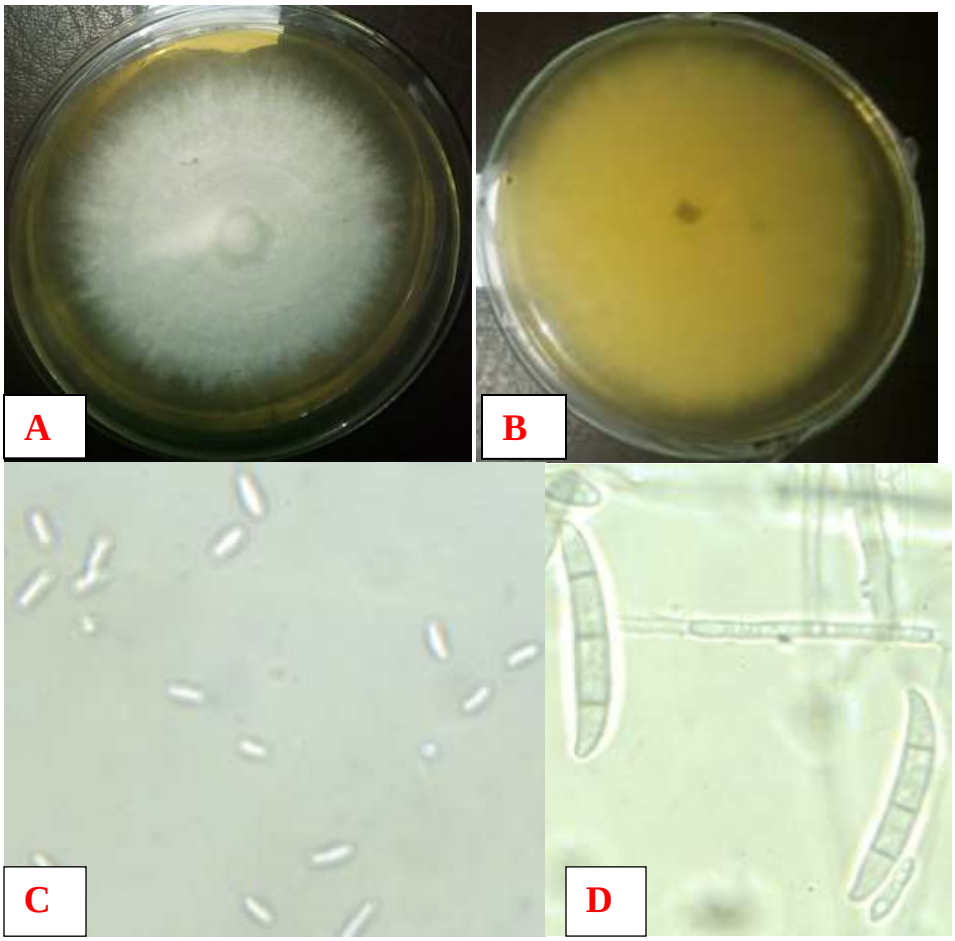


Figure 4. Colony morphology: Surface (A), Reverse (B); Micro conidia (C) and Macro conidia (D).

4.3. Evaluation of Chickpea Varieties against*Fusarium oxysporum* f.sp. *ciceris* in Siyadeberna Wayu Woreda

The current study was conducted to identify resistant varieties against chickpea *Fusarium* wilt in the study areas. The use of resistant cultivars appears to be the most practical and economically efficient control measure for management of chickpea *Fusarium* wilt. Resistant chickpea cultivars represent a key component in integrated disease management (IDM) programs that involve the use of additive or synergistic combinations of biotic, cultural, and chemical control measures. Chickpea varieties grown in the study areas were screened for their reaction against

Fusarium wilt (*Fusarium oxysporium* f.sp *ciceris*) at Siyadebrna Wayu under naturally infested field. Infected chickpea plants showed wilting and yellowing symptoms in the screening field as compared to the healthy ones (Figure 5).

Figure 5. Healthy (A): Diseased (B) plant samples collected from Natoli and Kassech varieties, respectively inthe study areas.

4.3.1. Disease Assessment

4.3.1.1. Disease Incidence

The analysis of variance in the field experiment revealed that there was highly significant difference ($P<0.05$) in the disease incidence among the screened chickpea varieties due to their genetic composition. The highest percentage of disease incidence (40.3%) was recorded from the variety Kassech while the lowest incidence (13.3%) was recorded from the variety Natoli from the Desi type chickpea. From the tested varieties Minijar, Yelbe and Kassech were susceptible to the disease, whereas the chickpea variety Kutaye was moderately resistant (Table 5). Iftikhar *et al*., (1997) also screened 31 chickpea germplasm lines received from ICARDA and based on disease incidence, they found that all of the varieties tested were highly resistant to wilt disease. Whereas, Bajwa *et al.,* (2000) found that out of 32 chickpea genotypes only one line was resistant, 4 lines were tolerant, and 27 were susceptible to highly susceptible against *Fusarium*

