CHOCOLATE SPOT EPIDEMICS ON DIFFERENT FABA BEAN VARIETIES AND CHARACTERIZATION OF SOME *Botrytis fabae* ISOLATES COLLECTED IN DAWURO ZONE, SOUTHWEST ETHIOPIA

M.Sc Thesis

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Jimma University

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By
Mesele Haile

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APPROVAL SHEET

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As thesis research advisor, I hereby certify that I have read and evaluated this thesis prepared, under my guidance, by **Mesele Haile Onu** Entitled: **Chocolate Spot Epidemics on Different Faba Bean Varieties and Characterization of Some *Botrytis fabae* Isolates Collected in Dawuro Zone, Southwest Ethiopia,** I recommend that it be submitted as fulfilling the Thesis requirement.

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As member of the Board of Examiners of the M.Sc Thesis Open Defense Examination, We certify that we have read, evaluated the thesis prepared by **Mesele Haile**, and examined the candidate. We recommended that the thesis could be accepted as fulfilling the thesis requirement for the Degree of Master of Science in **Plant Pathology**.

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External Examiner Signature Date

DEDICATION

This thesis manuscript is especially dedicated to my son Yididiya Mesele and my wife Tigist Bezuneh for their love and dedicated partnership in the success of my life.

STATEMENT OF AUTHOR

First, I declare that this thesis is my bonafide work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for M.Sc Degree at the Jimma University and is deposited at the University Library to be made available to borrowers under rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

The author, Mesele Haile, was born on 3rd of July 1981 in Mareka District of Dawuro Zone, Southwest Ethiopia. He attained his junior elementary School at Kawuka Elementary School, junior secondary school at Tocha and his secondary school at Waka Comprehensive Secondary School from 1988 to 2000. He joined then Ambo College of Agriculture in 2001 and graduated with Diploma in General Agriculture in July, 2002 and he also awarded B.Sc in Horticulture from Jimma University in November, 2008 in advance standing program. After graduation he was employed by the Ministry of Agriculture, Ethiopia where he served as agronomist and team leader within different districts of Dawuro Zone until he joined the School of Graduate Studies at Jimma University, College of Agriculture and Veterinary Medicine in April, 2010 to pursue his M.Sc study in Plant Pathology.

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LISTOF ACRONYMS AND ABBREVIATIONS

^0C	Degree Celsius
AARC	Adet Agricultural Research Center
ANOVA	Analysis of Variance
AUDPC	Area under the disease progress curve
CSA	Central Statistical Authority
CV	Coefficient of variation
DAP	Days after planting
DSI	Disease Severity Index
DZAD	Dawuro Zone Agricultural Department
EARO	Ethiopian Agricultural Research Organization
FAO	Food and Agriculture Organization of the United Nations
FBDA	Faba Bean Dextrose Agar
GLM	Generalized Linear Model
GPS	Global Positioning System
ha	Hectare
HSW	hundred-seed weight
ICARDA	International Center for Agricultural Research in the dry Areas
IR	Infection rate
M.a.s.l	Meters Above Sea Level
mm	Millimeter
MoARD	Ministry of Agriculture and Rural Development
NS	Non significant
PA	Peasant association
RCBD	Randomized Complete Block Design
RH	Relative humidity
SAS	Statistical Analysis System
SNNPRS	Southern Nations, Nationalities and Peoples' Regional State

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ABSTRACT

Chocolate spot caused by the fungus Botrytis fabae is the major disease threatening faba bean (Vicia faba L.) production in Ethiopia. However, the intensity and importance of this disease are not well studied in the faba bean production areas of southwest Ethiopia. The present study was conducted to determine the distribution, epidemics of chocolate spot on different faba bean varieties and to characterize some B. fabae isolates in major agro-ecologies of Dawuro Zone. A total of 44 faba bean fields were surveyed in 11peasant associations (PAs) of the zone, and all of the fields were infested with chocolate spot. The disease severity indices (DSI) varied among PAs, altitude range and crop management practices. For surveyed fields, the mean DSI ranged from 33.4% in Dali to 69.4 % in Waka PA. Logistic regression analysis showed that PA, crop variety, crop history and altitude were significantly associated with DSI in a multiple variable model. Higher DSI was significantly associated with high altitude (>2200). The field experiments carried at three locations and experimental plots arranged in RCBD with three replications. The progress of the symptoms evolved slowly on certain varieties but at much greater rates on other varieties depending on location. DSI scores almost for all assessment dates and the AUDPC values varied considerably among the varieties and significant genotypic differences were observed at all locations. The varieties Hachalu, Kuse, Tumsa, Mesay, Moti, Gebelcho, Walki, Nc-58 and local cultivar were suffered from the disease (highest AUDPC). Conversely, CS20DK, Degaga, Bulga-70, Tesfa and Kasa were rated moderately resistant and developed the least symptoms at all locations. Highly significant differences were observed for number of pods per plant, seed yield and hundred seed weight among varieties. CS20DK (2100kg/ha), NC-58 (2027kg/ha), Moti (1973kg/ha) and Degaga (1910kg/ha) under Tocha conditions where as Degaga (1327kg/ha), CS20DK (880kg/ha) and NC-58 (870kg/ha) under Mari conditions were the top yielding varieties. The correlation between seed yield and DSI for all assessment dates were negatively and highly significant ($p < 0.01$) having correlation values ranging from -0.592 to -0.361 at Tocha and Mari. Significant differences were observed in the frequency of isolates among PA with colony color (X^2=35.94, df = 2, $p < 0.05$) and colony growth rate (X^2=38.7, df = 2, $p < 0.01$). According to morphological characteristics all isolates were identified as B. fabae species (11-14 × 7-10 µm, mean 12.5×7.8 µm). In greenhouse, all isolates showed typical chocolate spot lesions and differed in their aggressiveness (27% more, 64% medium and 9% less aggressive). The study revealed high occurrence and importance of chocolate spot (B. fabae) in the major faba bean growing areas located at high altitudes and integrated disease management options like use of tolerant and high yielding varieties with appropriate cultural practices like timely weeding, optimum seeding rate, repeated ploughing, fallow cropping or crop rotation with cereals are recommended.

Key words: Faba bean, Varieties, chocolate spot, epidemics, *Botrytis fabae*, Aggressiveness

1. INTRODUCTION

1.1. Background of the Study

In Ethiopia, faba bean (*Vicia faba* L.) is grown in the highland area with an altitude ranging from 1800 to 3000 m.a.s.l. It is believed that the crop was introduced to Ethiopia from the Middle East via Egypt around 5000 B.C., immediately after domestication (Yohannes, 2000). Ethiopia is now considered as one of the centers of secondary diversity for faba bean. The crop occupies the largest area in Ethiopia among other pulses (CSA, 2009). Currently, the total area under cultivation is estimated to be about 512,067 ha from which 200,000 metric tonnes are produced (FAO, 2010).

Faba bean is a multi-purpose crop that plays an important role in the socioeconomic life of farming communities mainly grown as a valuable source of protein (24 - 30 %) and energy for both human food and animal feed (Sahile *et al.*, 2011). In addition, it is an excellent candidate crop to provide nitrogen input into agricultural systems; and it makes a significant contribution to soil fertility restoration as a suitable rotation crop that fixes atmospheric nitrogen (Noorka *et al.*, 2009; Rubiales, 2010). Despite its wide importance, the average yield of faba bean is still far below the crop's potential (>3 tonnes per hectare) (EARO, 2004), because of many biotic and abiotic constraints (Agegnehu *et al*., 2006; Sahile *et al*., 2008b).

Diseases are among the most important biotic factors causing faba bean yield reduction. Such diseases are chocolate spot (*Botrytis fabae* Sardina), bean rust (*Uromyces viciae-fabae*), black rot (*Fusarium solani*), aschochyta blight (*Ascochyta fabae*) and faba ban necrotic yellow virus (Abraham *et al.*, 2000; Yohannes, 2000; Sahile *et al*., 2010).

Chocolate spot is one of the important biotic factors threatening the production of faba bean (Stoddard *et al.*, 2010). It is caused by three species of *Botrytis*, is incited by the fungus *Botrytis fabae* Sard. (Sardina, 1929), *B. cinerea* (Harrison, 1988) and *B. fabiopsis* (Zhang *et al*., 2010). *B. fabae* is the only causal agent of chocolate spot in Ethiopia (Dereje, 1999; Sahile, 2008;Sahile *et al.,* 2012) and the most widespread and destructive, causing yield loss up to 61% on a susceptible genotype (Dereje and Yaynu, 2001). However, complete crop loss (100%) may occur under prolonged conducive environment for its development (Torres *et al.*, 2006; Ahmed *et al*., 2010). It mainly infects the leaf tissue but in severe cases, petioles, stems flowers and seeds are also infected and its main survival structures are sclerotia (Harrison, 1988; Ayman *et al.,* 2009).

Several mechanisms could facilitate gene flow between populations of *B. fabae*, apart from the air dispersal of presumed spores that are dispersible by windblown rain and strong air currents (Harrison, 1988). Infected faba bean seed (Hawthorne, 2004) and infected stubble or debris (Dereje, 1999) have long been recognized as important sources of chocolate spot inoculum and effective means of spreading the disease.

Botrytis classification is largely based on morphological and cultural characteristics. But many species are morphologically similar and growing conditions significantly influence variation (Beever and Weeds, 2004). Most their species of the genus have a more restricted host range (Domsch *et al*., 1993). Most restricted host specificity occurs on members of eudicot families like Fabaceae (Jarvis, 1977). Despite the importance of this pathogen, there have been any studies in southwest Ethiopia, especially regarding its taxonomy.

Since awareness of the existing species is essential for effective disease management.Several authors have reported differences in virulence among isolates (Hutson and Mansfield, 1980; Hanounik and Maliha, 1986). Hanounik and Maliha (1986) reported the first evidence of races in *B. fabae* populations. Besides, the confirmation of race existence is needed from tests of all sources of inoculum on the supposed host in the same environment (Bond *et al.,* 1994). Also wide variation in pathogenicity, cultural characteristics, sclerotial production and infection of different faba bean genotypes exists among isolates of *B. fabae* from other regions of Ethiopia (Dereje, 1996, Sahile *et al.,* 2012).

Farmers in developing countries, particularly resource-poor farmers, are the most affected by this disease. For instance, chocolate spot may cause 100 % yield loss under favorable conditions and many farmers have dumped faba bean production due to this disease (Hanounik and Hawtin, 1982). Aggressive stage of this disease can reduce yield by 50 to 100 % and destroy a crop within hours (Torres *et al.,* 2006). In Ethiopia and other African countries, losses in yield due to chocolate spot disease can reach 60 to 80 % among susceptible cultivars and up to 34 % among tolerant cultivars (Bouhassan *et al.,* 2004; ICARDA, 2006; Sahile *et al.*, 2008b).

Many methods of control are practiced for chocolate spot such as the integration of genetic resistance (Stoddard *et al*., 2010), adopting various cultural control strategies such as early sowing (Dereje, 1993), deep ploughing (Dereje, 1999), crop rotation, burying of crop residues and timely application of appropriate fungicides (El-Sayed *et al.*, 2011).

1.2. Statement of the Problem

The extent of the damage and genetic diversity of the causal agents of chocolate spot in southwestern parts of Ethiopia is not described and this created gaps to direct integrated management of the disease for faba bean producers. Until now the productivity of faba bean under traditional farming system is found to be around 0.9 tonne per hectare (CSA, 2009) in Dawuro Zone, which is very low. However there is a possibility to improve the situation using improved varieties (1.8 - 4 tonnes per hectare, which can give better yield than locally grown land races (EARO, 2010).

Many reasons have been given for the decline in the production as susceptibility to biotic (Sillero *et al.,* 2009) and abiotic stresses (Link *et al.,* 2010). As preliminary survey carried out by Dawuro Zone Agriculture Department for three successive years (2008 to 2010), chocolate spot was found to be the primary bottle-neck problems constraining the production and adoption of improved faba bean varieties that has been challenging to increase per capita of individual farmers and food self-sufficiency (DZAD, 2010).

Also crop production under varied agro-ecological conditions of the country would require modern varieties that fit to diverse ecologies. The existing breeding and seed multiplication capacity does not fully meet the critical constraints of varieties and seeds leading to stagnated production and decline in per capita food availability (Mulualem *et al.,*2012). Farmers as well as seed producer cooperatives are highly demanding on resistant (tolerant) and better yielding varieties to maximize their product, and improve the livelihood of their families. However, unaccessibility of approved varieties for specific production areas was also attributed to low crop production.

Therefore, the use of resistant / tolerant and high yielding varieties for subsistence farming systems considered as the only solution to maximize their likelihood. As it provides practical, long-term and environmentally friendly being means of limiting the damage from the diseases (Wang *et al*., 2001). Most studies carried out on the resistance to foliar diseases like chocolate spot in faba bean crop have concentrated on just evaluation or assessment of the crop genotypes resistance to diseases under diseased conditions (Daniel *et al*, 2008).

Little is known about the disease distribution, intensity and epidemics, and the fungus population in the study area. Therefore, this study helps to insight the intensity and importance of the pathogen, and resistance levels of selected varieties under varying agro-ecologicay of the southwest Ethiopia.

1.3. Objectives of the Study

1.3.1. General Objective

To study and determine the distribution and epidemics of chocolate spot on different faba bean varieties in major agro-ecologies of Dawuro Zone, southwest Ethiopia.

1.3.2. Specific objectives

The specific objectives of the study are:

1. to determine severity and prevalence of chocolate spot (*Botrytis fabae*) in major agro-ecologies of faba bean at Dawuro Zone.
2. to study the epidemics of chocolate spot on different faba bean varieties under field conditions, and
3. to characterize some isolates of *Botrytis fabae* collected from different agro-ecologies of Dawuro Zone, southwest Ethiopia.

2. LITERATURE REVIEW

2.1. Faba Bean

2.1.1. Taxonomy and ecology of faba bean

Taxonomically, faba bean belongs to Kingdom: Plantae; Division: Magnoliophyta; Class: Magnoliopsida; Order: Fabales; Family: Fabaceae; Subfamily: Faboideae; Tribe: Vicieae Genus: Vicia; Species: *V. faba*; Binomial name: *Vicia faba* L., Synonyms Faba sativa Moench (Anonymous, 2012). *Vicia faba* has a diploid (2n) chromosome number of 12 (Terekhina, 2009).

Vicia faba is an annual legume with one or more rigid, hollow and erect stems (McVicar *et al*., 2009). Faba bean is 0.5-1.7 m tall with a square cross-section stem, and has a strong tap root. The leaves are 10-25 cm long, pinnate with 2-7 leaflets, and do not have tendrils for climbing over other vegetation. The flowers are 1-2.5 cm long with five petals such as one standard petal white, two wing petals white with a black spot (true black, not deep purple or blue), and two keel petals white (Mussa *et al*., 2008). A flower cluster may produce one to four pods. The pods are large and green, turning dark at maturity (McVicar *et al*., 2009). Three to four oblong/oval seeds are contained within each pod.

Faba bean is grown in temperate regions, subtropics, and tropics. It is not suited to the lowland tropics, where it may flower well but usually does not produce pods. The optimal temperature for plant growth is 15 - 20 ^{0}C and flowers will abort if temperatures exceed 27 ^{0}C (Anonymous, 2012). The crop grows with rainfall of 700 - 1000 mm per annum (Mussa *et al*., 2008). Faba bean is best suited to well-structured soils. It prefers clay and loamy fertile soils with neutral or sub-acidic pH levels and high water-retention ability (Terekhina, 2009). Faba beans are vulnerable to soil compaction and hard pans. The common sowing dates are mid-June in mid-altitude areas and late June to early July in high-altitude areas.

2.1.2. Economic importance

Faba bean is a major grain legume used as alternative crops in the changing environments (Ahmed *et al*., 2010). It is a "break" crop, which enhances cereal yield because it decreases the occurrence of take-all and cereal cyst nematode, which affect cereals (Anonymous, 2012). Bean is a good honey plant (Terekhina, 2009). Due to its nitrogen fixing capacity, faba bean is used in crop rotation with the nationally important cereal crops like wheat, teff, and barley (Jarso and Keneni, 2006; Mussa *et al*., 2008). This unique ability reduces the dependence of farmers on extensive use of chemical fertilizers protecting soil and water quality (Sillero *et al*., 2009).

2.1.3. Production constraints

The total area in the world dedicated to faba bean cultivation is declining (Jensen *et al*., 2009). One of the main reasons is the unreliable yields, mainly due to susceptibility of the crop to Chocolate spot (*Botrytis fabae* and *B. cinerea*), Ascochyta *fabae* (*Ascochyta fabae*), rust (*Uromyces viciae-fabae*), Downy mildew (*Peronospora viciae*), Cercospora leaf spot (*Cercospora zonata*), Alternaria leaf spot (*A. alternate* and *A. tenuissima*), wilt (*Fusarium oxysporum)*, root rot (*Rhizocotonia solani)*, stem nematode (*Ditylenchus dipsaci*), root-knot nematode (*Meloidogyne incognita and M. javanica*), weeds, viruses, water logging, cold and poor soil fertility (Mussa *et al*., 2008; Sahile *et al.*, 2010; Stoddard *et al*., 2010). Chocolate spot (*Botrytis fabae*) is one of the economically important diseases that damages the leaves and reduces faba bean production globally including Ethiopia (Torres *et al*., 2006; Samuel *et al*., 2008a).

2.2. The Genus *Botrytis*

Taxonomically, the genus *Botrytis* belongs to division: Deutromycotina (imperfect fungi), class: Hyphomycetes, order: Hyphales (Moniliales), family: Moniliaceae. The fungus is usually referred to by its anamorph (asexual) form because the sexual phase is rarely observed or unknown (Pradier *et al*., 2007).

Botrytis and its teleomorph *Botryotinia* Whetzel comprise 22 species and 1 hybrid species (Yohalem *et al.*, 2003). *Botrytis* classification is largely based on morphological and cultural characteristics. Species of *Botrytis* have been named based on host association (Jarvis, 1977). Features such as sclerotial size and form and conidium size are useful in delimiting some species, but many species are morphologically similar and growing conditions significantly influence these characters (Beever and Weeds, 2004). Most species have a worldwide distribution or occur wherever their host crops are grown (Staats, 2005). The genus *Botrytis* comprises one generalist, *B. cinerea*, infecting over 200 eudicot hosts, especially senescing or otherwise weakened or wounded plants. All other species are considered specialists with a narrow host range.

Sardina (1929) in Spain was the first to associate *Botrytis* with the chocolate spot disease. He considered a new species, *Botrytis fabae* Sard., to be a cause of chocolate spot but that infection by *B. cinerea* Pers. could be similar although the lesions are not identical. Zhang *et al.* (2010) found that inoculation of broad bean leaves with conidia of *B. fabiopsis* caused typical chocolate spot symptoms with a similar disease severity to that caused by *B. fabae* but significantly greater than that caused by *B. cinerea* in China. He was also able to distinguish or identify three species of *Botrytis* based on morphology of colonies, sclerotia and conidia. At 20 ^{0}C *B. fabiopsis* grew on potato dextrose agar (PDA) at 12–13 mm d^{-1}, similar to *B. fabae* (13 mm d^{-1}), but slower than *B. cinerea* (17–19 mm d^{-1}). It forms pale gray colonies with short aerial mycelia and gray to black sclerotia in concentric rings on PDA. *B. fabiopsis* producs greater numbers of sclerotia than *B. cinerea* but fewer than *B. fabae*. Conidia produces by *B. fabiopsis* on broad bean leaves are hyaline to pale brown, elliptical to ovoid, wrinkled on the surface and are larger than conidia of *B. fabae* and *B. cinerea*. In pathogenicity tests, representative isolates caused typical chocolate spot symptoms and were reisolated from infected leaves, indicating that *B. fabae* is the causal agent of chocolate spot in Ethiopia (Samuel *et al.*, 2012).