wilt. Iqbal *et al.,* (2005) also reported the sources of resistance against *Fusarium* wilt in chickpea germplasm originating from national and international research institutes. According to Chaudire *et al.,* (2006), among 414 varieties/germplasm accessions evaluated for *Fusarium* wilt, 35 test lines were resistant, 208 intermediate, 77 susceptible and 94 highly susceptible. Chaudire *et al.,* (2007) screened 196 chickpea germplasm lines for resistance to wilt disease. None of the test lines was found immune or highly resistant to the disease. The present study was in line with, Thaware *et al.,* 2017 who screened 50 chickpea varieties against Fusarium wilt and all the chickpea varieties tested exhibited different reactions against *Fusarium oxysporum* f. sp. *ciceris.* Demisew (2010) reported that newly released varieties have good genetic potential for disease resistance than old varieties. Thaware *et al.*,(2017) screened chickpea varieties /cultivars against *F. oxysporum* f. sp. *ciceris*under natural epiphytic condition during spring2013-14 (50 entries) and during 2014-15 (48 entries) and reported that during summer 2013-14, all the 50 chickpea entries exhibited different reactions against *F. oxysporum* f. sp. *ciceris*. However, six test entries were found highly resistant (Vishal, BCP-10, BCP-21, BCP-49, BCP-60 and BCP-61), thirty one were resistant, eight were moderately resistant (BDNG 9-3, BDNG-2003-1, JAKI- 9218, BDNG-2010-1, BDNG- 801, AKG-12009, PKV Kabuli-2 and BCPK-3), two were moderately susceptible (PKV Kabuli-4 and Virat) and three were highly susceptible (JG-62, BDNGK-807 and AKG-1207). In the Rabi 2014-2015, a total 48 entries exhibited different reactions against *Fusarium* wilt of chickpea. Single test entry was found highly resistant (PG- 8108), twenty one were resistant, eight were moderately resistant, ten were moderately susceptible, five were susceptible and 32 were highly susceptible.

Table 7: Mean values of disease incidence and reaction of varieties tested

Treatment varieties (Not tested in the area)	Disease incidence (%)	Disease reactions*
Kassech	40.3[a]	S
Minjar	35.3[a]	S
Yelbe	32.0[a]	S
Kutaye(control)	23.0[b]	MR
Arerti	19.6[bc]	R
Mastewal	16.0[bc]	R
Natoli	13.3[bc]	R
LSD(0.05)	8	
CV (%)	10.79	

Note: - Means followed by similar letters in a column are not significantly different at 5% LSD.
*S: Susceptible; MR: Moderately Susceptible; R: Resistant

The differences in the reaction of different chickpea varieties were due to the mechanism of host resistance by producing chemicals such as Peroxidases (PO), root exudates and phytoalexin accumulation. PO in resistant variety acts as barriers against pathogen invasion by enhancing phenol and lignin accumulation (Okay *et al*., 1997). The root exudates of four cultivars of chickpea which varied in their resistance to two races of FOC were **antifunga**l by the presence of higher concentration of medicarpin and maackian (Stevenson *et al*., 1995)

4.3.1.2. DiseaseSeverity

The result of the present study revealed that there was highly significant difference ($p<0.05$) in percentage severity index in each variety for each consecutive week. The percent disease severity index ranging from 39.3 to 82.1% was recorded for the susceptible varieties Kassech. The maximum disease severity index was recorded from variety Kassech and the minimum was recorded from variety Natoli (Table 6). The percentage of disease severity index increased from week one to week four.

The disease development was rapid on the susceptible varieties and had relatively slow progress on resistant varieties. The area under disease progress curve (AUDPC) value varied significantly ($p<0.05$) between the varieties. The highest AUDPC (1186.9%-day) was recorded from the susceptible varietiesKassech whilethe lowest AUDPC (942.7%-day) was recorded from resistant varieties Natoli(Table 6). There was no significant difference ($P<0.05$) in AUDPC between the varieties that are grouped in the same disease rating but there was significant difference amongvarieties grouped in the different disease rating scale. The result was supported by Awoke *et al*., 2019 who found that the percentage of disease severity increased with time. This result is also in agreement with the findings of Maitlo *et al.*, (2005) who reported that the degree of disease severity of Fusarium wilt of chickpea increases from seedling to flowering stage and the highest severity was recorded at podding stage for susceptible.

Table 8: Percentage Severity Index (PSI) and Area under Disease Progress Curve (AUDPC)

Variety	Percent Severity Index				AUDPC 4thwks.
	Week one	Week two	Week three	Week four	
Kassech (S)	39.3^{a}	49.6^{a}	60.0^{a}	82.1^{a}	1186.9^{a}
Minjar (S)	39.3^{a}	49.6^{a}	58.5a^{b}	77.9^{a}	1129.6^{b}
Yelbe (S)	37.8^{ab}	48.1^{a}	57.0^{bc}	73.1^{b}	1125.7^{b}
Kutaye (MR)	36.3^{ab}	48.1^{a}	55.6^{bc}	69.0^{b}	1104.6^{c}
Arerti (R)	36.3^{ab}	45.2^{a}	52.^{6c}	64.1^{c}	1052.6^{c}
Mastewal (R)	36.3^{ab}	45.2^{a}	52.^{6c}	64.1^{c}	1036.7^{c}
Natoli (R)	33.3^{b}	37.8^{b}	48.1^{d}	60.0^{c}	942.7^{d}
LSD (0.05)	5.22	4.51	3.98	4.47	49.31
CV (%)	7.95	5.48	4.08	3.59	2.56

Note: Means with the same letters in a column are not significantly different.

4.3.1.3. Grain Yield

The analysis of variance that there was significantly higher (P<0.05) in grin yield between the varieties tested (Appendix Table 2). The maximum yield was obtained from the resistant variety Natoli whereas the minimum yield was recorded from the susceptible varietyKassech with the yield range of 202.7 Kg/ha (Appendix Table 2). This result is also supported by Goa *et al*., (2016) which reported significant differences among chickpea varieties in yield, plant height, number of pods, and hundred seed weight.

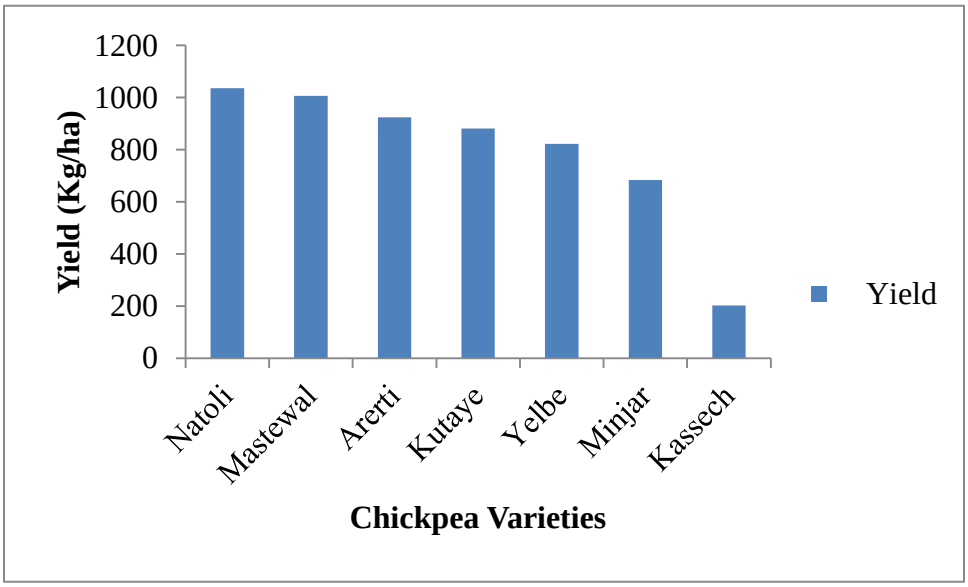


Figure 6. Bar Graph for average yield (kg/ha) of treatments (varieties)

4.3.1.4.Correlation between Grain Yield and Disease Parameters

Grain yield was affected by different disease t parameters like disease incidence, percent severity index and Area under Disease Progress Curve as indicated in table below. It is negatively correlated to all disease t parameters. Grain yield had strongly negative correlation with disease incidence, percent severity index and area under disease progress curve (Table 9). Each disease parameter and grain yield was positive correlated with itself.

Table 9. Simple linear Correlations (Pearson) of grain yield with disease assessment parameters.

	DI	PSI	AUDPC	GYLD
DI	1.0000			
PSI	0.655**	1.0000		
AUDPC	0.7950**	0.8572**	1.0000	
GYLD	-0.7664**	-0.7832**	-0.8336**	1.0000

Where DI=Disease Incidence, PSI=Percent Severity Index, AUDPC=Area under Disease Progress Curve and GYLD=Grain Yield

4.4. Pathogenicity Test Result

4.4.1. Disease Assessment

The first symptoms appeared one week after inoculation. The result of this experiment was similar with Asma Al-Jaradi *et al.,*2018, whostatedthat inoculated seedlings showed disease symptomsseven days after inoculation. The symptoms observed on inoculated plants were identical to the originalsymptoms observed in the field during the survey i.e. Described in the field experiment as shown in (Figure 7B) below. There wasyellowing and wilting of plants sown in the greenhouse. This result was also similar with Chickpea Fusarium wilt symptoms described by Million Eshete (from Debre Zeit Agricultural Research Center) and Asnake Fikre (from Ethiopian Institute of Agricultural Research) in 2017. They reported that the fungus infects chickpeas via the roots system and moves throughout the plant's vascular system. The cell wall starts degrading by the enzymes produced by the pathogen. The pathogen then forms the gels that block the plant's transport systems and cause yellowing and wilting of the plant. Vascular discoloration occurs on the roots and then towards the young stems, followed by yellowing and wilting of the leaves before final necrosis. The seedlings that are affected with Fusarium, first show dropping of the leaves and then finally collapse.