2.2.1. The pathogen *Botrytis fabae*

This species closely resembles *B. cinerea* but is a specialized pathogen of *Vicia faba*, distinguished by higher pathogenicity, somewhat larger spore size, tendency to produce small sclerotia in culture and protein electrophoresis patterns (Harrison, 1988; Sahile *et al.*, 2012). Hutson and Mansfield (1980) explored the pathogenicity of 15 different macrocondial lineages from one parent, and found a two-fold difference in lesion diameter hinting at the possibility the original strain was a heterokaryon or heteroplasmon.

2.2.2. Host range

Botrytis fabae was thought to be a host specialized pathogen but has now been recorded as a cause of disease on *Vicia* spp., *Pisum* spp., Lens spp., and *Phaseolus* spp., all belonging to *Fabaceae* (Staats *et al.*, 2005; Tivoli *et al.* 2006), and *Trifolium dasyurum* (You *et al.*, 2008).

2.2.3. Symptoms and aggressiveness

Leaves, stems, flowers and pods can all be infected (Gaunt, 1983), with flowers and pods being the most susceptible parts (Griffiths and Amin, 1977). On flowers tiny red brown spots appear (MacLeod and Sweetingham, 2006) while in severe infections the pod might split and small red brown spots can be seen on the outer surface of the seed. If the disease becomes severe at an early stage of growth before the fruit matures, the entire crop can be lost (Matthews, 2003).

Aggressiveness is a property of the pathogen reflecting the relative amount of damage caused to the host without regard to resistance genes and it is description of the rate at which a level of disease is reached, with more aggressive pathogens reaching this level faster where as virulence is the genetic ability of a pathogen to overcome genetically determined host resistance, which is effective against other races of that pathogen, and cause a compatible disease (Shaner *et al*., 1992). According to Richardson and Horsham (2008), two stages of the chocolate spot disease are usually recognized i.e a non-aggressive phase followed by an aggressive phase. The terms "aggressive" and "nonaggressive” describe rapidly expanding and limited lesions, respectively. The non-aggressive lesions, which either do not expand at all or do so only slowly, are discrete reddish-brown spots over the leaves and stems, and limited. Under conditions of continuous high humidity, limited lesions become aggressive, dark in color and rapidly increasing in size and coalesce to form larger grey-brown target spots that eventually cover the entire plant and lead to loss.

2.2.4. Pathogenic variability

A wide variation in pathogenicity exists among isolates of *Botrytis fabae*. According to Dereje (1996) nine isolates of *B. fabae* from Ethiopia, with a similar conidial size, differed in their cultural characteristics, sclerotial production and infection of different genotypes. These variations resembled differences in cultural characteristics and virulence patterns in *B. fabae* collected from other locations (Mohammed *et al.*, 1981). Hanounik *et al.* (1984) observed that in a collection of different isolates, the more virulent isolates produced fewer conidia and formed larger sclerotia than the less virulent isolates. Variation in morphology and aggressiveness between isolates has also been reported by Hutson and Mansfield (1980). According to Sahile *et al*. (2012) a preliminary severity of disease test results with 76 isolates revealed that differences in virulence of the *B. fabae* isolates from faba bean fields in different agro-ecological zones of Northern Ethiopia. Gubalafto–Woldia district had the highest percentage of avirulent isolates, while Yilman– Densa, Gondar–Zuria, Wegera, Ambasel–Tehulederae and Kutaber districts had no avirulent isolates. Overall, the sub-mid north-east districts Ethiopia (Dessie Zuria, Kutaber and Ambasel–Tehulederae) had a higher frequency of virulent isolates than the moist, humid north-western districts of the country.

2.2.5. Disease epidemiology

Several mechanisms could facilitate gene flow between populations of *Botrytis fabae*, apart from the air dispersal of putative spores that are dispersible by windblown rain and strong air currents (Harrison, 1988). Infected faba bean seed (Bretag and Raynes, 2004; Hawthorne, 2004) and infected stubble or debris (Dereje, 1999) have long been recognized as important sources of chocolate spot inoculum and effective means of spreading the disease in Ethiopia.

Carry-over of *B. fabae* between crops occurs as mycelium in crop debris (Gaunt, 1983) or as sclerotia in the soil and in crop debris. Mycelium can survive for over a year in crop debris on the surface of the soil, but only four months when buried 20 cm (Dereje, 1999), while sclerotia can survive for up to one year without loss of viability (Harrison, 1978). Sclerotia buried in soil are less likely to survive (Harrison, 1978). This form of survival is particularly important when there is a long summer gap between host crops as in Mediterranean climates

(Jellis *et al.,* 1998). Alternative hosts and volunteer faba bean plants may also be important for survival of the fungus between cropping seasons. Seed infection may have a role in introducing the disease into new areas (Harrison, 1978).

The conidia are released as clumps or singly, and are generally wind-borne (Harrison and Lowe, 1987), but may be dispersed by rain or insect vectors, particularly thrips (Harrison, 1988). Also it spread by splash droplets (Jarvis, 1962). However, the numbers of conidia dispersed in splash droplets or released by the puff or tap mechanisms at the onset of rainfall were small by comparison with the diurnal maxima observed in infected crops in the experiments of Fitt, Creighton and Bainbridge (Fitt, et al., 1985). They concluded that wind was the main agent in spore dispersal, even during periods of rainfall, and that the development of chocolate spot epidemics was associated with wet weather because the high relative humidities favored sporulation, infection, and lesion development. These highlight the need to use appropriate sampling methods for splash-dispersed or dry dispersed inoculum and show how conclusions about methods of spore dispersal may greatly affect our understanding of the epidemiology of a this disease.

Chocolate spot development is highly dependent upon environmental factors. Frequently the disease is first noticed on isolated plants that act as foci from which the disease develops rapidly in humid weather. It spreads via airborne conidia within crops where relative humidity is above 70 % and at temperatures of 15 to 23 ^{0}C (Harrison, 1980; Dereje, 1993), especially in mornings, warm and rainy (frequent rain) weather conditions are favorable for the growth of chocolate spot epidemic. With this environment, the epidemic grows with apparent infection rates ranging from 0.142 to 0.164 disease units per day, which means several chocolate spot infections within a single growing season (Dereje, 1993). This is an indication for rapid and dangerous spread of the disease. Obviously, if the pathogen falls short of the above mentioned weather variables, it will be forced to have short infection period, and this is significant in spread of an epidemic.

Lesions expand slowly when relative humidity is below 66 % (Harrison, 1980). The optimum temperature for lesion growth is between 15 to 20 ^{0}C, with minimum of 4 ^{0}C and maximum of 30 ^{0}C. Neither light intensity nor water films on the leaves have an impact on lesion growth

(Harrison, 1980) though near ultra-violet light induces sporulation (Harrison and Heilbronn, 1988) and it is common practice to use alternating cycles of 12 h darkness and 12 h of near ultra-violet light to stimulate sporulation of *B. fabae* (Dhingra and Sinclair, 1995). Plants become more susceptible to chocolate spot as they age or suffer from freezing (Creighton *et al.,* 1985; Heilbronn and Harrison, 1989).

2.2.6. Economic significance of chocolate spot

Chocolate spot, caused by *B. fabae,* is one of the major diseases of faba bean nationwide (Dereje and Tesfaye, 1994; Sahile *et al.,* 2012). According to Sahile *et al.* (2010) chocolate spot disease has reduced the grain yield and quality by reducing 100 - seed weight. Dereje and Beniwal (1988) recorded yield losses of up to 61 % for susceptible varieties and 34 % for tolerant varieties in Ethiopia. Yet, complete crop failure due to the disease is commonly encountered when a long lasting favorable environment for disease development prevails in an area (Dereje *et al.*, 1994). In Tigray (Negash areas), seed yield loss of 62 % was estimated due to chocolate spot in 2000 (MARC, 2002).

2.2.7. Disease management

2.2.7.1. Cultural practices

Avoidance of factors that predispose the crop to disease epidemics can also operate chocolate spot epidemics. The use of crop rotation to avoid infected debris and volunteer plants, burning of infected plant debris, burying or grazing stubble and removal of volunteer plants will minimize the risk of chocolate spot in subsequent faba bean crops (Liang, 1989). Deep plowing of faba bean fields with high chocolate spot infection immediately after harvest reduces the risk of disease development (Dereje, 1993). High plant densities that provide a high humidity environment should be avoided and in Europe it is recommended that early sowing be avoided to prevent spring frost damage and hence predispose the crop to the disease (Jellis *et al.,* 1998). The association between early sowing and chocolate spot was also noted in Syria (Hanounik and Hawtin, 1982). In Ethiopia considerable hold-up and shortening of chocolate spot epidemics and there by reduction of attack can be achieved by late sowing of faba bean as the conditions suitable for development of the disease do not exist for a sufficiently long period of time (Dereje, 1993). Planting faba bean in mixture with field pea in a ratio of 1:2 drastically reduces epidemic development of chocolate spot in faba bean (Dereje, 1999; Amare, 2009).

Adequate fertilizer, in particular potassium and nitrogen, and good soil drainage is necessary to prevent early senescence, which would provide dead leaves upon which the fungus sporulates (Liang, 1989; Jellis *et al*., 1998). According to El-Bramawy *et al.* (2010) potassium is an important element for agronomic characters improvement and for enhancing the resistance to rust and to chocolate spot diseases. The results showed that the potassium soil + foliar application (PSFA) at level 3 (171.36 + 3.4 Kg K_2O/ha) followed by potassium as foliar application (PFA) at level 3 (3.40 Kg K_2O/ha), increased significantly the majority of studied characters including the agronomic characters and/or increase resistance to foliar diseases. A higher incidence of chocolate spot occurred in plants grown on acidic soils (Elliott and Whittington, 1978) and with increased nitrogen fertilizer (Hegab and Beshir, 1994).

2.2.7.2. Chemical control

There are a range of fungicides available to control *Botrytis,* and selection of the most appropriate fungicide could depend on the level of disease pressure present. However, several applications of fungicide are generally required in situations where the environment remains favorable for disease development. In Ethiopia many fungicides, both systemic and protectant were tested for control of chocolate spot in the past (Habtamu and Dereje, 1986; Sahile *et al.*, 2010), among these chlorothalonil and benomyl with menacozeb were effective against chocolate spot. Chlorothalonil completely protected faba bean plants from infection when applied at weekly intervals.

In Australia it is recommended that fungicide be applied from flowering onwards, depending on conditions. Carbendazim or procymidone are recommended in high disease situations, while benomyl, chlorothalonil, mancozeb, tebuconazole or copper products are also available (Panagiotopoulos *et al.,* 2002). Carbendazim was better than mancozeb or chlorothalonil for control of chocolate spot (Bretag *et al.*, 2002). In Europe benomyl, or tank mixes of carbendazim plus chlorothalonil or iprodione or vinclozolin are used from early flowering and

3 - 4 weeks later (Jellis *et al.,* 1998). Bainbridge *et al.* (1985) were able to reduce an early chocolate spot epidemic by using seed treated with benomyl plus thiram. In China, sprays of carbendazim or thiophanate-methyl at podding or flowering were found to be effective (Liang, 1989).

Late epidemics appear not to affect yield and fungicides are not warranted in these conditions. The best indication to farmers of a potentially damaging epidemic appears to be the build-up of chocolate spot on lower leaves before or during flowering (Creighton *et al.,* 1985). Resistance to benzimidazole fungicides, such as carbendazim, is a major problem when they are used frequently (Jellis *et al.,* 1998). According the concept of integrated disease management, chemical treatment should only be applied as supplement to combat chocolate spot risk. It is worth remembering that these products are protectants and are most effective if applied before disease development.

2.2.7.3. Biological control

There is promise in using biological control agents to control chocolate spot diseases; nevertheless, this strategy has not been fully exploited. Isolate of *Trichoderma ovalisporum* and *Trichoderma longibrachiatum* as effective antagonists of *B. fabae* for the first time (Sahile *et al.,* 2011). *Trichoderma viride* was found effective against chocolate spot in 1990 (Jellis *et al.,* 1998). Jackson *et al.* (1997) reported that some isolates of *Penicillium chrysogenum* inhibited growth and development of *B. fabae in vitro* and showed the development of chocolate spot under glasshouse conditions was substantially reduced by this agent. Similar results were obtained with *Penicillium brevicompactum* and *Cladosporium cladosporioides* (Jackson *et al.,* 1997).

Fourteen *Bacillus* isolates were found to be antagonistic to *B. cinerea* and *B. fabae* and reduced development of chocolate spot symptoms. However, they were not effective in the field (Sharga, 1997). In Ethiopia, study has revealed that the biological control agents for chocolate spot of faba bean and *Bacilli* are natural residents of faba bean leaves. Thirty isolates of *Bacillus* spp. were tested for their effects on *B. fabae* by dual culture technique on potato dextrose agar. Sixteen isolates produced 5 mm or higher inhibition zone and out of these, two isolates were the most effective having inhibition zone of 8 and 7 mm. Isolates reduced the growth of the pathogen colony in dual culture by 23 - 64%. Four Isolates proved most effective in retarding disease development on two susceptible and one tolerant cultivar and can be further explored for commercial use for management of chocolate spot disease of faba bean (Sahile *et al.,* 2009). Another antagonistic bacterium, *Erwinia herbicola*, which was isolated from the phylloplane of a chocolate spot-resistant genotype, was able to significantly reduce disease severity when used as a foliar spray (Abd-El-Moity *et al.,* 1990).

2.3.7.4. Host resistance

The major constraint for faba bean breeding for chocolate spot resistance is the lack of good sources of resistance. Some resistant germplasm has been reported but most of it has yet to be introduced into the agricultural market. So far, total resistance has not been reported and only incomplete resistance is being used (Jensen *et al.,* 2010). The level of resistance to chocolate spot in most faba bean cultivars is low although varying degrees of resistance have been reported (Bouhassan *et al.,* 2004; Tivoli *et al.,* 2006; Villegas-Fernandez *et al.,* 2009).

Based on an analysis of variance of host and pathogen effects, Van der Plank (1984) also determined that resistance in a host could be classified as either horizontal (race nonspecific) or vertical (race specific) and that corresponding pathogenicity in the pathogen was classified as aggressiveness and virulence, respectively. Horizontal resistance and aggressiveness were defined as the main effects of the host and pathogen, respectively. Vertical resistance and virulence were defined as the interaction effect among host genotypes and pathogen races.

Vertical resistance is also known in plant breeding as “specific”, and is determined by one or a few genes that impart complete resistance to a certain pathogen. This type of resistance is also known as “short-term” resistance, and also known as qualitative, monogenic, or strain specific resistance (Gair *et al*., 1987). Horizontal resistance, in turn, is usually associated with several genes and imparts an incomplete type of resistance; the host is attacked by the pathogen in a higher or lower degree, depending on the number and type of genes involved in the resistance. This type of resistance is also known as general resistance, field resistance, non-specific, quantitative, or polygenic resistance (Robert *et al*., 2000).

Resistance is thought to be based on the ability of faba bean to produce, as a post-infection defence response against *B. fabae*, low-molecular-weight secondary metabolites, such as furanoacetylenic phytoalexins (Ingham, 1982). In contrast, the greater ability of *B. fabae* to colonize broad bean tissues seems to be related to its capacity to detoxify broad bean phytoalexins and to reduce their toxic effects (Madeira *et al*., 1993). *B. fabae* is able to produce pectin degrading enzymes, such as polygalacturonase, during development of chocolate spot (Harrison, 1988). The possible dual role of polygalacturonase isoenzymes from *B. fabae* as defense responses elicitors during broad bean colonization (Buzi *et al*., 2003). It is very clear that *Botrytis* is well equipped for the necrotrophic lifestyle and may use different approaches to disease development depending on the strain or strain–host combination (Hammerschmidt, 2010).

Different approaches may be taken in order to control chocolate spot (Stoddard *et al.,* 2010). The use of resistant varieties to chocolate spot is one of the management options. In order to identify the resistant varieties, the test genotype has to be subjected to a uniform and heavy disease pressure obtained through natural happening (hot spot), and artificial inoculation (Mussa *et al*., 2008).

Faba bean collections have been screened for response to chocolate spot and evaluation methods improved (Bouhassan *et al*., 2004). The International Centre for Agriculture Research in Dry Areas (ICARDA) has incorporated resistance into local germplasm, so new genotypes have been introduced in Australia, Egypt and Ethiopia, among other countries (Stoddard *et al*., 2010). So far several chocolate spot resistant genotypes have been reported (Table 1). Source of resistance for chocolate spot (BPL-1179-A-1, BPL-1802-1 and BPL-1179-2) have been identified from introductions of ICARDA to Ethiopia (Mussa *et al*., 2008). From the landrace collections, a number of varieties, namely CS20DK, NC-58, Bulga 70 (coll 111/77), Wayu (Wayu 89-5), Selale, Lalo, Dagm and Adet Hana have been nationally or regionally released for different recommendation domains (Mussa *et al*., 2008).

Table 1. Faba bean germplasm resistant to chocolate spot (*Botrytis fabae*).

Accession name	Material	References
LPF 44, LPF 237, LPF 05, LPF 113	Breeding lines	Rhaiem *et al*. (2002)
ILB 1414, ILB 6561	Breeding lines	Bayaa *et al.* (2004)
FRYM167, FRYA58	Breeding lines	Bouhassan *et al.* (2004)
BPL 260, BPL 2282, L82010, L83106, L83108, L83109, L83117, L83129, L83149, B8838, B8839	Breeding lines	Khalil *et al.* (2004)
Reina Blanca	Cultivar	Khalil *et al.* (2004)
Icarus, Nura	Cultivars	Raynes (2008)
Gladice	Cultivar	INRA/DPE (2008)
BPL710, BPL 1763, ILB 4726, LPF 120, ILB 4709, ILB 5284, Sel.97 Lat.97 132-1, Sel.97 Lat.97 132-3	Breeding lines	Villegas-Fernandez *et al.* (2009)

2.2.7.5. Integrated disease management

These are controlled through the use of Integrated Disease Management (IDM), which can be defined as the use of a range of disease management practices to reduce the impact of plant diseases. Used alone, an individual management practice may not reduce the level of disease to an acceptable level, whereas the additive effect of several practices will be done. An integrated approach is the key to successful management of chocolate spot in faba bean. Mixed cropping of faba bean with cereal crops and mancozeb spray reduced the disease and increased the grain yield, as well as the seed weight over sole and mixed cropping with field pea (Sahile *et al.*, 2008b; Sahile *et al.*, 2010). Similarly Agegnehu *et al.* (2006) and Gemechu *et al.* (2006) also found that cereal mixing with faba bean has advantages over faba bean mixing with field pea. According to Fernandez-Aparicio *et al*. (2011) chocolate spot was significantly reduced when faba bean was intercropped with cereals, but not when intercropped with legumes. Suppressive effects can be ascribed to a combination of host biomass reduction, altered microclimate and physical barriers to spore dispersal. Also creation of a physical barrier in the form of non-host plants prevents some of the dispersed spores from being deposited on the host tissue by interception (Khalil *et al.*, 2011).

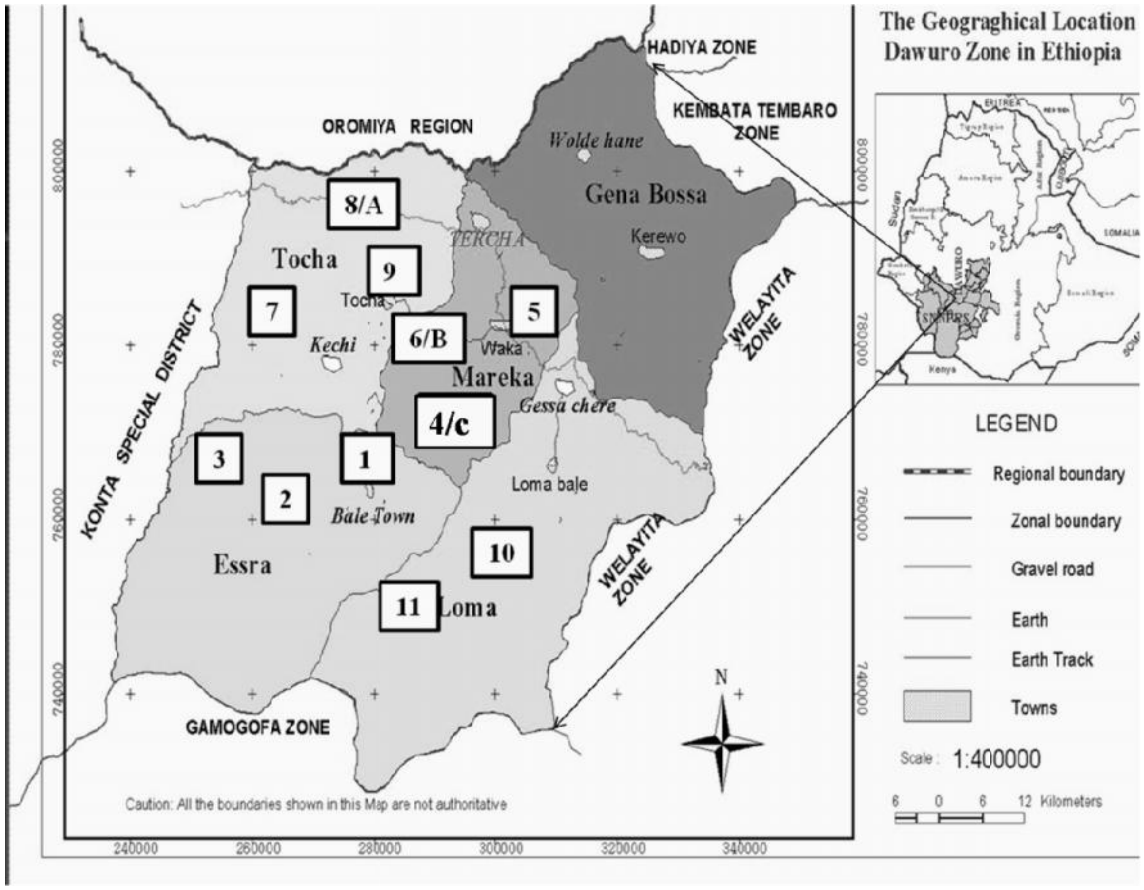
Integrated disease control for faba bean studies conducted on farmers' fields under different environmental conditions showed that newly released varieties with resistance to chocolate spot responded less to fungicidal applications. These findings led to the development of improved disease control packages. The use of new, resistant varieties has reduced the use of chemicals drastically (Khalil and Erskine, 2001). In addition, early sowing, use of improved varieties and fungicide protection avoid the occurrence of chocolate spot disease at epidemic proportions (Dereje, 1993).

3. MATERIALS AND METHODS

3.1. Geographic Location Location of Dawuro Zone

Survey and field experiment were carried out in the 2011 crop growing season in Dawuro zone, Southern Nations, Nationalities and Peoples' Regional State (SNNPRS). Dawuro zone lies in between 6^0 36' to $7^0$21' north latitudes and $36^0$68' to 37^0 52' east longitudes. It is one of the 13 zones of the region situated between Omo river in the north to south and Gojeb river in the north to northwest. Tercha is the zone capital, 486 km from Addis Ababa through Jimma road, and 282 km from Hawassa. The zone is subdivided into five woredas (local districts) with varying altitudes ranges from 730 to 2850 m.a.s.l. The area has mountainous topography. The zone receives a total annual rainfall of 1200 to 1800 mm with mean monthly temperature varying from 15 to 27.5 ^{0}C.

The area under cultivation is estimated to be 100,395 ha of the total 446, 082 ha of the zone and out of which 38 % is lowland (500 – 1500 m), 41 % mid altitude (1501 - 2200 m) and 21% (> 2200m) highland. The dominant crops grown in the study areas include Enset, Legume crops (faba beans, chickpeas, haricot beans, lentils and field peas), Cereal crops (wheat, maize, teff, sorghum and barley), and Root crops (taro, sweet potatoes and cassava).



Note: 1=Bale, 2=Dali, 3=Sengetsi, 4 = Gozoshasho, 5=Waka, 6=Mari, 7=Kechi, 8= Medanalem, 9=Botori, 10=Gessachare and 11= Tulema indicates surveyed peasant associations; where as A=Tocha, B=Mari and C=Turi indicates field experimental sites.

Figure 1. Geographic location of Dawuro Zone in Ethiopia.

3.2. Description of the Study Areas

Field surveys were conducted in major areas of faba bean production areas of eleven peasant associations (Bale, Dali, Sengetsi, Waka, Mari, Gozoshasho, Medhanalem, Botori, Kechi, Gessachare and Tulema) in four districts (Essera, Mareka, Tocha and Loma) of Dawuro zone during 2011 growing season (Figure 1). These focused administrative units were selected based on secondary data obtained from Dawuro Zone Agricultural department, conveniently with the assistance of development agents and local administrators, and measuring the location of fields using GPS. The selected study sites were stratified to include two agro-ecological zones, "*Wayina Dega*" Zone (mid-altitude 1800 to 2200 m.a.s.l, with sub-humid and warm climate) and "*Dega"* Zone (high land > 2200 m.a.s.l, with humid and cool climate) (Appendix Table 1A). From each PAs four faba bean fields, a total of 44 fields were surveyed from September 15 to October 5, 2011. Depending on their relative faba bean production, 36 % were in mid-altitude and 64 % in highland altitude. During surveyed period, the faba bean fields were at pre-podding and podding stages (at about 81 to 100 days after planting) at this growth stage chocolate spot severity expected maximum (Sahile *et al.*,2008a).

The field experiments were carried at farmers training centers (FTC), farm of Dawuro Zone Agricultural Department at Tocha (2658 m.a.s.l), Mari (2444 m.a.s.l) and Turi (1805 m.a.s.l) under rain fed conditions. These experimental sites had been planted with faba bean in the past years and indigenous chocolate spot populations were considered adequate for disease development. Soil physico-chemical prosperities for field experimental sites were peresented in Appendix Table 1B.

3.3. Disease Survey and Assessment Methodology

Selection of surveyed faba bean fields within each agro-ecological zone was varying at least 5 km intervals along main and feeder roads depending on the availability of the farm. In each sample field, three quadrants (1 m x 1 m) of at least 10 m apart were sampled at diagonal transects. Eight faba bean plants were assessed for disease severity along an X-shaped transect were taken as the sample unit. In each quadrate of assessed plants for chocolate spot severity, averages were taken for each field. Disease symptoms were scored on the basis of a 1 – 9 class visual scale where, 1: no lesions or covering up to 1 % of leaf surface; 3: lesions covering 1 – 2 % of leaf surface; 5: lesions common (3 – 5 mm in diameter), covering 2 – 5 % of leaf surface; 7: lesions that cover 5 – 10 % of leaf surface; 9: extensive lesions, covering more than 10 % of the leaf surface. Disease severity scores were converted into disease severity index (DSI) for the analysis (Hanounik, 1986; Ayman *et al.*, 2009; Abo-Hegazy *et al.*, 2012).

$$\text{Dissease severity index (DSI)\%} = \frac{\sum(\text{NxV})}{9\text{N}} \text{x}100$$

Where: N = number of infected leaves, V= numerical grade, and 9 = Higher degree in the category.

During the surveyed period, the plant population (m^2) in each quadrant was counted and the mean crop population was obtained by averaging the crop population in the three quadrants, altitude (m) from GPS readings, slope (%) of the farm using Clinometer's reading, and weed condition of the farm were recorded for each sampled field. Growers were asked information on their attitude towards faba bean production, type of varieties they planted, and cultural practices (time of planting, frequency of ploughing their farm, previous crop history, and disease control practices) employed.

3.4. Disease Sample Collections

Representative samples of leaves, stems, pods and seeds showing symptoms of chocolate spot were collected from all the surveyed areas. For each sample reference number, sampling date, locality and altitude were recorded. Then disease specimens were carefully wrapped individually in newspaper and taken to Plant Pathology Laboratory of Jimma University, kept at 4 ^{0}C in a refrigerator.

3.5. Field Experiment

3.5.1. Plant material

Thirteen improved varieties released in Ethiopia, up to 1.5 kg of each were obtained from Holeta Agricultural Research Center (HARC) and a local cultivar being used by farmers in the study area were assessed for their chocolate spot disease reaction and yield performance. These varieties incorporated source of resistance for chocolate spot and released for different recommendation domains. About the information obtained from MoARD (2009) and EARO (2010), varieties are given in Table 2.

Table 2. List of faba bean varieties used for the study and their agronomic traits

Variety	Pedigree name	Year of Release	Altitude (m)	Maturity days	Yield (qt/ha)		TSW
					Station	On-farm	
Tumsa	EH9965-3	2010	2050-2800	121-176	25-69	20-38	737
Hachalu	EH960091-1	2010	1900-2800	122-156	32-45	24-35	890
Walki	Bulga-70 x ILB4615	2007	1800-2800	133-146	24-52	20-42	676
Gebelcho	Tesfa x ILB4726	2006	1800-3000	103-167	25-44	20-30	797
Moti	ILB4432 x Kuse 2-27-33	2006	1800-3000	108-165	28-51	23-35	781
Degaga	R-878-3	2002	1800-3000	116-135	25-50	20-45	517
Tesfa	75TA26026-1-2	1995	1800-2400	125-135	20-40	20-45	441
Mesay	74TA12050 x 74TA236	1995	1800-2400	120-130	25-50	20-45	428
Bulga-70	Coll 111/77	1994	2300-3000	143-150	20-40	15-35	440
Kasa	NA	1980	1900-2300	120-135	45-55	25-40	428
Kuse	Kuse 2-27-33	1979	2300-3000	135-150	20-35	15-25	393
NC-58	NC-58	1978	1900-2300	118-132	20-40	15-35	449
CS20DK	CS20DK	1977	2300-3000	145-160	20-40	20-40	476
Local[a]	NA	NA	-	-	-	-	-

NA = information not available; Local cultivar= obtained from farmers in the study area; TSW=1000 seed weight

3.5.2. Experimental design and management

The experimental fields were prepared by using local oxen drown plough (*maresha*) according to farmers conventional farming practices. The fields were ploughed three times, the first at the beginning of June 2011, the second after three weeks and the third during the first week of July 2011 before planting. In accordance with the specifications of the design, a field layout was prepared and each treatment was assigned randomly to experimental units within a block. Sowing was done on July 16, July 18 and July 20, 2011 at Tocha, Mari and Turi experimental sites, respectively. Two seeds per hill were planted manually to ensure germination and good stands of the varieties and then thinned to one plant 15 days after emergence to achieve 40 plants per plot.

The experimental plots were arranged in randomized complete block design with three replications. Each experimental plot had a total area of 1.6 m^2 (1 m x 1.6 m) with spacing of 40 cm between rows and 10 cm between plants to give a final population density of 250,000 plants per hectare (EARO, 2010). Between each block (0.5 m), there was a disease spreader row of local cultivars. This practice induces early disease development on local checks which can later serve to spread the inoculums to adjacent test entries (ICARDA, 1986). Local cultivar was also serving as a standard against which disease rating is done in the nursery for disease resistance. Each plot had 4 rows from which the central two rows of middle four plants from each were used for disease assessment and yield components determination where the other two outermost rows were kept as boarder plants.

3.5.3. Disease assessment

Chocolate severity expressed as the proportion of diseased tissue exhibiting chocolate spot symptoms, was recorded weekly for each plot using a 1 – 9 scale according to Hanounik (1986) as described in 3.2, starting from 7 days when first symptoms of chocolate spot were visible in the experimental plots and were repeated ten times every 7–10 days until the podding stage where the amount of chocolate spot disease was expected maximum (Sahile *et al.,* 2008b;Villegas–Fernandez *et al.,* 2012) from eight selected and tagged plants from middle two rows in each plot (excluding the two outer rows). Disease severity scores were converted into disease severity index (DSI) for the analysis as described in 3.2.

The response of varieties was expressed as the disease severity index (DSI) values grouped in six resistance levels described by Abo-Hegazy *et al.* (2012) as indicated in the Table 3.

Table 3. Six resistance levels used for classification of faba bean varieties.

Reaction group	DSI range
Highly resistant (HR)	0 and 2.0
Resistant (R)	>2.0-15.0
Moderately resistant (MR)	>15.0-40.0
Moderately susceptible (MS)	>40.0-60.0
Susceptible (S)	>60.0-80.0
Highly susceptible (HS)	>80.0-100.0

3.5.3.1. Disease progression and modeling

Disease progress curves (chocolate spot severity indices over time) were plotted for each variety for each location. These curves provide the basis for the three other epidemiology measurements: final DSI, area under the disease progress curve (AUDPC) and apparent infection rate (*r*).

AUDPC values were calculated for each plot according to the mid-point rule using the following formula:

$$\mathbf{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{Yi + (Yi + 1)}{2} \right) T(i + 1)\_Ti)$$

Where *Yi* is the Disease severity index (DSI) of chocolate spot at i^{th} assessment, *Ti* is the time of the i^{th} assessment in days from the first assessment date and *n* is the total number of disease assessments. Because severity was expressed in percentage and time in days, AUDPC was expressed in %-days.

The apparent infection rate, expressed in disease units per day, was calculated by first converting DSI values to a proportion on a scale of 1 to 9 and then transforming the data using Logistic, ln [(Y/1-Y)], (Van der Plank, 1963) and Gompertz, -ln [-ln(Y)], (Berger, 1981) models were used to compare estimation of disease progression parameters**,** where *Y* represents the proportion of infected plants and *1-Y* represents the proportion of healthy plants remaining in the plot. The transformed values (*y*) were regressed against time (*x*) to determine the model (Campbell and Madden, 1990). The goodness of fit of the models was tested based on the magnitude of the coefficient of determination (R^2). The appropriate model was then used to determine the apparent rate of disease increase (r) and the intercept of the curve.

3.5.4. Yield and yield components

Separating harvesting (boarder and central rows) was made from the middle of November to first week of January, 2011 depending on the maturity period of each variety. After exposed to sun drying, the harvested products were threshed and yield and hundred seed weight were measured from central rows of each plot (Khalil *et al.*, 2011).

At harvest, number of pods/plant, number of seeds/pod, seed yield per hectare and weight of 100 seeds per plot were recorded in the following manner.

1. Number of pods per plant (NPPL): Eight plants were tagged for chocolate spot severity from each plot was used and their pods were counted and averaged.
2. Number of seeds per pod (NSPL): The seeds of five randomly taken pods from each of eight randomly pre-tagged plants was counted and averaged.
3. Seed yield (SYD): For seed yield two central rows of each plot were harvested, sun-dried, threshed, cleaned and weighed with an electronic balance in gram then converted to kilogram per hectare according to the following equation (Khalil *et al.*, 2011).

   $$\text{Yield (kg per ha)} = \frac{\text{Grain weight (g)}}{\text{No. of rows (2) X R} - \text{R Distance (0.4m) X Row length (0.5 m)}}$$
4. Hundred seed weight (HSW): The 100 seed-weight of randomly taken seeds from each variety at 10% moisture content weighed with electronic balance.

3.5.5. Weather parameters

Monthly total rainfall (mm), relative humidity (%) and the daily minimum and maximum temperature (^{0}C) for the experimental periods were obtained from the nearby weather stations in Dawuro zone.

3.6. Studies in the Laboratory

3.6.1. Media Preparation

Botrytis fabae was isolated on faba bean dextrose agar (FBDA). Two hundred gram of faba bean seeds was weighed out in a 1.5 liter flask to which 1liter of distilled water was added and boiled for 30 minutes, and also sterilized at 121 ^{0}C for 30 minutes in a pressure cooker. Then after, the extract was separated. Eighteen gram of agar and 5 g of dextrose were added to the extract, stirred till dissolved and made up the volume to 1 liter with additional water. Then, this preparation was sterilized at 121 ^{0}C for 30 minutes in a pressure cooker, cooled and poured into Petri dishes (Dereje, 1996).

3.6.2. Isolation of the pathogen

Samples of naturally infected faba bean tissues with symptoms of chocolate spot disease were cut into small pieces, each with a single lesion. Plant parts, which were not used for the isolation of the fungus were cut off and discarded (Amare, 2009). Each piece of specimen were sterilized by soaking in 5 % sodium hypochlorite for 2 to 3 min, rinsed with distilled water, dried on sterilized filter paper. Five pieces were placed onto each culture placed on FBDA plate, and the plates were incubated at room temperature five to seven days for emerging fungal colonies.

3.6.3. Hyphal tip isolation and preservation

Pure isolates were obtained using hyphal-tip techniques (Hanounik and Robertson, 1988). When the fungal colonies developed from plant pieces, each colony was transferred to another FBDA plate, by stabbing a flamed loop into the medium, touching the mycelium on growing edge of a colony and then streaking onto the fresh plate. It was repeated until pure colonies were obtained. Isolates of each location were maintained on FBDA in sterile screw-capped test tubes at 4 ^{0}C until used for further study. Identification was done using microscopic examination, comparisons with reference slides with *B. fabae* obtained from HARC and identification keys (Marthin and Panela, 1985).

3.6.4. Cultural and morphological characterization of the *B. fabae* isolates

The colony appearance (texture, form and pigmentation), growth rate, and conidial morphology were studied to determine variations among the field collected isolates of *B. fabae* from different agro-ecologies. Single colonies of each isolate were initiated by displeasing a 5-millimeter mycelial plug from the advancing edge of the fungal culture onto the centre of each Petri dishes containing FBDA amended with 0.01% streptomycin (Sahile *et al.,* 2012). The plates were incubated at room temperatures under alternative 12 hr white fluorescent light/12 hr dark cycle. The plates arranged in completely randomized design and for each isolate three petri-dishes were used as replicate, the colony diameter in two perpendicular directions were recorded starting from second days on wards, daily until the colony fully covered the petri dish and used to compute the radial growth rates of the isolates. On the day that the fungal colony fully covered plate, the color of each isolate colony was described using Rayner's (1970) mycological color chart. Texture of aerial mycelium, the nature of colony edges and zonation were also described (Mirzaei *et al.,* 2007).