Figure7.Un Inoculated (control) plants (A) and inoculated plants (B)

1. **Disease Incidence**

The disease incidence from the pathogenicity test was significantly($p<0.05$) differentfor the different treatments tested i.e. SW, EN, MJ and MW while H_2O as control. It ranged from 7 to 50.333% and the highest disease incidence was recorded from EN2 isolate while the lowest was recordedfrom the control treatment.The result of this experiment showed that the different FOC isolates differed significantly in causing wilt disease in chickpea under greenhouse condition (Figure 8).

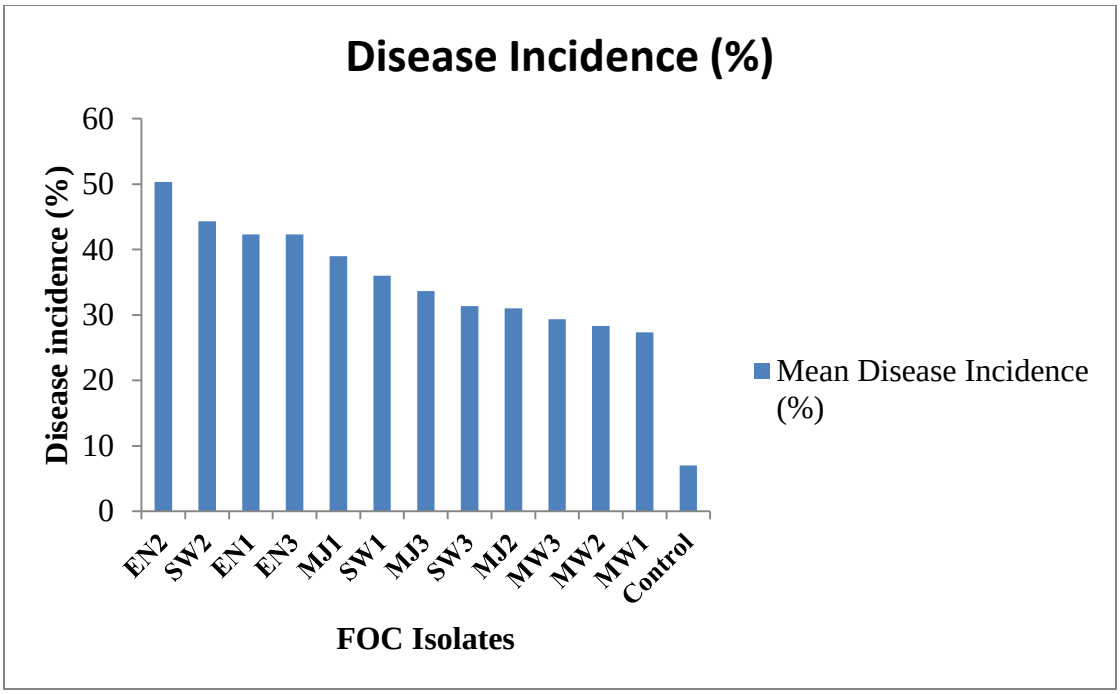
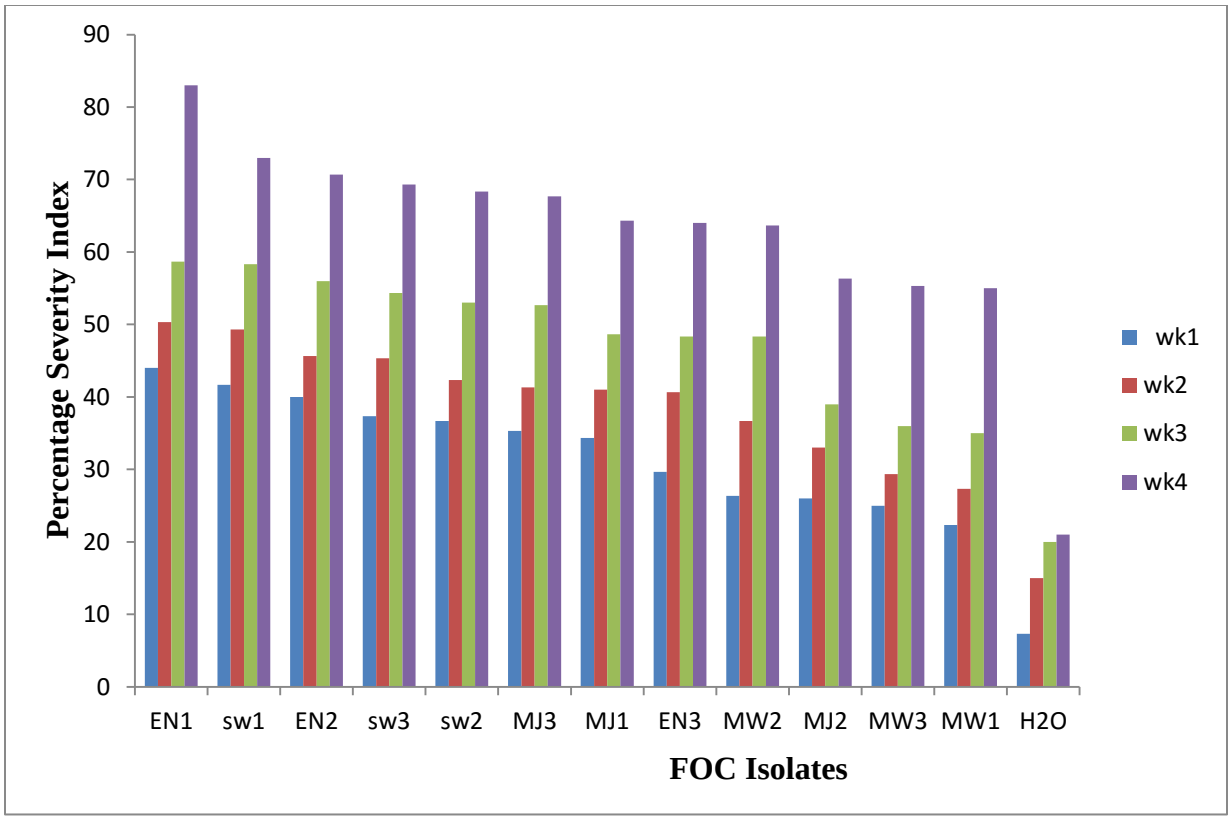