Colony texture was recorded as either appressed with sparse aerial mycelium, flocculose with raised and slightly dense aerial mycelium, or floccose with raised and dense aerial mycelium. Colony color was described as white, white and tending towards gray (grayish white), or gray. Colony shape was either uniform with smooth edges, irregular with rough edges or banded with sectors consisting of thin expansive mycelium. Isolates that grew at > 9 millimeter /day were considered fast growing; those that grew at between 8 and 9 mm /day were considered medium-growing, while those that grew at < 8 millimeter /day were slowing-growing.

Conidial morphology was studied from ten-days-old cultures of all isolates. Slide preparations of the conidial suspensions were subsequently made using cotton–blue lactophenol. Conidial size for each isolate was determined by measuring the length and width of 20 randomly chosen conidia using an eye-piece micrometer (Sahile *et al.,* 2012).

3.6.5. Propagation of the Inoculum

Mass spore production of *B. fabae* was carried out on a natural medium of chrysanthemum (*Chrysanthemum sinense* Sabine) flower petals (Beniwal and Gorfu, 1989). Twenty five gram of chrysanthemum flower petals were weighed out and enriched with 5g of dextrose in a flask and sterilized at 121 ^{0}C for 30 minutes in a pressure cooker. A pure culture of *B. fabae* grown on FBDA was inoculated into the cool medium and kept under room temperature for 15 days and then spore concentration ($5 \times l0^5$ spores /ml) was determined using haeymacytometer from sporulating media. After the spore concentration was standardized, inoculation test were carried out.

3.7. Aggressiveness of *B. fabae* isolates

Seeds from tolerant variety CS20DK (moderately resistant in field evaluation) was sown in 30-cm-diameter pots filled with arable soil, peat and sand (3:1:1; v: v: v) (Bouhassan *et al.*, 2004), and germinated seedlings were thinned to four plants per pot in the greenhouse. Forty-five days after sowing, the upper side of the leaves was inoculated with 1.5 ml of a spore suspension containing about 5×10^5 per ml spores of *B. fabae,* one droplet on each half of leaflet by using micropipette and amended with Tween 20® (1.2 % v/v) and covered with polyethylene bags for 24 h to maintain a high relative humidity (Tivoli *et al*., 2006). The pots were arranged in completely randomized design and replicated three times (three plants or leafletes per isolate). Plants sprayed with distilled water were used as a control. The inoculated and uninoculated plants were maintained under greenhouse conditions.

3.7.1. Disease assessment

Incubation period was determined as the time (in days) between inoculation and the appearance of first disease symptoms. Disease severity was recorded by calculating the proportion of leaf surface lesions rated using a 1– 4 scale, where 1: no infection or very small flecks (1–25% necrosis); 2: necrotic flecks with few small lesions (26–50% necrosis), and very poor sporulation; 3: medium coalesced lesions (51–75% necrosis) with intermediate sporulation; 4: large coalesced lesions (76–100% necrosis) with abundant sporulation. Isolates were classified in to 1-3 aggressiveness groups: less = Disease severity ≤ 50% necrosis; medium = 50 < Disease severity < 75; more = Disease severity ≥ 75% (Tivoli *et al*., 2006).

3.7.2. Reisolation of the pathogen

The pathogen was reisolated from the leaf lesions. Leaf lesions were cut in to pieces and surface sterilized with sodium hypochlorite for 2 minute and rinsed thrice with sterile water in Petri plates. Pieces dried with sterile filter paper and plated on FBDA medium and incubated at room temperature for 7 days. The fungus was sub cultured to purify, and identification was by comparison with the respective parent stock culture.

3.8. Data Analysis

For disease survey, primarily descriptive statistics was performed on data collected from each field. In addition, analysis was conducted to describe the prevalence and association of chocolate spot severity in relation to independent variables. Contingency X^2 tests were used to compare the frequencies of diseased fields between PAs based on the proportion of fields that were assigned to a given disease severity class. Where significant difference for disease severity existed, the mean disease differences were separated using the T-test at $P \leq 0.05$ (Zewde *et al.*, 2007).

In the second analysis, the variables classes were categorized based on the frequency of fields. Disease severity (the response variable) was classified into a distinct class of bi-variate qualitative data using the SAS software of the univariate procedure of disease severity as variable. Selected class boundaries were $p \leq 45\%$ and > 45%. Tables for variable classes were constructed to represent the bivariate distribution of the fields (Fininsa, 2003; Zewde *et al.*, 2007). The value corresponding to each independent variable represents the frequency of fields falling in the disease range. The associations of chocolate spot severity with the independent variables and variables classess were analyzed using a logistic regression model with the SAS procedure of the GENMOD. The GENMOD procedure estimates the parameters of the model numerically through an iteractive fitting process and it fits a generalized linear model to the data by maximum likelihood estimation of the parameter.

For field experiment, AVOVA model for individual location articulated the observed response (yij) of the variety in the replication and was expressed as:

$$yij = \mu + Vi + Bj + \acute{e}ij$$

Where; μ was the mean across replicates, Vi was effect of variety , Bj was the effect of replicate, and êij was the random error assumed to be normally and independently distributed as mean zero and variance equal to δ^2.

The ANOVA model of the combined data expressed the observed mean response over replicates (Y_{ij}) of the i^{th} variety in the j^{th} location with r replicates in each of the $(i\times j)^{th}$ cells was expressed as:

$$\text{yij} = \mu + \text{Vi} + \text{Lj} + (VL)ij + Eij$$

where μ was the grand mean across all varieties and locations, Vi *was* the additive effect of the variety, L_j was the additive effect of the j^{th} location, VL_{ij} was the VLI component for the i^{th} variety in the j^{th} location, and E_{ij} was the random error assumed to be normally and independently distributed as $(0, \sigma^2/r)$ (where σ^2 was the within locations error variance, assumed to be constant.

Pearson's correlation coefficients were calculated to test the strength of relationships among the parameters. Disease parameters showing significant negative correlation with the yield and yield components were used in regression analysis.

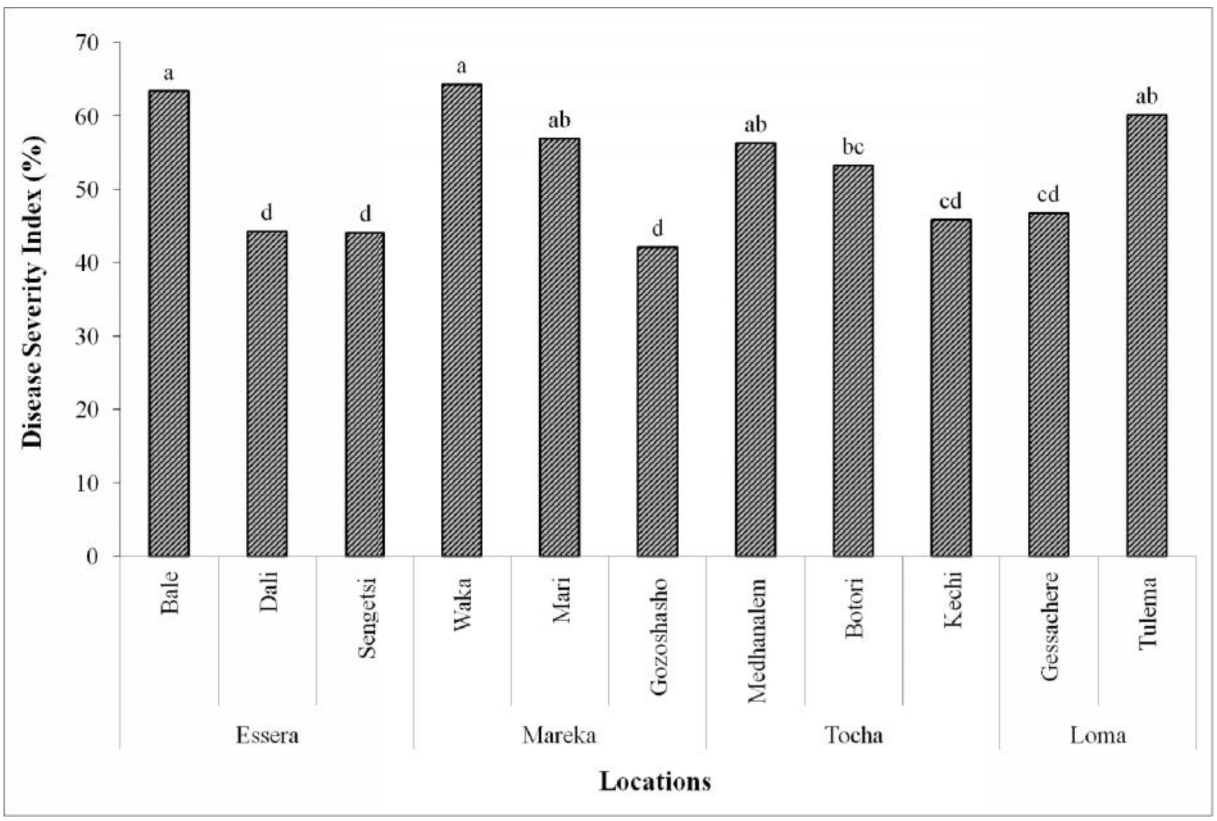
Chi Square for independence of frequencies in a variable was used to analyze the collected data for laboratory and greenhouse experiments.

Computer based SAS statistical software version 9.2 (SAS Institute, 2008) was used for analysis and a significant level of $\alpha \leq 0.05$ was used.

4. RESULTS

4.1. Prevalence and Severity of Chocolate Spot in Dawuro Zone

All surveyed faba bean fields were infected with chocolate spot. The results indicated that, the total fields (44) surveyed were infected with chocolate spot with different levels of severity. The highest mean chocolate spot severity ranged from 45.8 to 69.4 % at Waka, Bale, Tulema, Mari, Medhanalem, Botori, Gessachere and Kechi peasant associations (PAs) and lowest ranged from 33.4 to 44.2 % in Dali, Gozoshasho and Sengetsi PAs (Figure 2).



Bars followed by similar letters are not significantly different ($p < 0.05$)

Figure 2. Mean severity index of chocolate spot in farmers' field, 2011 cropping season.

There was significant difference ($p < 0.05$) in the mean disease severity among the peasant associations (Appendix Table 11). Among all PAs, the highest disease severity was observed at Waka and Bale with 64.4 and 63.3 %, respectively. In most cases disease severity varied between altitude ranges within district and it was higher in fields more than 2200 m.a.s.l (Table 4).

Table 4. Mean severity index of faba bean chocolate spot for different independent variables in 2011 cropping season for surveyed fields.

Variable	Variable class	Mean DSI (%)	SD (±)
PA	Bale	63.3	8.1
	Dali	44.2	2.6
	Sengetsi	44	4.4
	Waka	64.2	6.9
	Mari	56.8	6.0
	Gozoshasho	42	2.6
	Medhanalem	56.2	3.5
	Botori	53.2	6.8
	Kechi	45.8	2.6
	Gessachere	46.7	6.0
	Tulema	60.1	2.5
Crop variety	Improved	47	9.8
	Local	53.2	9.1
Crop density	≤55plants /m^2	49.8	9.4
	>55 plants /m^2	53.3	9.0
Planting date	After July,15	50.7	7.2
	Before July,15	55.8	12.4
Weed mg't	Weeded	43.9	6.4
	Intermediate	50.3	8.7
	Not weeded	54.9	8.7
Altitude	> 2200 m	57.5	7.4
	≤2200 m	43.5	4.5
Previous crop	Cereals	53.7	9.6
	Legumes	50.8	7.5
	None (fallow)	40.4	4.0
Land preparation	2xploughing	53.8	9.3
	3xploughing	47.6	8.1
Field size	≤ 0.5 ha	49.7	6.8
	>0.5 ha	56.7	11.4
Growth stage	Pre-podding	49.9	7.9
	Podding	55.1	10.2
Slope	>8 %	52.3	7.4
	≤ 8 %	52.4	10.5

DSI (disease severity index), SD (standard deviation)

The severity of chocolate spot was higher in local cultivar planted fields and with two times ploughing than in improved planted and three times ploughed fields. From the cropping history, it was observed that when bean was planted in rotation with other crops (non leguminous species) the chocolate spot was less compared with faba bean continuously grown on the same field of the total fields surveyed; seven fields were continuously planted with faba bean. Fields with good weed management practices had lower chocolate spot severity (Table 4). It appeared that the status of weed infestation had an influence on chocolate spot severity (54.9 %) was recorded in not weeded fields.

4.2. Association of Faba Bean Chocolate Spot with Independent Variables

The association of variables is presented in Table 5. The independent variables such as peasant association, crop variety, planting time, altitude and crop history were significantly associated with chocolate spot severity when entered into the logistic regression model as a single variable. However, when all variables entered last into the regression model, only peasant association, crop variety, altitude and crop history remained significant in their association with chocolate spot severity. Among the independent variables, peasant association (X^2 = 1819.0 and 413.37, 10 df), altitude (X^2 = 6.75 and 10.91, 1 df) and crop history (X^2=6.81and 8.29, 2df) were the most important variable in its association with severity when entered first and last in to the model. Planting time lost their importance when entered into reduced variable model.

Table 5. Independent variables used in logistic regression analysis and likelihood ratio statistics for eleven variables entered first and last into a model.

Independent variable	df	Type 1 analysis [a]		Type 3 analysis [b]	
		DC	PR>X^2	DC	PR>X^2
PA	10	1819.0c	0.000	413.37	0.000
Crop Variety	1	11.59	0.001	4.23	0.04
Crop density	1	2.22	0.136	0.25	0.614
Planting time	1	6.25	0.012	0.86	0.353
Weed condition	2	0.41	0.815	3.56	0.169
Altitude	1	6.75	0.009	10.91	0.001
Crop history	2	6.81	0.033	8.29	0.016
Field size	1	0.15	0.699	0.00	0.998
Land preparation	1	0.01	0.924	0.01	0.923
Growth stage	1	0.03	0.865	0.31	0.580
Slope	1	2.73	0.098	2.73	0.098

Dependent Variable=Disease severity index
Model=Peasant association, Crop Variety, crop density, planting time, Weed condition, Altitude, Crop history, Field size, land preparation, Growth stage and Slope.
df, degrees of freedom; DC, deviance change; PR, probability of a chi square value exceeding the deviance; LRT, likelihood ratio test.
[a] *Type 1 analysis =variable entered first in to the model.*
[b] *Type 3 analysis = variable entered last in to the model.*

The results for analysis of deviation for variable and variable class are presented in Table 5. The independent variables such as peasant associations, crop variety, crop density, altitude, weed managements and slope were tested in a reduced multiple variable models. The deviation analysis of these variables in a reduced multiple variable models showed the significance of their association with disease severity.

The parameter estimates, standard error and odds ratio presented in Table 6 indicates: probability of lowest severity (≤ 45) was highly associated with the mid-altitude PAs (≤ 2200 m.a.s.l) (Dali, Sengetsi, Kechi and Gozoshasho), good weed management practices and use of improved varieties. High chocolate spot severity had high probability of association to high-altitude (> 2200 m.a.s.l) (Bale, Waka, Botori and Mari) PAs, none weeded fields and use of local varieties. The severity was greater in none weeded fields than weeded ones. All other variables such as crop density, field size, and slope of farms, planting time, previous crop and ploughing frequency did not show significance association on the severity of chocolate spot.

Table 6. Analysis of deviance, natural logarithms of odds ratio and standard error of the selected independent variables in a reduced model analyzing chocolate spot severity.

Independent variable [a]	df	RD	LRS [b] DC	LRS [b] $PR>x^2$	Variable class	Estimate [c]	SE	Odds ratio [d]
Intercept	0	0.083	-	-	-	0.11	0.37	1.11
Crop variety	1	4.41	12.88	0.036	improved	-0.34	0.16	0.71
					Local	0*	0*	1
Crop density	1	0.3	2.47	0.584	≤55plants /m^2	-0.11	0.20	0.90
					>55 plants /m^2	0*	0*	1
Altitude	1	14.31	10.96	0.000	≤2200m	-0.61	0.16	0.54
					>2200m	0*	0*	1
Weed mg't	2	3.38	0.34	0.07	Weeded	-0.019	0.15	1.33
		0.79		0.37	Intermediate	-0.466	0.16	0.87
					Not weeded	0*	0*	1
Cropping history	2	8.82	9.67	0.003	Cereals	1.07	0.36	2.91
		11.53	1.156	0.001	Legumes	1.16	0.34	3.18
					Fallow	0*	0*	1
Slope	1	3.09	3.09	0.079	>8%	-0.20	0.11	0.82
					≤8%	0*	0*	1
PA	10	4.662	35.12	0.000	Tulema	0.15	0.22	1.16
					Dali	-0.28	0.26	0.75
					Sengetsi	-0.09	0.26	0.91
					Waka	0.02	0.24	1.02
					Mari	-0.07	0.21	0.93
					Gozoshasho	-0.45	0.39	1.57
					Medhanalem	-0.22	0.21	0.80
					Botori	0.02	0.22	1.02
					Kechi	-0.19	0.35	1.21
					Gessachere	0.03	0.30	1.03
					Bale	0*	0*	1

*df, degrees of freedom; DC, the changes in deviance; $PR>x^2$,probability of a chi square value exceeding the deviance; SE, standard error of the estimate; *Reference group.*
[a]*Independent variables added in to the reduced model; RD = residual deviance(Unexplained variations after fitting the model);* [b]*Likelihood ratio statistics.*
[c]*Estimates from the model with the independent variables added in to a reduced model.*
[d]*Exponentiating the estimates.*

4.3. Chocolate Spot Epidemics and Progression

4.3.1. Disease onset and symptoms observation

At both at Tocha and Mari, chocolate spot was first observed on the local cultivar in spreader rows around the end of July at about second to third leaf emergence and it was recorded on the experimental plots on first to second week of August, 2011. The disease was observed on the improved varieties at 20 days after planting (DAP) at these two experimental sites, while it occurred at later (after 27 DAP) at Turi.

After the establishment on all experimental plots, it spreads rapidly within a crop and at 4 - 5 days of infection spores formed on infected tissue and initiated secondary spread of the disease.