Figure 8: Bar graph for Disease Incidence (%) of susceptible variety kassech against different FOC isolates.

2. Disease Severity

There was significant ($p<0.05$) difference in percentage severity index among the different FOC isolates during all the four consecutive weeks disease assessment. Chickpea Fusarium wilt has progressively increased as the day increased. The isolates obtained from different location varied in their aggressiveness as shown in (Figure 9 and Appendix Table 5) below.The highest percent severity index was recorded from the isolate EN1; while the lowest was recorded from the control treatment throughout the four week disease assessment periods. Some isolates like SW1, SW2, EN2, MJ1, MW1 and MW2 werecaused in total death of the plants in green house as shown in the figure 7B.



Where wk1=week one, wk2=week two, wk3=week3 and wk4=week 4

Figure 9.Bar Graph for percentage severity index of susceptible variety (kassech) against different FOC isolates at four consecutive weeks.

4. 4.2.Re-isolation and Identification of Fungi

Re-isolation of fungi was carried out from the inoculated symptomatic chickpea plants grown in the greenhouse to test the pathogenicity of the FOC isolates. The re-isolated fungus had similar morphological characteristics with the original fungus isolated from wilt diseased chickpea plants; the fungus produced abundant white mycelium with no pigmentation on agar (Figure 10A and B). It produced macro conidia and micro conidia. The macro conidia was falcate shaped i.e. straight to slightly curved with 3 septation(Figure 10D). While the micro conidia was oval shaped with no septation as illustrated in (Figure 10C). The re-isolated fungus was confirmed to be *Fusarium oxysporium* f.sp. *ciceris* which caused chickpea wilt by comparing its

morphological features with the original culture isolated from wilt diseased chickpea plants using the *Fusarium* laboratory manuals (Nelson *et al*., 1983;Leslie andSummer ell, 2000 and Burgess *et al*., 1994) full filling the requirement for Koch's postulates.