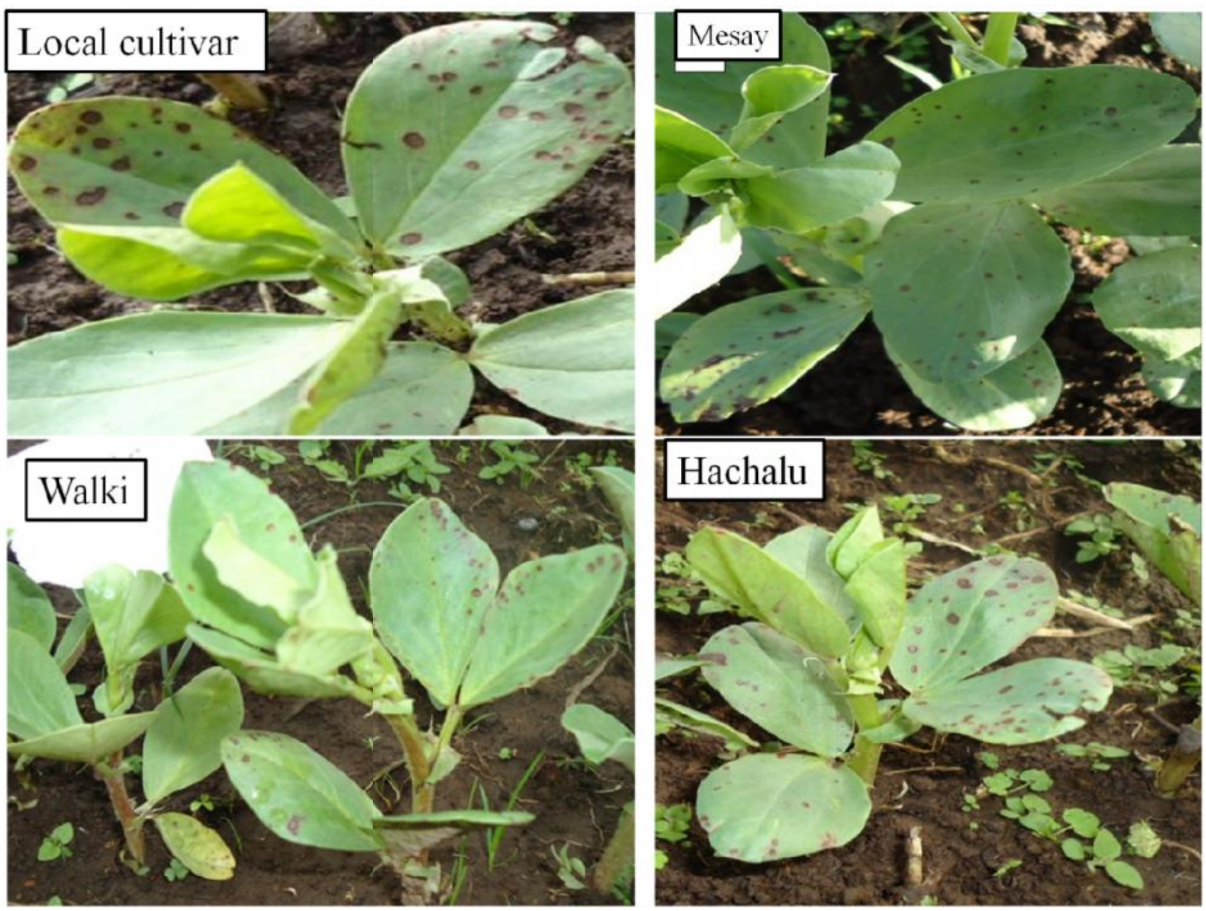
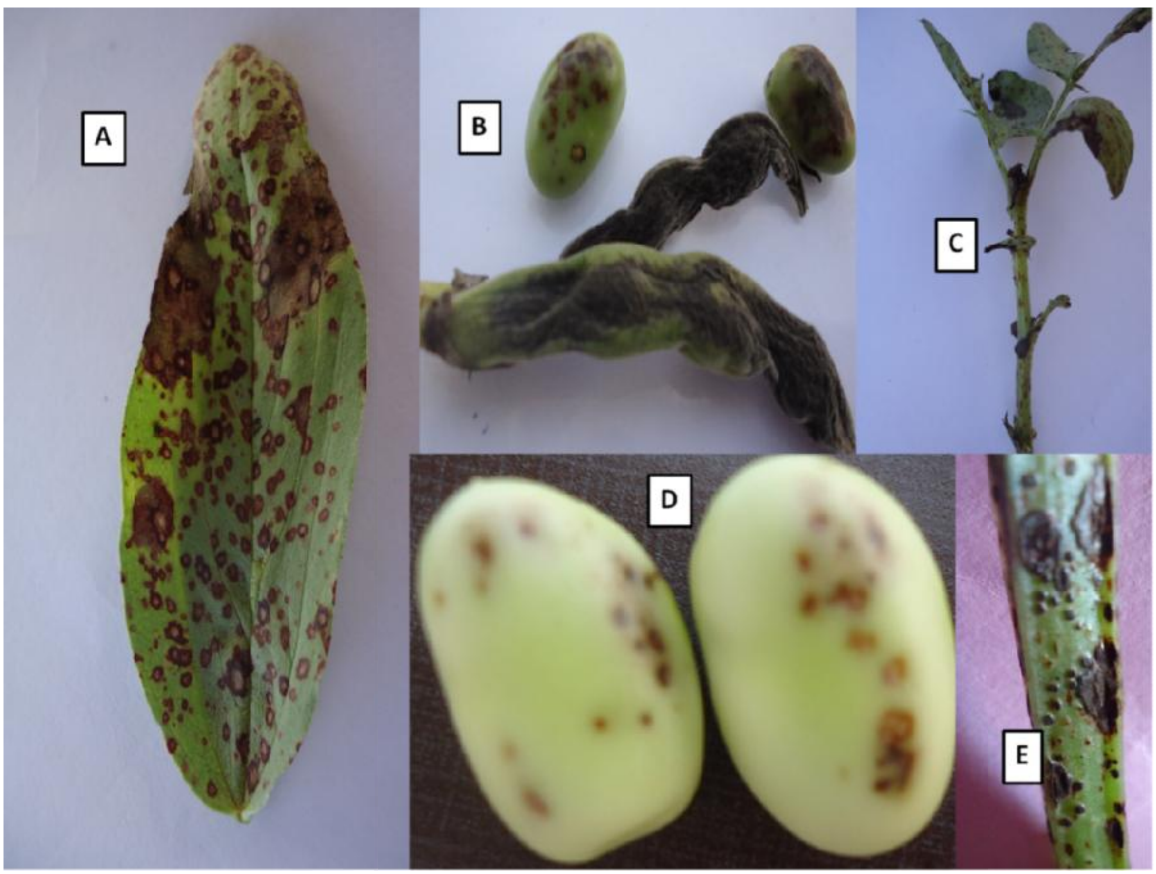


Figure 3. Early infection of chocolate spot on faba bean varieties, 30 Days after planting.

Chocolate symptoms are varied, and range from small discrete red-brown lesions (1 - 3 mm) on the leaves to complete blackening of the entire plant. Leaves are the main part of the plant affected at early stage of growth (Figure 3), under high altitude ranges (Tocha and Mari); it also spreads to stems, flowers, pods and seeds (Figure 4B-E). Two stages of the disease are frequently recognized. First, a non-aggressive phase, when discrete reddish-brown spots over the leaves and stems, and then an aggressive phase, when spots darken in color and coalesce to form larger grey-brown target spots that may eventually cover the entire plant. On stems, dark brown streaks may become noticeable on the badly diseased plants (Figure 4E). Infection of stems can lead to lodging. Indeed, infected flowers and pods may abort (Figure 4C).



Note: A =Leave areas die leading to defoliation; B and D = Pods and Seeds – discolorations; C= Abnormal leaf fall and abortion of infected flowers and pods; E=Sclerotia of Botrytis fabae on faba bean stems

Figure 4. Aggressive stages of chocolate spot symptoms or signs on local cultivar.

When the crop was infected at early growth stage (before flowering), aggressive development of infection late in the season can cause the poor pod set, the crop to lodge and drop of leaves in the vicinity of the plant and whole plant death was observed. Also the secondary branches may severely attacked and becomes stunted and weak (not vigor) on plots of certain varieties such as Local, Mesay, Kuse and Moti.

4.3.2. Analysis of variance for location

The results of analysis of variance for disease reaction and yield related traits at each location showed highly significant variations among faba bean varieties .These are an indication that the responses of the varieties differ across location for chocolate spot infections and yield related traits under consideration (Appendices Table 3 - 10).

4.3.3. Chocolate spot progress at test locations

The chocolate spot severity indices recorded at 35, 42, 49, 56, 63, 70, 77, 84, 91 and 98 days after planting revealed significant ($P < 0.01$) effects of the tested varieties at all assessment dates after disease appearance on experimental plots across location except for Mari at 35 DAP; and Turi at 35 and 42 DAP confirming differences in disease reaction among the varieties (Appendix Table 3). Maximum chocolate spot severity, up to 46.3 % was recorded on the local cultivar plots at Tocha while a lower chocolate spot severity of 23 % was recorded on Degaga at Turi 98 DAP (Table 7).

At Tocha, after 63 DAP onward, the chocolate spot severity was varied on CS20DK, Degaga, Tesfa and Bulga-70 and began to show significantly lower levels up to 98 DAP. Severity levels in plots of the Local cultivar, Kuse, Hachalu, Gebelcho were statistically at far with that of the CS20DK, Degaga, Bulga-70 and Tesfa during all the successive assessments of the disease onward 63 DAP (Figure 5).

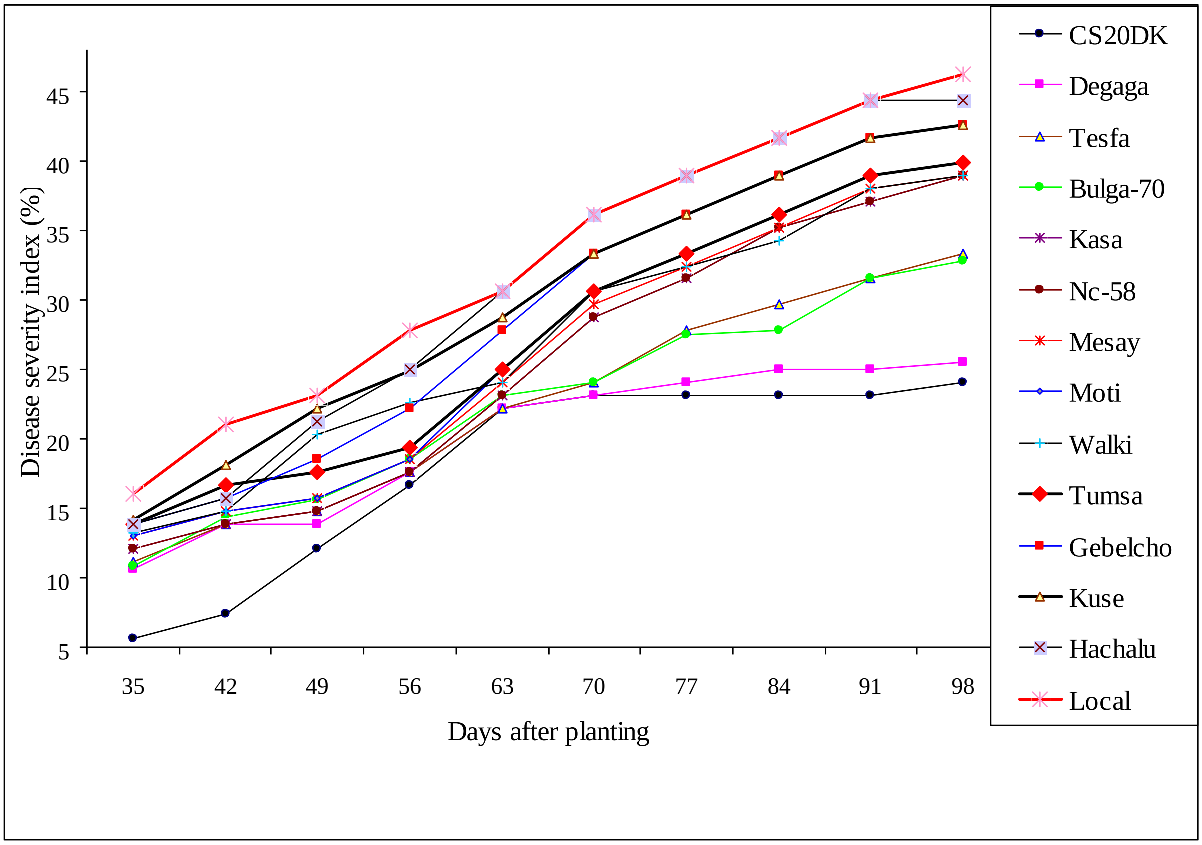


Figure 5. Comparison of disease progress curves for epidemics of chocolate spot on faba bean varieties at Tocha, 2011 growing season.

At Mari, severity levels in plots of the variety CS20DK, Degaga, Bulga-70, Tesfa and Kasa began to show significantly lower levels of disease severity after 56 to 83 DAP compared with severity levels in the plots of local, Hachalu Gebelcho, Kuse, Moti, Mesay, NC-58, Tumsa and Walki during all the successive assessments of the disease. But after 84 DAP variety Bulga-70 showed significant increment of severity as those varieties of high infection rate while Kasa decreases as 98 DAP. In contrast, Disease severity was constantly lowered on CS20Dk, Degaga and Tesfa (Figure 6).

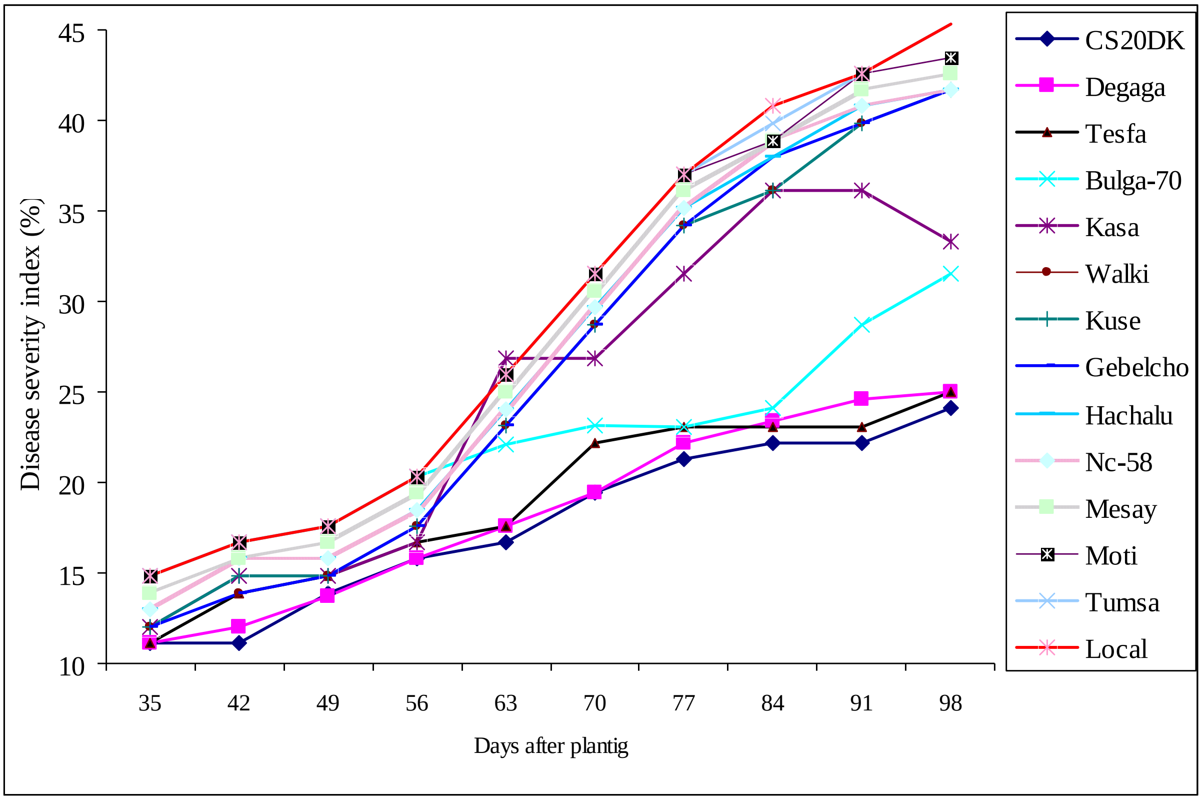


Figure 6. Comparison of disease progress curves for epidemics of chocolate spot faba bean varieties at Mari, 2011 growing season.

Also at Turi, the varieties showed different levels of chocolate spot severity starting from the 4th disease assessment (at 56 DAP).The final levels of chocolate spot severity were significantly different ($P < 0.05$) among varieties but they were lower by comparison to the severity levels at Mari and Tocha (Figure 7).

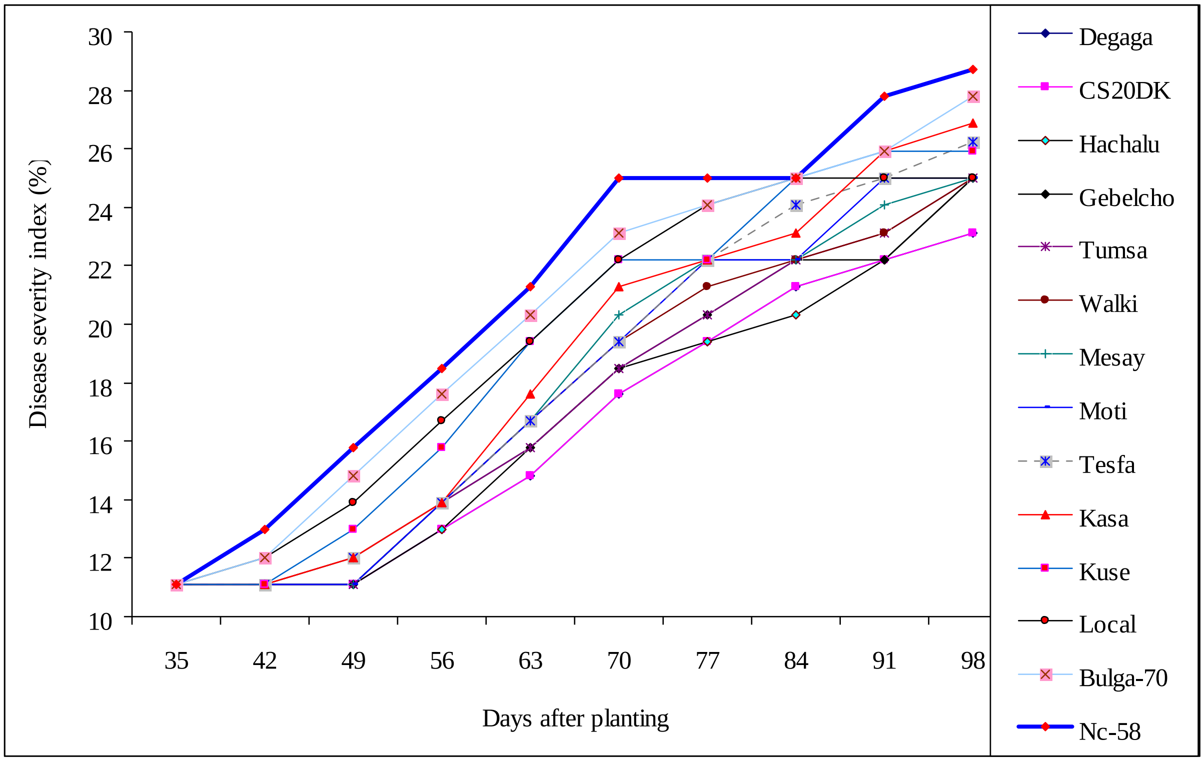


Figure 7. Comparison of disease progress curves for epidemics of chocolate spot on faba bean varieties at Turi, 2011 growing season.

Mean disease severity index at 98 DAP ranged from 24.1 to 46.3% in experimental plots of both Tocha and Mari in comparison with 23.1 to 28.7 % in Turi. According to terminal DSI values, the five faba bean varieties namely Bulga-70, CS20DK, Degaga, Kasa and Tesfa showed the moderately resistance to chocolate spot at Tocha and Mari where high disease pressure was observed. But at Turi the maximum chocolate spot severity was 28.7 % recorded from NC-58 and it was not significantly different from other varieties except from the CS20DK and Degaga. All the tested varieties showed moderately resistance to chocolate spot at Turi and rated low disease severity (Table 7).

Table 7. Grouping of 14 faba bean varieties into reaction groups at three test locations, 2011growing season.

Variety	Tocha	Mari	Turi
Bulga-70	32.8[e]	31.5[c]	24.1[bc]
CS20DK	24.1[f]	24.1[d]	23.1[c]
Degaga	25.5[f]	24.7[d]	23.0[c]
Gebelcho	42.6[bc]	41.7[b]	25.0[bc]
Hachalu	44.4[ab]	41.7[b]	25.0[bc]
Kasa	38.9[d]	33.3[c]	27.0[ab]
Kuse	42.6[bc]	42.0[b]	26.0[a-c]
Local	46.3[a]	45.0[a]	25.0[bc]
Mesay	38.9[d]	43[ab]	25.0[bc]
Moti	39.8[cd]	44.0[ab]	25.0[bc]
Nc-58	38.9[d]	42.0[b]	28.7[a]
Tesfa	33.3[e]	25.0[d]	26.0[a-c]
Tumsa	39.8[cd]	45.0[a]	25.0[bc]
Walki	38.9[d]	42.0[b]	25.0[bc]
Mean	37.6	37.4	25.2
SD (±)	6.45	7.8	1.7
CV (%)	2.8	2.9	4.5
p-value	<0.0001	<0.0001	<0.0001

Similar letter(s) down the columns are not significantly different ($P < 0.05$)

DSI =disease severity index assessed at 98 days after planting.

The cut-off values for moderately resistant ranging between >15.0 - 40.0, and moderately susceptible ranging between >40.0 - 60.0 as expressed by Abo-Hegazy et al. (2012)

4.3.4. Area under the disease progress curve (AUDPC)

Areas under the disease progress curve (AUDPC) for chocolate spot were significantly different (P < 0.01) among the varieties tested in all locations (Appendix Table 4). The highest AUDPC was observed at Tocha and Mari experimental plots (1289 to 2387 % - days), on CS20DK and local cultivar respectively, compared with at Turi (1195 to 1539 % - days on CS20Dk or Degaga and NC-58, respectively (Figure 8 - 10).

At Tocha, according to their mean AUDPC values, tested varietis were categorized into five groups, the first group were local and Hachalu, the second group were Kuse and Gebelcho; the third group were Tumsa,Walki, Moti, Nc-58,Mesay and Kasa ;the fourth group were Bulga-70 and Tesfa; and the last with least AUDPC was Degaga and CS20DK (Figure 9).

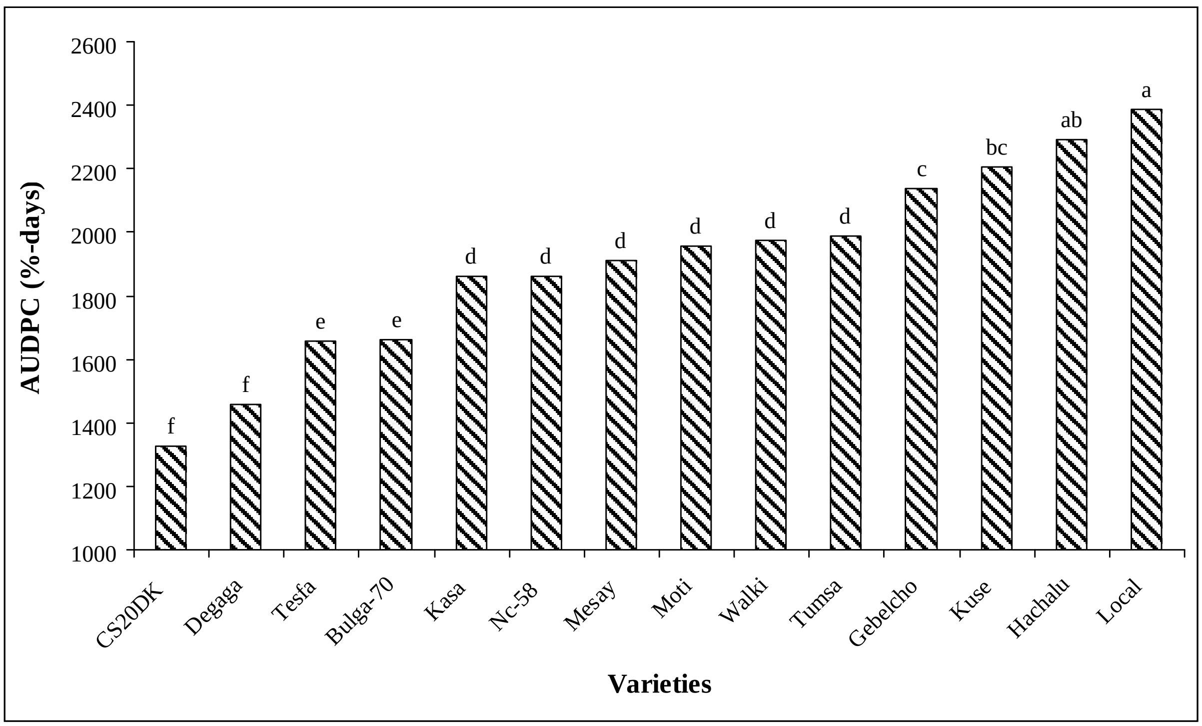


Figure 8. Classification of 14 varieties of faba bean according to AUDPC values determined at Tocha, 2011 growing season.

Bars with similar letters are not significantly different (P < 0.05).

At Mari, based on their mean AUDPC values tested varieties were classified into four groups, the first group were Local and Tumsa, Moti, and Mesay, the second group were Nc-58,Hachalu,Gebelcho, Kuse ,Walki and Kasa ; the third group was Bulga-70;the fourth group and the last with least AUDPC were Tesfa ,Degaga and CS20DK (Figure 9).

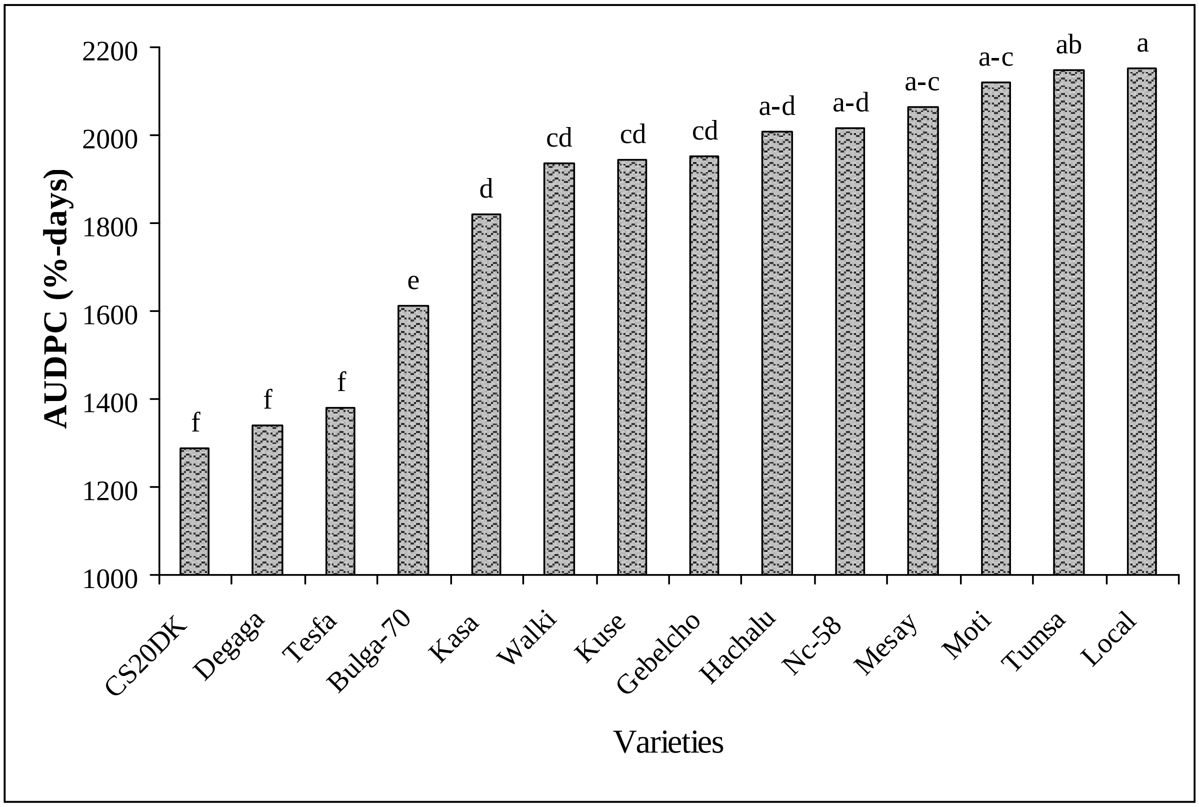


Figure 9. Classification of 14 varieties of faba bean according to AUDPC values determined at Mari, 2011 growing season.

Bars with similar letters are not significantly different ($P < 0.05$).

Also at Turi, highly significant ($P < 0.01$) difference with AUDPC values were realized (Appendix Table 4) but the least AUDPC values were obtained compared with other test locations. Among varieties Nc-58, Bulga-70 and local cultivar had higher AUDPC that of Degaga and CS20DK. Others categorized as similar trends with each other (Figure 10).

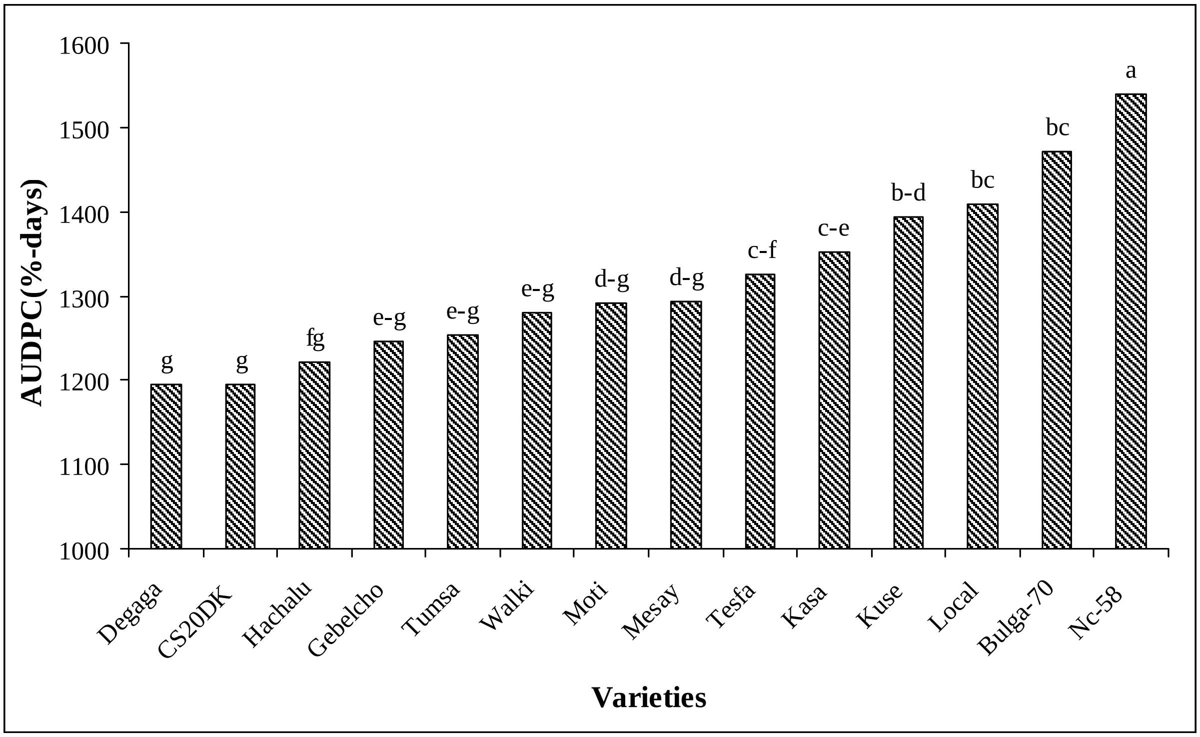


Figure 10. Classification of 14 varieties of faba bean according to AUDPC values determined at Turi, 2011 growing season.

Bars with similar letters are not significantly different ($P < 0.05$).

4.3.5. Chocolate spot infection rate

Disease progress data were fitted to logistic and Gompertz growth models. Based on coefficients of determination (R^2), standard errors of the estimate, and examination of residuals, the logistic model was found to provide the best fit and, therefore, this model was used to quantify disease progression to make compressions among the fourteen varieties.

ANOVA for apparent infection rates (IR) were significantly different ($p < 0.05$) for tested varieties for each of the test locations (Appenidix Table 5). The mean IR ranging from 0.118 to 0.207 disease units per day. Apparently low rate of chocolate spot infection was quantified for Degaga (0.118 disease units per day) at Tocha, CS20Dk, Degaga and Tesfa (0.110, 0.118 and 0.104 disease units per day at Mari, respectively) and CS20Dk and Degaga (0.118 disease units per day) at Turi compared with other varieties within each location. The acceptable regression equations with coefficient of determinations (R^2) ranging from 73.8 to 97.3% were produced when chocolate spot severity was regressed over time in days after planting for all plots. The other varieties did not affect the infection rate of chocolate spot significantly within location. The rates of infection on those plots were ranged from 0.122 to 0.196 disease units per day, at Turi and Mari, respectively (Table 8).

Table 8. Infection rates of chocolate spot on 14 faba bean varieties at three locations in Dawuro zone, 2011 growing season.

Variety	Tocha				Mari				Turi			
	IR	S	I	R^2	IR	S	I	R^2	IR	S	I	R^2
Bulga-70	0.151[b]	0.47	-2.11	93.8	0.131[bc]	0.32	-1.82	87.6	0.122[def]	0.39	-2.22	92.1
Cs-20Dk	0.181[ab]	0.64	-2.62	73.8	0.110[c]	0.34	-2.18	87.6	0.118[f]	0.37	-2.31	93.1
Degaga	0.118[c]	0.39	-2.07	83.9	0.118[c]	0.37	-2.18	87.6	0.118[f]	0.37	-2.31	93.6
Gebelcho	0.185[a]	0.57	-1.97	96.3	0.207[a]	0.64	-2.25	97.1	0.124[c-f]	0.39	-2.30	94.7
Hachalu	0.192[a]	0.6	-1.91	94.8	0.195[a]	0.60	-2.13	96.7	0.122[ef]	0.38	-2.32	93.1
Kasa	0.189[a]	0.58	-2.20	96.6	0.170[ab]	0.55	-2.11	86.6	0.139[a]	0.44	-2.30	93.4
Kuse	0.167[ab]	0.52	-1.81	95.4	0.175[a]	0.62	-2.25	97.3	0.134[a-c]	0.42	-2.23	92.8
Local	0.167[ab]	0.51	-1.69	96.0	0.192[a]	0.59	-2.00	96.7	0.123[c-f]	0.39	-2.14	91.2
Messay	0.181[ab]	0.56	-2.11	96.4	0.170[ab]	0.59	-2.06	96.6	0.131[a-e]	0.41	-2.30	93.2
Moti	0.186[a]	0.58	-2.13	95.9	0.186[a]	0.57	-1.98	96.5	0.133[a-d]	0.41	-2.31	94.7
Nc-58	0.189[a]	0.58	-2.20	96.6	0.196[a]	0.61	-2.13	96.5	0.131[a-e]	0.41	-2.09	91.8
Tesfa	0.187[a]	0.43	-2.25	84.9	0.104[c]	0.34	-2.06	87.1	0.136[ab]	0.42	-2.31	95.5
Tumsa	0.171[ab]	0.52	-1.98	96.8	0.191[a]	0.59	-2.00	97.1	0.126[b-f]	0.39	-2.31	95.0
Walki	0.165[ab]	0.52	-1.96	92.9	0.178[a]	0.63	-2.27	97.3	0.124[c-f]	0.39	-2.27	94.9
Mean	0.173				0.166				0.127			
CV	10.7				15.3				5.15			
SD	0.025				0.034				0.009			
p-value	0.0031				<0.0001				0.0113			

Means in a column with the same letter are not significantly different ($P < 0.05$).
IR =infection rate; S= standard error of infection rate; I=intercept; R^2 =Coefficient of determination for the logistic model (%).
Intercept and infection progress rate represent the equation of the line.

4.4. Yield and Yield Components

4.4.1. Number of pods per plant (NPP) and number of seeds per pod (NSP)

Analysis of variance showed highly significant difference ($p < 0.01$) for NPP among varieties at three locations but significant difference for NSP ($p < 0.05$) was realized only at Turi (Appendix Table 6 and 7).

The mean values of number of pods per plant and number of seeds per pod are presented in Table 10. Mean values ranged from 5.6 for (Gebelcho) to 17 for (Degaga) at Tocha; 8.6 (Hachalu) to 15.7 (NC-58) at Mari; and 9 (Gebelcho and Hachalu) to 15.7 (NC-58) at Turi. Nine varieties namely Walki, Tesfa, Nc-58, Messay, Kuse, Kasa, Degaga, CS20Dk and Bulga-70 had high NPP than the average at each location (Table 9).

Among the varieties for SNP the highest SNP is 4 at Turi for Bulga-70, Hachalu and Tumsa while the lowest is 2.7 for varieties Walki at Tocha and Gebelcho at Mari and Turi. Four varieties namely Bulga-70, Hachalu, Tumsa and Moti had high NSP than the average at Turi (Table 9).

Table 9. Mean values of number of pods per plant and number of seeds per pod of faba bean varieties tested at three locations in Dawuro zone, 2011 cropping season.