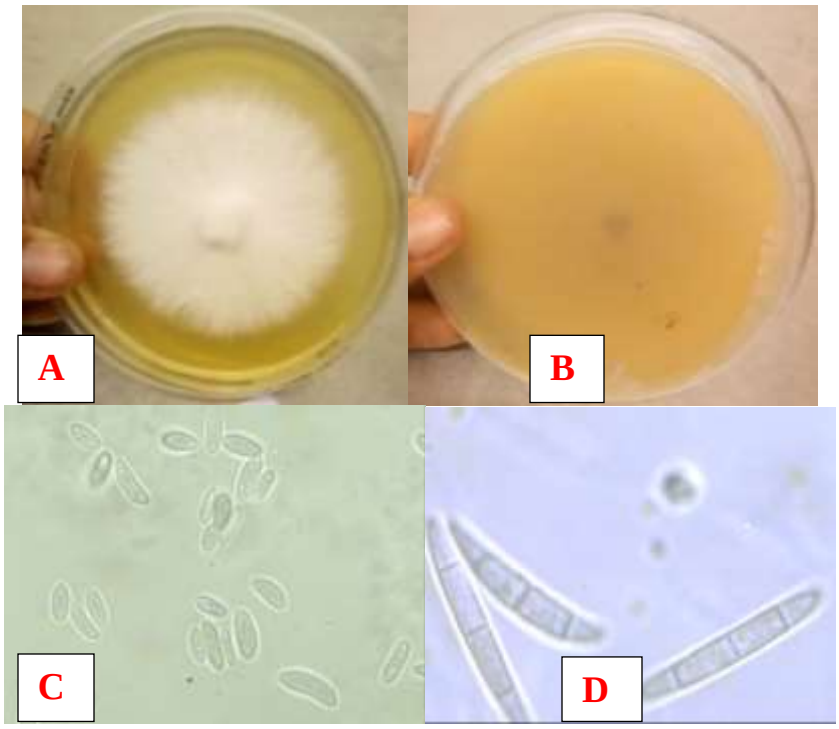


Figure 10. Colony morphologyof re-isolated fungus: Surface (A), Reverse (B); Micro conidia (C) and Macro conidia (D).

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

Chickpea is an annual legume crop which is mainly grown as source of human food and animal feed. In addition, chickpea returns significant amount of nitrogen to soil and improves soil fertility and break the disease cycles of important cereal pathogens and for this main reason, it is used in rotation with several cereals like teff and wheat on heavy soils. Chickpea production and productivity is affected by several biotic and abiotic factors. Chickpea *Fusarium* wilt caused by *Fusarium oxysporum* f.sp.*ciceris* is one of the most important biotic constraints of chickpea production. In extreme cases where no management measures are taken, it can lead up to 100% crop loss in the field. This study was conducted to determine the status of *Fusarium* wilt of chickpea at selected Woreda of North Shewa zone and screen chickpea varieties for their reaction against Fusarium wilt (*Fusarium oxysporium* f.sp *ciceris*).

The result of the survey showed that above 50% of chickpea farms surveyed in each woreda were infected by *Fusarium* wilt with mean disease incidence of 31.75%. Intensity of Chickpea *Fusarium* wilt was highest at Siyadebrna Wayu woreda with mean prevalence and incidence of 100% and 36.55%, respectively. Whereas, it was lowest in Moretna Jiru Woreda with prevalence of 85% and incidence of 27.8%.

The wilt causing pathogenic fungi was isolated from wilt infected roots of chickpea. The wilt causing fungi in chickpea was identified as *Fusarium oxysporum* f. sp.*ciceris*using field symptoms and morphological features of the fungi (colony morphology and spore structure) using *Fusarium* identification manuals.

Use of resistant chickpea varieties is one of the best approaches in the management of the disease, as it is cheaper and safer to environments. All the tested chickpea varieties showed symptoms of Chickpea *Fusarium* wilt (CFW) disease at different assessment periods. Therefore from this finding, it can be concluded that there was no highly resistant chickpea variety against CFW disease among the test varieties but relatively resistant. However, some chickpea varieties showed some degree of resistance against the disease. Among the screened seven chickpea varieties three varieties were resistant, one variety was moderately resistant and three varieties were susceptible. Natoli was comparatively resistant against CFW infection followed by Mastewal and Arerti. While varieties like Yelbe, Minijar and Kassech were highly susceptible to the disease as compared to the other varieties. Maximum disease incidence and severity were recorded in Yelbe and Kassech whereas; the minimum was noted in Natoli and Mastewal.

5.2. Recommendations

Based on the results of this study, the following recommendations are made.

- Creating continues awareness about use of resistant varieties as the one component of integrated disease management is essential.
- The current study identified three relatively resistant varieties to the pathogen among the tested varieties. Therefore, use of these comparatively resistant varietiesintegrated with cultural practices demonstrated and recommended for the farmers.
- Chickpea plant is genetically diverse legume crop in North Shewa Zone of Amhara region and in this study only seven chickpea varieties were evaluated.
    - Therefore, it is recommended that all chickpea varietiesfound in this zone should be evaluated for their reaction to CFW.

- The current study was based on only morphological identification of the causal pathogen. However, as there are several fungi associated with chickpea wilt disease, it is essential to do further research on molecular identification of the fungus associated with chickpea wilt.

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APPENDIX

Appendix I

Tables in the Appendix

Appendix table 1: ANOVA for disease incidence for field experiment

Source	DF	SS	MS	F	P
Rep	2	341.24	170.62		
Trt	6	1545.81	257.64	21.36	<.0001
Error	12	144.76	12.06		
Total	20	2031.81			
Grand Mean	26.762 CV				12.98

Appendix table 2: Mean Yield of chickpea varieties tested against Fusarium wilt disease

Variety	Yield(Kg/ha)*
Natoli	1036.2[a]
Mastewal	1006.3[b]
Arerti	924[c]
Kutaye	880.6[d]
Yelbe	822[e]
Minjar	683[f]
Kassech	202.7[g]
LSD (0.05%)	1.5
CV (%)	0.31

*Means with the same letters in a column are not significantly different at 5% level of probability.