Variety	Number of pods per plant			Number of Seeds per Pod		
	Tocha	Mari	Turi	Tocha	Mari	Turi
Bulga-70	13.0^{b-d}	14.0^{ab}	14.0^{ab}	3.3^{a}	3.3^{a}	4.0^{a}
CS20Dk	12.0^{cd}	12.7^{abc}	12.7^{a-c}	3.3^{a}	3.0^{a}	3.0^{ab}
Degaga	17.0^{a}	13.7^{ab}	12.7^{a-c}	3.3^{a}	4.0^{a}	3.0^{ab}
Gebelcho	5.7^{h}	9.0^{de}	9.0^{d}	3.0^{a}	2.7^{a}	2.7^{b}
Hachalu	8.3^{f-h}	8.7^{e}	9.0^{d}	4.0^{a}	3.3^{a}	4.0^{a}
Kasa	15.3^{ab}	14.7^{a}	14.0^{ab}	3.0^{a}	3.0^{a}	3.0^{ab}
Kuse	13.0^{b-d}	12.3^{a-d}	12.3^{a-d}	3.0^{a}	3.0^{a}	3.0^{ab}
Local	9.0^{efg}	10.7^{b-e}	11.0^{b-d}	3.3^{a}	3.0^{a}	3.0^{ab}
Messay	14.3^{a-c}	13.7^{ab}	13.7^{ab}	3.3^{a}	3.0^{a}	3.0^{ab}
Moti	8.0^{f-h}	10.0^{cde}	10.0^{cd}	3.7^{a}	3.3^{a}	3.3^{ab}
Nc-58	15.0^{a-c}	15.7^{a}	15.7^{a}	3.3^{a}	3.3^{a}	3.0^{ab}
Tesfa	14.3^{a-c}	12.3^{a-d}	12.3^{a-d}	3.0^{a}	3.0^{a}	3.7^{ab}
Tumsa	10.7^{d-f}	9.3^{cde}	9.3^{cd}	3.0^{a}	3.3^{a}	4.0^{a}
Walki	7.0^{gh}	15.0^{a}	14.7^{a}	2.7^{a}	3.0^{a}	3.0^{ab}
Mean	11.62	11.46	12.16	3.24	3.17	3.26
SD(±)	3.57	2.48	2.36	0.48	0.49	0.54
CV (%)	9.2	9.29	9.9	11.95	14.93	11.95
p-value	<0.0001	<0.0001	<0.0001	0.1174	0.26	0.0011

Means in a column with the same letter are not significantly different ($P < 0.05$).

4.4.2. Seed Yield (SYD) and Hundred Seed Weight (HSW)

The analysis of variance displayed significant differences among varieties for SYD (P < 0.01) (Appendix Table 8) and hundred seed HSW (P < 0.01) (Appendix Table 9). Mean values of seed yield and hundred seed weight are presented in Table 10.

At Tocha, SYD ranged from 2100 kg/ha (CS20Dk) to1305 kg/ha (Mesay); at Mari1327 kg/ha (Degaga) to 533 kg/ha (local) and at Turi 1221 kg/ha (Degaga) to 697 kg/ha (local). All varieties produced 1305 kg/ha and above at Tocha and only Degaga yielded more than 1305 kg/ha at Mari. But, at Turi all varieties yielded less than 1305 kg/ha (Table 11). CS20DK (2100 kg/ha), NC-58 (2027 kg/ha), Moti (1973 kg/ha) and Degaga (1910 kg/ha) were highest seed yield performer at Tocha where as Local cultivar (1873 kg/ha), Bulga-70(1500 kg/ha) and Kasa (1520kg/ha) are low yielders. Similarly, at Mari high SYD (kg/ha) of 1327,880 and 870 was obtained from Degaga, CS20Dk and NC-58 respectively. Variety Degaga (1221 kg/ha) was high yield performed at Turi where as local cultivar (697 kg/ha), Tumsa (860 kg/ha) and Walki (873 kg/ha) produced low yield.

The mean for HSW ranged 99.1g (Hachalu) at Tocha to 28.6g (Kuse) at Turi (Table 10). At Tocha the Varity Hachalu (99.1g), Moti (91.3g), Gebelcho (86.3g) and Tumsa (86.3g) were produced heavier seeds where as Kuse (38.6g), Local (38.6g) and Bulga-70 (44.1g) produced small seeds. Similarly at Mari heavier seeds were recorded from Hachalu (95.6g), Moti (83.7), Tumsa (73.7g), Gebelcho (72.7g) and Walki (65.3g) where as lighter seeds was obtained from variety Kuse (35.7g), Local (36.3g), Tesfa (38.3g) and Bulga-70 (39.3g). Also at Turi; Moti (84g), Hachalu (82.8g) and Gebelcho (77.1g) were produced heavier seeds and Kuse (28.6g), Local (44.5g) and Tesfa (39.6g) produce smaller seeds.

Table 10. Mean values of seed yield (kg/ha) and hundred seed weight (g) of faba bean varieties, 2011cropping season.

Variety	Seed yield (kg/ha)			Hundred Seed weight (g)		
	Tocha	Mari	Turi	Tocha	Mari	Turi
Bulga-70	1500^{g}	813^{bc}	963^{bc}	44.1^{gh}	39.3^{g}	42.3^{gh}
CS20Dk	2100^{a}	880^{b}	1043^{ab}	55.3^{ef}	57.7^{e}	49.0^{f}
Degaga	1910^{b-d}	1327^{a}	1221^{a}	62.4^{d}	58.7^{e}	53.7^{e}
Gebelcho	1810^{c-e}	803^{bc}	1013^{bc}	86.3^{b}	71.2^{cd}	77.1^{b}
Hachalu	1890^{b-e}	840^{bc}	937^{bc}	99.1^{a}	95.7^{a}	82.8^{a}
Kasa	1520^{g}	813^{bc}	1027^{bc}	58.5^{de}	54.0^{e}	72.3^{c}
Kuse	1570^{fg}	863^{bc}	977^{bc}	38.6^{h}	35.7^{g}	28.6^{i}
Local	1873^{b-e}	533^{d}	697^{d}	38.6^{h}	36.3^{g}	40.8^{h}
Messay	1305^{h}	737^{c}	1050^{ab}	50.7^{fg}	47.2^{f}	44.5^{g}
Moti	1973^{a-c}	760^{bc}	970^{bc}	91.3^{b}	83.7^{b}	84.0^{a}
Nc-58	2027^{ab}	870^{b}	993^{bc}	56.7^{d-f}	53.3^{ef}	48.0^{f}
Tesfa	1840^{c-e}	790^{bc}	903^{bc}	46.7^{g}	38.3^{g}	39.6^{g}
Tumsa	1773^{de}	833^{bc}	860^{cd}	84.5^{b}	73.7^{c}	66.1^{d}
Walki	1727^{ef}	820^{bc}	873^{b-d}	44.1^{c}	65.3^{d}	69.8^{c}
Mean	1772.74	834.5	996.24	63.27	57.9	57.04
SD (±)	225	165.3	125.05	19.78	18.06	17.55
CV (%)	3.8	5.08	6.13	3.35	3.53	1.77
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means in a column with the same letter are not significantly different ($P < 0.05$).

4.4.2. Performance of tested faba bean varieties across location

The analysis of variance for yield and yield components was combined after Bartlett's test for homogeneity of error variance. The test identified the error variance as homogeneous for only number of seeds per pod at Tocha and Mari (Appendix Tables 6 - 9).

However, combined analysis of variance was recommended to be done even if the test is found significant by excluding the data of locations where coefficient of variation for the trait analyzed is greater than twenty (Gomez and Gomez, 1984). Therefore, in the present study, the coefficients of variation at all locations were less than twenty for all traits. The analysis showed that the effects due to all components namely variety, location, and variety - location interaction were highly significant ($p < 0.01$) for yield and related traits except for pod number per plant for location effect, and seeds per pod for interaction effect (Appendix Table10) . This indicated the presence of variability among the varieties and locations and emphasized consideration of variety - location interaction when selecting faba bean varieties for production.

The mean SYD of the varieties ranged between 1486 kg/ha (Degaga) to 845kg/ha (Local). High yielding varieties were Degaga (1486), CS20Dk (1341kg/ha), Nc-58 (1297 kg/ha) and Moti (1234 kg/ha); while Local (845kg/ha), Bulga-70 (1092 kg/ha) and Kasa (1120) were low yielding varieties (Table 11). When locations were compared, the highest SYD (1772.7 kg/ha) was obtained at Tocha which is followed by Turi (966.2 kg/ha), while Mari (834.5 kg/ha) was poor yielding (Table 12).

There was significant ($p < 0.05$) variation for HSW across locations. High value of HSW was recorded for the varieties Hachalu (92.5g), Moti (86.3g), Gebelcho (78.2g), Tumsa (74.8g) and Walki (69.4g) which had large seed size. Kuse had low (34.3g) HSW (Table 11). When locations were compared, the highest SYD (1772.7 kg/ha) was obtained at Tocha which is followed by Turi (966.2 kg/ha), while Mari (834.5 kg/ha) was poor yielding (Table 12).

Table 11. Combined mean values for chocolate spot severity and yield related traits of tested faba bean varieties.

Variety	NPP	SNP	SYD (kg/ha)	HSW(g)
Bulga-70	13.7^{a-c}	3.6^{ab}	1092^{f}	41.9^{j}
CS20DK	12.4^{c}	3.1^{a-c}	1341^{b}	54.0^{h}
Degaga	14.4^{ab}	3.4^{a-c}	1486^{a}	58.2^{g}
Gebelcho	7.9^{e}	2.8^{c}	1209^{c-e}	78.2^{c}
Hachalu	$8.7d^{e}$	3.8^{a}	1222^{c-e}	92.5^{a}
Kasa	14.7^{ab}	3.0^{bc}	1120^{f}	61.6^{f}
Kuse	12.6^{c}	3.0^{bc}	1137^{ef}	34.3^{l}
Local	10.2^{d}	$3.1\ ^{a-c}$	845^{g}	38.6^{k}
Mesay	13.9^{a-c}	$3.1\ ^{a-c}$	1220^{c-e}	47.5^{i}
Moti	$9.3d^{e}$	$3.4\ ^{a-c}$	1234^{cd}	86.3^{b}
Nc-58	15.4^{a}	$3.2\ ^{a-c}$	1297^{bc}	52.7^{h}
Tesfa	13.0^{cb}	$3.2\ ^{a-c}$	1178^{d-f}	41.5^{jk}
Tumsa	9.8^{d}	$3.4\ ^{a-c}$	1156^{d-f}	74.8^{d}
Walki	12.2^{c}	2.9^{bc}	1140^{ef}	69.4^{e}
Mean	12.02	3.2	1191	59.4
SD (±)	2.9	0.50	451.8	18.6
CV (%)	9.4	13.4	4.55	3.2
p-value	<0.0001	<0.0001	<0.0001	<0.0001

Means in a column with the same letter are not significantly different ($P \leq 0.05$). NPP=number of pods per plant, NSP=number of seed per pod, SYD=seed yield in kg per hectare, HSW=hundred seed weight in gram.

Table 12. Combined mean values for yield and yield components of the tested faba bean varieties across locations.

location	NPP	SNP	SYD (kg/ha)	HSW (g)
Tocha	11.6[b]	3.2[a]	1772.7[a]	63.3[a]
Mari	12.3[a]	3.2[a]	834.5[c]	57.9[b]
Turi	12.2[ab]	3.3[a]	966.2[b]	57.0[b]
Mean	12.0	3.2	1191.0	59.4
SD(±)	2.8	0.5	451.8	18.5
CV (%)	9.4	13.4	4.55	3.2
p-value	<0.001	<0.0001	<0.0001	<0.0001

Means with in a column with the same letter are not significantly different (P < 0.05). NPP=number of pods plant^{-1}, NSP=number of seeds per pod, SYD=seed yield in kilogram per hectare, HSW=hundred seed weight in gram.

4.5. Relation of Chocolate Spot to Faba Bean Yield Components

There was variation between epidemiological measurements and yield and yield related components in their associations. But all three epidemiological measurements (final disease severity, area under the disease progress curves and infection rate) were significantely ($p < 0.05$) associated with grain yield at all test locations (Appendix Table 12). Chocolate spot severity was significantly associated ($p < 0.05$) at Tocha and Mari and negatively related to seed yield of faba bean starting from the first date until the last date of the assessment (98 DAP). Disease severity from 35 to 56 DAP at Tocha experimental site and from 35 to 98 DAP in Mari experimental site was negatively correlated and highly significant with seed yield ($p < 0.01$) having correlation values ranging from -0.592 to -0.361. But in Turi disease severity was negatively correlated and not significant ($p < 0.05$) except at 84 DAP with correlation value -0.335 (Table 13).

Table 13. Coefficient of correlation between seed yield and chocolate spot severity for assessment dates, 2011 growing season.

DAP	Tocha		Mari		Turi	
	r	P-value	*r*	P-value	*r*	P-value
35	-0.453	0.0026	-0.394	0.010	_	_
42	-0.592	< 0.0001	-0.524	0.000	-0.149	0.347
49	-0.505	0.0006	-0.418	0.006	-0.262	0.094
56	-0.491	0.001	-0.477	0.001	-0.261	0.095
63	-0.361	0.0189	-0.493	0.001	-0.295	0.058
70	-0.324	0.0364	-0.563	0.000	-0.245	0.118
77	-0.369	0.0163	-0.504	0.001	-0.268	0.086
84	-0.362	0.0185	-0.499	0.001	-0.335	0.030
91	-0.386	0.0116	-0.474	0.002	-0.166	0.293
98	-0.439	0.0037	-0.528	0.000	-0.153	0.333

DAP =days after planting; r =Coefficient of correlation

The seed weight correlation with disease severity followed a different trend in the experimental plots, with the exception of Tocha and Mari locations (Table 14).

Table 14. Coefficient of correlation between 100 seed weight and severity of chocolate spot for assessment dates, 2011 growing season.

DAP	Tocha		Mari		Turi	
	r	p-value	*r*	p-value	*r*	p-value
35	-0.353	0.02	-0.166	0.29	_	_
42	-0.262	0.09	-0.212	0.18	0.285	0.07
49	-0.454	0.001	-0.171	0.28	0.404	0.01
56	-0.502	0.001	-0.190	0.23	0.305	0.05
63	-0.527	0.001	-0.134	0.40	0.377	0.01
70	-0.577	<0.0001	-0.304	0.05	0.369	0.02
77	-0.536	0.001	-0.298	0.06	0.293	0.06
84	-0.502	0.001	-0.272	0.08	0.255	0.10
91	-0.519	0.001	-0.272	0.08	0.352	0.02
98	-0.512	0.001	-0.327	0.04	0.293	0.06

DAP =days after planting; r =Co-efficient of correlation

The linear regression of AUDPC better described the relationships between faba bean pod number and seed yield with disease severity in Tocha and Mari compared to severity values of each assessment dates . The relationship described by the model accounted 22.4 to 64.8% for pod number per plant and 21.7 - 60.8 % for seed yield of the variance. The estimated slope of the regression line obtained were -0.805 and -2.1298 , -0.6402 and -0.3267, and 1.0809 and -1.3279, for pod number per plant and seed yield (kg/ha) at Tocha, Mari , and Turi, respectively (Figure 11A and B).

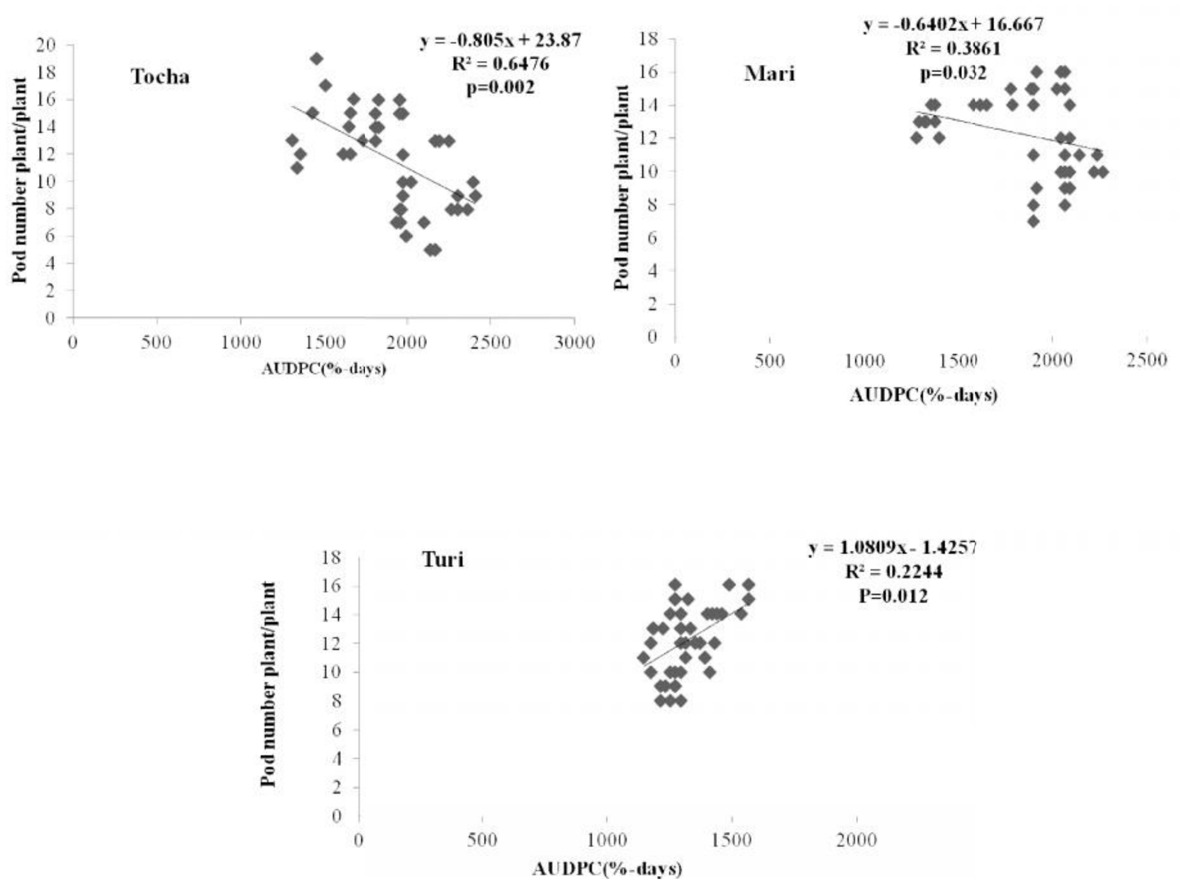


Figure 11A. Linear regression relating AUDPC with pod numbers per plant of faba bean under three locations, 2011cropping seasons.