Appendix table 3: ANOVA for chickpea yield for field experiment

Source	DF	SS	MS	F	P
Rep	2	**77**	38		
Trt	6	1472539	245423	347570.20	<.0001
Error	12	85.08	6.077		
Total	20	1472624.17			
Grand Mean	793.54CV	0.11			

Appendix table 4. ANOVA for disease severity for field experiment

Source	DF	One	two	three	Four	AUDPC
Variety	6	12.86ns	52.05**	50.167**	193.91***	19042.16***
Rep	2	0.94ns	0.94ns	2.82ns	1.345ns	164.25ns
Error		8.62	6.43	5.02	6.31	768.18
CV (%)		7.95	5.48	4.08	3.59	2.56

AppendixTable 5. Percent severity index (% PSI) for the different FOC isolates

	Percent Severity Index (PSI)			
Isolate	wk1 (%)	wk2 (%)	wk3 (%)	wk4 (%)
EN1	44[a]	50.333[a]	58.667[a]	83[a]
SW1	41.667[a]	49.333[a]	58.333[a]	73[ab]
EN2	40[ab]	45.667[ab]	56a	70.667[b]
SW3	37.333[abc]	45.333[ab]	54.333[a]	69.333[b]
SW2	36.667[abcd]	42.333[abc]	53[a]	68.333[b]
MJ3	35.333[abcd]	41.333[abc]	52.667[a]	67.667[bc]
MJ1	34.333[abcd]	41[abc]	48.667[ab]	64.333[bcd]
EN3	29.667[bcde]	40.667[abc]	48.333[ab]	64[bcd]
MW2	26.333[cde]	36.667[bcd]	48.333[ab]	63.667[bcd]
MJ2	26[cde]	33[cd]	39[bc]	56.333[cd]
MW3	25[de]	29.333[d]	36[c]	55.333[d]
MW1	22.333[e]	27.333[d]	35[c]	55[d]
H2O	7.3333[f]	15[e]	20[d]	21[e]
LSD(0.05)	11.831	10.940	10.360	11.992
CV	15	14.04	13.19	11.44

*Means with the same letters in a column are not significantly different at 5% level of probability.

Where wk1=week one, wk2 =week two, wk3=week three and wk4=week four

Appendix Table 6. ANOVA for disease severity for Green house experiment from week one up to week four labeled as (Table A, B, C, & D)

A. Completely Randomized AOV for wk1

Source	**DF**	**SS**	**MS**	**F**	**P**
trt	12	3554.92	296.244	5.96	<0.0001
Error	26	1292.00	49.692		
Total	38	4846.92			
Grand Mean	31.231		CV	12.57	

B. Completely Randomized AOV for wk2

Source	**DF**	**SS**	**MS**	**F**	**P**
trt	12	3548.77	295.731	6.96	<0.0001
Error	26	1104.67	42.487		
Total	38	4653.44			
Grand Mean	38.256		CV	17.04	

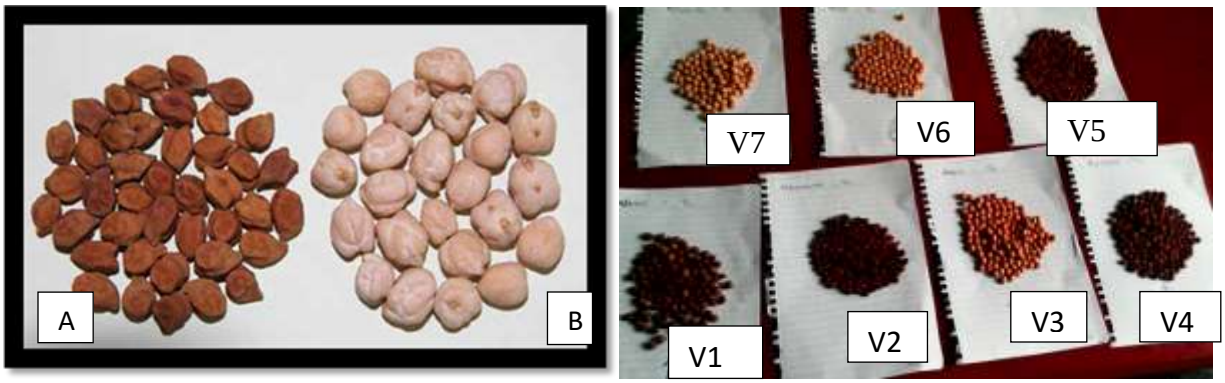
C. Completely Randomized AOV for wk3

Source	**DF**	**SS**	**MS**	**F**	**P**
trt	12	4593.69	382.808	10.05	<0.0001
Error	26	990.67	38.103		
Total	38	5584.36			
Grand Mean	46.795		**CV**	13.19	

D. Completely Randomized AOV for wk4

Source	**DF**	**SS**	**MS**	**F**	**P**
trt	12	7738.26	644.855	12.63	<0.0001
Error	26	1327.33	51.051		
Total	38	9065.59			
Grand Mean	62.436		CV	11.44	

Figures in the Appendix



Appendix Figure 1.Sampling of chickpea seed [Types of chickpea (Desi A& Kabuli B) and Chickpea Varieties used for treatment where V1-Natoli, V2-Mastewal, V3-Areriti, V4-Kutaye, V5-Minijar, V6-Yelbe and V7-Kassech in North Shewa Zone of North Eastern Ethiopia

Appendix Figure2: Interview (A), Field Observation (B), Plant Sample Collection(C),and Laboratory Experiment (D) in North Shewa Zone of North Eastern Ethiopia and Pots Used In the Plastic Green House for Pathogenicity Test (E)

Appendix II

A RESEARCHQUESTIONNAIRE

Dear respondent! This survey questionnaire is designed with the objective of collecting Information on the technology adoption of farmers. It therefore meant only for research Purposes. For this purpose your genuine responses to each of the survey questions are highly useful. There is no “right” or “wrong” answers. Your responses will be confidentially used for this research purpose only. We highly appreciate for your willingness to participate as a respondent in this survey.

For all closed type questions please put <X> mark where appropriate and please strictly, follow the instruction given in each part of the questionnaire.

- Interviewer (Enumerator) Name: _____
- Tell: _____
- Name of respondent: _____
- Questionnaire No.: _____
- Date of Interview:___/___/_____
- ID of Interviewer:

I. Demographic information of respondents

1. Sex: Male---------- Female----------

2. Marital status: Married..... Divorced.....Unmarried..... Widowed.....

3. Age of the household head:_____

4. Education level of the respondent:

A. No education (illiterate)_____

B. Traditional education (Mosque or church education)_____

C. Elementary education (1-6 grades) ----------

D. Junior level education (6-8 grades)----------

5. Woreda in the Zone: [Location of the farmer (town/village)]: _____

A. Mojana Wodera

B. Ensaro,

C. Sayadebrena Wayu

D. Moretina Jiru

I. INFORMATION WITH REGARDING TO THE CHICKPEA PRODUCTION AND IT'S CONSTRAITS IN THE STUDY AREA

6. Responsibility of the respondent in the farm: A= Owner / B = Chief Manager

7. Do you produce chickpeas?

A. Yes B. No

8. If your answer for Q7 is yes, for how many years have you been producing it? _____

9. What is the typical planting period of chickpea (month/ week) in your area?
.....

10. Which type of chickpea is most common in your area?

A. Desi

B. Kabuli

11. The type of chickpea production in this area is:

A. Rain fed

B. Irrigated

12. What kind of production problem do you face?

A. Biotic

B. Abiotic

C. Socio Economic

D. Management related

E. Others

13. If your answer for Q12 is biotic, which biotic constraint is common?

A. Diseases

B. Insects

14. If your answer for Q13 is diseases, what are the symptoms?

A. Wilting

B. Root rot

C. Yellowing

D. All

E. If any others mention?

15. When does the disease start to appear in your farm?

……..

16. Which weather condition is favorable for the disease?

A. Drought

B. Excess rain

C. Low temperature

D. High temperature

17. What are the management options you use? (Discuss about your answer).

A. Cultural/agronomic practice

B. Chemical

C. Biological

D. Resistant variety

E. Integrated disease management

18. Which management option is more effective?

……

19. Is there any training given by agricultural extension worker about the disease?

A. yes

B. No

20. Did the agricultural extension workers observe your farm problem?

A. Yes

B. No

21. If your answer to Q20 is yes, what did they tell you about the disease name, causal agent, and ways of transmission?

……

22. What do you call the disease locally? …….

23. How many chickpea varieties are found in your area? (List them)

……

24. Which variety is more resistant against Fusarium wilt and more adopted by the farmers in your area?

……

25. Incidence data collection from surveyed fields.

No	Quadrants (Q)	No. of	No.of	Total	% Incidence

	/field	normal plants	diseased plants	plants
1	Q_1			
2	Q_2			
3	Q_3			
4	Q_4			
5	Q_5			

BIOGRAPHY SKETCH

The author was born at Rema Ena Direa Kebele, Mida Woremo Woreda, North Shewa Zone, Amhara Regional State, on 29December 1994. She attended her elementary and junior education at Rema Primary School from 1999-2006. She attended secondary and preparatory education at Mida Model Service Secondary and Preparatory School from 2007-2010. Upon passing the Ethiopian Higher Education Entrance Examination in 2010, she joined Dilla University in 2011 and graduated with BSc degree in Plant Science in 2013. After her graduation, she joined Dilla University and served as Graduate Assistant (GA) for two years from September 2013 until August 2015. She was married on 8 January 2016. Then she joined the Postgraduate Programs Directorate (PGPD) at Hawassa University in November 2016 to pursue her study leading to MSc Degree in Crop Protection (Plant Pathology).