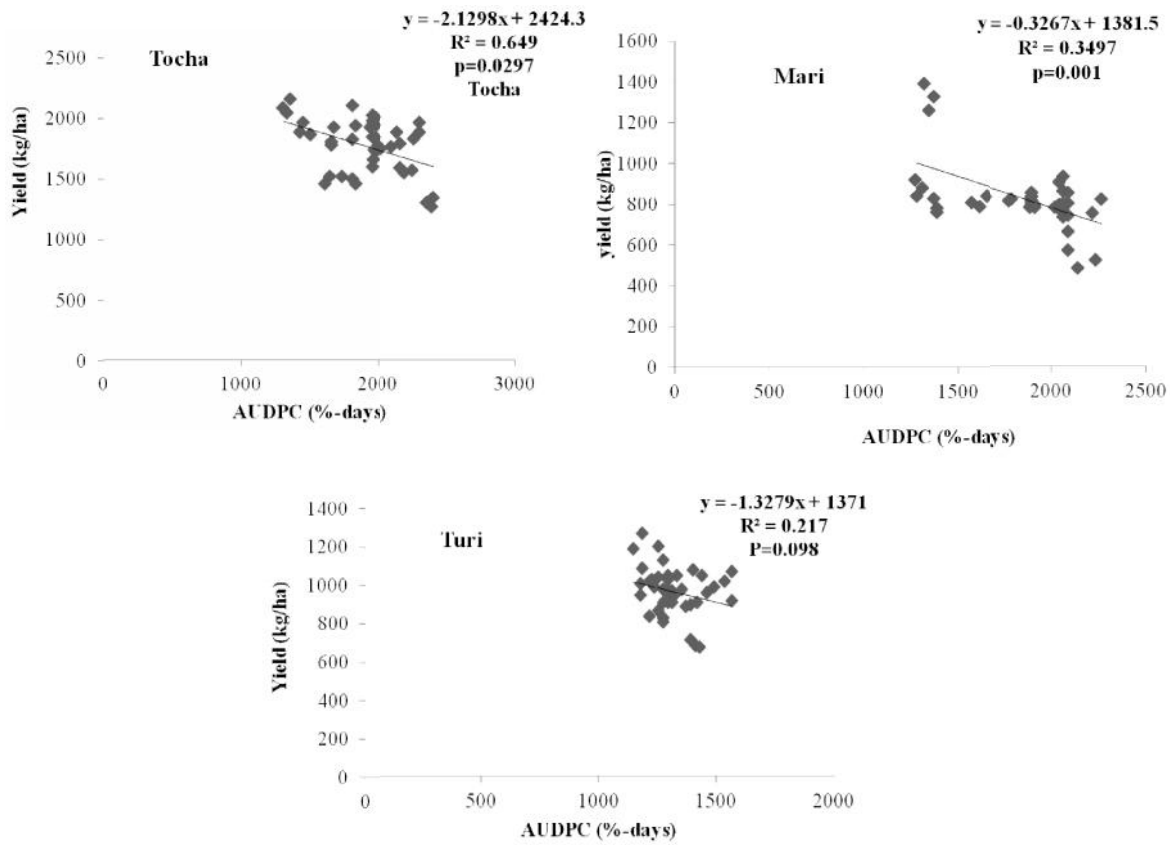


Figure 11B. Linear regression relating chocolate spot AUDPC with seed yield of faba bean under Tocha, Mari and Turi experimental sites, 2011cropping seasons.

4.6. Morphological and Cultural Characterization

All Botrytis isolates examined in this study, size of conidia had a maximum length of 11 to 14 *μm* and mean width of 7 to 10*μm* (mean 12.5 × 7.8 μm). No Significant differences were observed in the frequency of isolates with specific morphological or cultural traits in the high-altitude locations compared to the mid-altitude parts of the study area. But there was significant difference observed in the frequency of isolates among PAs. This was the case with colony color and colony growth rate. Where 48% of the isolates were grayish white, 27% gray and 25% white; and while 45% of isolates were fast-growing, 39% moderate growth rate, and 16 % had a slow growth rate compared among all isolates (Table 15).

Table 15. Morphological characteristics *B. fabae* isolates from faba bean fields in Dawuro zone, southwest Ethiopia (n=44).

PA	Colony color			Colony shape			Colony texture			Growth rate		
	W	GW	G	Un	Ir	Sr	Ap	Fcu	Flco	S	M	F
Bale	2	1	1	2	2	0	1	2	1	2	2	0
Dali	2	2	0	2	2	0	0	2	2	0	2	2
Sengeti	2	0	2	0	3	1	0	2	2	1	1	2
Waka	2	0	2	1	2	1	0	2	2	0	2	2
Mari	2	1	1	1	3	0	0	4	0	1	3	0
Gozoshasho	0	2	2	0	4	0	0	2	2	1	3	0
Medanalem	1	3	0	1	3	0	0	2	2	0	3	1
Botori	0	3	1	2	1	1	0	1	3	0	0	4
Kechi	0	4	0	1	3	0	1	1	2	2	0	2
Gessachare	0	3	1	2	2	0	0	2	2	0	0	4
Tulema	0	2	2	2	2	0	0	2	2	0	1	3
Total	11	21	12	14	27	3	2	22	20	7	17	20
	X^2=35.94,df=2,p<0.05			X^2=17.7,df=2,ns			X^2=14.5,df=2,ns			X^2=38.7,df=2,p<0.01		

PA=peasant association, W=white, GW=grayish white, G=gray, U= uniform, Ir=irregular,
Sr = Sectoring, AP = Appressed, Fcu =Flocculose, Flco= Floccose, S=Slow, M=medium, F=fast, X^2= *chisquare*, *df =degree of freedom*, *ns*=non- significant difference in frequency of isolates.

4.7. Aggressiveness of the *Botrytis fabae* isolates

In the greenhouse test, 12 (27%) isolates produced the highest leaf necrosis 87.04 - 92.2% of DSI and lowest 2.61 days of incubation period and grouped as more aggressive, while 28 (64 %) of the isolates induced leaf necrosis of 45. 7% to 62.4% and 1.5 to 1.78 incubation period belonged to moderately aggressive. The rest four 4 (9 %) isolates induced 1 to 12 % necrosis and longest 4 to 6.5 days of incubation period and grouped as less aggressive (Table 16). Esera and Mareka districts had the highest frequency of more aggressive isolates while isolates from Tocha and Loma districts were moderately aggressive without or weakly aggressive isolates. Mostly, isolates obtained from Bale, Waka and Mari had a higher frequency of more aggressive isolates than others. These three PAs accounts 75 % of more aggressive isolates.

Table 16. Aggressiveness of *B. fabae* isolates against faba bean under greenhouse conditions.

District	PA	Number of isolates with aggressiveness group		
		More	Moderate	Less
Essera	Bale	3	1	-
	Dali	2	1	1
	Sengetsi	-	2	2
Mareka	Waka	3	1	-
	Mari	3	1	-
	Gozoshasho	1	2	1
Tocha	Medhanalem	-	4	-
	Botori	-	4	-
	Kechi	-	4	-
Loma	Gessachere	-	4	-
	Tulema	-	4	-
	Total (%)	12 (27)	28 (64)	4(9)

PA = Peasant association, 2= Aggressive was determined using the1 – 3 rating scale of ICARDA (1986). Less = DS ≤ 3; Medium = 3 < DS < 7; more = DS ≥ 7

5. DISCUSSION

This study has provided information on the distribution and epidemics of chocolate spot on different faba bean varieties in production areas, and characteristics of *B. fabae* isolates of Dawuro zone, southwest Ethiopia. Chocolate spot is a damaging disease of faba bean throughout production areas; during 2011 growing season, it was found widely distributed in both surveyed faba bean fields and experimental plots with variations in their severity. The highest mean chocolate spot severity ranged from 45.8 to 69.4 % at Waka, Bale, Tulema, Mari, Medhanalem, Botori, Gessachere and Kechi and the lowest ranged from 33.4 to 44.2 % in Dali, Gozoshasho and Sengetsi peasant associations (PAs). Generally, disease severity was relatively higher above 2200 m.a.s.l by 6.4 – 34.6 % than mid altitudes (< 2200m) (Figure 2). In the 2011 cropping season, the weather conditions were more favorable for disease development at high altitude parts. These situations were attributed to the higher rainfall and lower temperature, where the rainfall is more than sufficient for crop growth, whereas at the mid-altitudes there is a scarcity of rainfall and relatively high temperature (Appendix Table 2). On average, the temperature ranged from 14.9 to 21.6 ^{0}C and 27 rainy days per month for these particular locations during the growing season. This suggests that moist and mild climate is more favorable for chocolate spot development and epidemics. This fact extensively studied by others, demonstrating that several cycles of infection may take place in a short period of time, particularly when mild temperature giving rise to extended necroses that can frequently cause defoliation and even death of whole plants (Assefa and Gorfu, 1985, Harrison, 1988; Villegas-Fernandez *et al.,* 2010; Fernandez-Aparicio *et al.*, 2011).

Farmers interviewed for their seed source, most farmers (86 %) were used local cultivar maintained from previous seasons, which favored the occurrence of chocolate spot. It has been reported by Entwistle (1990) that the inoculum levels will inevitably be within the crop, or debris from a previous crop in the vicinity. Also it had been observed that higher severity of infected plants with chocolate spot symptoms was recorded in densely populated fields than in sparsely populated fields. Although the data was not significantly different, it might be due to secondary infections in the fields. It has been reported that in a dense population of faba bean plants, the severity increases due to more plant to-plant spread of the *Botrytis fabae* inoculum. According to Khalil *et al.* (2011), plant density is affecting phonological development, source sink relationship and assimilates partitioning of faba bean. Dense plant density can cause less light penetration in the crop canopy, reduce photosynthetic efficiency and may lead to chocolate spot epiphytotics.

In the same way, chocolate spot severity was observed more in unweeded fields where there was competition for soil nutrients, spacing and moisture and as a result, the faba bean plants were weak and more prone to the disease due to reduction in plant vigor. Again outbreaks of chocolate spot are highly dependent on microclimate within crops, such as reduced temperature and increased moisture (wet) condition that favoring the disease development.

There was a strong association established between the severity of chocolate spot and PAs located at high altitude ranges, where farmers experienced continues faba bean production year after year. This continued growing may increases the inoculum level and could result in rapid intensification of chocolate spot. The inoculum may carry-over from one season to the next on infected faba bean seed, stubble and volunteer plants. Hawthorne (2004) mentioned that, continuous growing of faba bean leads to accumulation of the chocolate spot sclerotia in the soil that increases occurrence of chocolate spot. A similar association had been reported for faba bean cercospora leaf spot and other disease (Vereijssen *et al.,* 2006; Kimber, 2011). Therefore, if the level of initial inoculums is high and conditions are favorable for primary infection, disease may be severe even when few secondary cycles occur (Paul and Munkvold, 2005). Our experiments conducted in the field was also suited at previous fields of faba bean confirmed that chocolate spot infested from debris remaining on the soil surface as a predominant factor in the availability of *B. fabae* inoculum to infect faba bean planted in succeeding seasons.

Faba bean fields with fallow- cropped had lower chocolate spot severity. This might be due to the low inocula effect of the fallowed fields and the reduced source of inoculum during the fallow periods. Harrison (1988) reported the significant role of the amount and quality of inoculum delivered to the crop canopy as well as the time of arrival of inoculum in relation to the stage of the crop development on the increase of the disease.

In the field experiment, the epidemics of chocolate spot were successful as all plants were infected with occasional hot – spots simulating the action of a natural outbreak. Furthermore, Investigations of the effect of environmental conditions on the severity of chocolate spot also provided a greater understanding of the dynamics of disease. Differences in disease onset and development with earlier and higher disease spread at Tocha and Mari could be explained by lower daily average temperature associated with prolonged precipitation favorable to chocolate spot, leading to rapid expansion of lesions on infected faba bean leaves and premature defoliation. For instance, in August, where early chocolate symptoms observed, 26 days having rain presented with an average temperature below 20 ^{0}C. There was late appearance of the disease at Turi that related to unfavorable weather conditions particularly the absences of precipitation in mid July till early August. Vandenberg (2006); Villegas-Fernandez *et al.* (2009) and El-Sayed *et al.* (2011) mentioned that, chocolate spot infection and development is favored by mild and moist conditions. Dry conditions are known to hamper the initiation and development of chocolate spot epidemics since leaf wetness is needed for successful infection and disease establishment (Bretag and Raynes, 2004).

Based on their final disease severity indices (DSI), great difference was observed as different varieties planted at three locations, the higher percentage on local cultivar, the lowest on CS20DK rated at Tocha and Mari locations (Table 8). This indicate that the disease development and spread of the pathogen was higher on the plants sown at high altitude ranges, where the mean temperature of growing season was still low 17.4 ^{0}C, than on the plants sown at Turi, where the temperature increased up to 24 ^{0}C. This result supports our survey results in that why chocolate spot is favored at high altitude ranges rather than mid altitude. In warm conditions chocolate spot is typically important later in the season during flowering and after canopy closure (Table 13) where significant ($p < 0.05$) and negatively correlated results of DSI with seed yield at 83 DAP.

However, during longer periods of wet weather the fungus thrives and the leaf is killed (RHS, 2012). The most important damage usually occurs when plants are flowering as that is when the environmental conditions are often more conducive to disease development, decaying flower petals are available for growth of the fungus and yield reductions may be serious due to spread of the pathogen from the flowers into the developing pods (Stoddard *et al.,* 2010).

Harrison (1981) also suggested that, at any stage of plant growth, the rate of aggressive lesion progress was linear and proportional to the optimum temperature between 6 to 20 ^{0}C, and neither light intensity nor a film of free water affected lesion development. Tocha and Mari locations had extended rainfall and were favorable for chocolate spot development. Hanounik (1979) and Dereje *et al*. (1994) reported prolonged rainfall is conducive for chocolate spot development leading to complete crop loss.

The linearization of disease progress curve was essential to determine epidemic speed, to project future disease and to estimate initial disease. However, disease progress curves indicated that epidemics on tested varieties progressed slowly at early growth stage, and then increased rapidly mostly at Tocha and Mari 56 DAP onwards until late assessment date, after which epidemics slowed as fewer healthy plants remained to be infected. The chocolate spot progress curves for these two locations are generally attained typical sigmoid shape except for the moderately resistant plots of few varieties (Figure 5 and 6). At Turi, the chocolate spot growth curves did not attain the sigmoid shape due to the reduced disease development (Figure 7). The chocolate spot development revealed that growth curves vary depending on the resistance level of the variety and environmental factors. The difference in shape and magnitude of chocolate spot progress curves at Tocha, Mari and Turi indicate the effect of environmental factors on disease progress and final disease development as stated by Campbell and Madden (1990). Epidemics that progressed in this fashion are best described by a logistic (or similar) growth function (Campbell and Madden, 1990) and have been termed as compound interest or polycyclic diseases (Van der Plank, 1963).

In describing the rate of chocolate spot infection, Logistic model was superior to Gompertz model. The coefficient of determination (R^2) was higher for Logistic model consistently for all assessment dates except for the terminal chocolate spot assessment in which Gompertz model was slightly better than Logistic model. This indicates that chocolate spot infection rate is apparently related to the logarithm of the ratio of the amount of diseased and healthy tissues present as described by Campbell and Madden (1990). However, chocolate spot progress was not significant for the weekly assessed plots of the tested varieties apparently except for Degaga (0.118 disease units per day) at Tocha, CS20Dk, Degaga and Tesfa (0.110, 0.118 and 0.104 disease units per day at Mari, respectively) and CS20Dk and Degaga (0.118 disease units per day) at Turi where retarded infection rate compared with other varieties. The other varieties did not affect significantly the infection rate of chocolate spot within test locations (Table 6). Nevertheless, infected faba bean plants with in each plot were contributing the new infected plants (Van der Plank, 1963). To lessen the epidemics of that increase logistically, it is important to reduce the rate of plant to plant spread of the pathogen within the plot, thereby allowing more time for plants to set high number of fruiting nodes**.** El-Bramawy *et al.* (2010) recommended that, use of appropriate agricultural practices for agronomic characters improvement and for enhancing the resistance to foliar diseases, which could be used to minimize the harmful effects of fungal pathogens and to increase the grain production. The moderately resistant faba bean varieties, for instance CS20DK, Degaga and Tesfa, which reduced the apparent infection rate of chocolate spot in this study by 13 - 50% compared to other tested varieties, may also provided a substantial benefit in terms of faba bean yield.

Host resistance to chocolate spot pathogen is another important factor that could slow epidemic. The tested varieties were significantly different ($p < 0.01$) for their reaction to the disease without expressing the extreme reactions (Table 7). For instance Mesay, Tumsa, NC-58 and Walki which recorded moderately resistant (MR) at Tocha and Turi turned to be moderately susceptible (MS) under Mari conditions. Local cultivar, Gebelcho, Hachalu and Kuse both at Tocha and Mari, and Mesay, Moti, NC-58, Tumsa and Walki at Mari rated to be MS to chocolate spot having the highest degree of infection. Other faba bean varieties recorded as moderately resistant (MR) to chocolate spot infection at all locations. Neither highly resistant nor highly susceptible varieties were rated by the disease severity.

Area under disease progress curve (AUDPC) is a resistance parameter calculated from the percentages of the leaf area affected. Calculation of the AUDPC as a measure of quantitative disease resistance entails repeated disease assessments at different times during the epidemic. The analysis of variance for AUDPC revealed highly significant differences ($p < 0.01$) among the tested varieties. Five varieties such as Bulga-70, Cs-20-Dk, Degaga, Kasa and Tesfa were significantly less affected by chocolate spot even at Tocha and Mari where the chocolate spot infection was higher. Moreover, AUDPC values were greater in MS varieties than in MR varieties (Figure 9 and 10). This indicates MS varieties rated higher AUDPC values were not limited disease spread as effectively as did MR varieties (Figure 5 and 6) and also this may reflect the incompetent of those varieties to slow down the disease at different phases of its progression. In general, the higher the AUDPC, the more susceptible is the variety. The loss of active leaf area results in less photosynthetic available region during the flowering and or pod setting stage which eventually results in producing less productive pods per plant. This reduction may eventually contribute to the overall yield losses. The advantage of using AUDPC was that it was simple to calculate and uses multiple evaluations which are often based on assumptions about the distribution of the data points (Campbell and Madden, 1990).

The use of AUDPC values rather than a single score of severity at a specific stage may prove the importance under fluctuating climatic conditions influencing the development of the disease. In addition, the stage after natural infection of the disease may coincide with a different seasonal period under different environmental conditions. The variability observed in this experiment showed that faba bean varieties possess remarkable genetic diversity for chocolate spot resistance. Similarly, Abo-Hegazy *et al.* (2012) found that thirteen faba bean varieties varied in their reactions to *Botrytis fabae* infection between indoor and outdoor experiments. Moreover, similar results were obtained by ICARDA (2006) in the field conditions for performance of 17 Iraqi faba bean genotypes in relation to infection with chocolate spot and other foliar diseases. Therefore, the present study and those of others show that faba bean varieties vary in their resistance to chocolate spot disease. In a recent report, Gebrehiwot (2011) indicated that 38 faba bean genotypes differed significantly in their reactions to *Botrytis fabae* isolates studied in the laboratory.

There were also the highly significant differences ($P < 0.01$) of the variety × location interaction indicate that fluctuation of varieties in their responses to the different environments (Appenidex Table 10). There was tremendous change in yield and related traits of the varieties across locations. Regarding the mean number of pods per plant (NPP) (Table 11) shows big ranges over the three locations 5.7 to 17. Degaga (17) both at Tocha and Mari produced greatest number of pods /plant, followed by Nc-58 (15.6), and Walki (14.6) at Turi. Nine varieties namely Walki, Tesfa, Nc-58, Messay, Kuse, Kasa, Degaga, Cs-20Dk and Bulga-70 had high NPP than the average (12.02) at each location (Table 9). These varieties also showed highly significant differences ($P < 0.01$) in grain yield performance (Appendix Table 8). Based on the means obtained over the three locations, it is obvious that the seed yield (SYD) range 1486 (Degaga) – 845 kg/ha (local), and with respect to hundred seed weight (HSW), it was ranged from 34.3 - 92.5g with mean of 57.04g (Table 11).

The most commonly used varieties in the area were local cultivar which produced low yields of 845 kg/ha. Hence, the result clearly showed that high yielding varieties such as Degaga (1486), Cs-20Dk (1341kg/ha), Nc-58 (1297 kg/ha) and Moti (1234 kg/ha) could be best substitutes the local cultivar and other tested varieties and can be introduced in seed production and distribution. When locations were compared, the highest SYD (1772.7 kg/ha) was obtained at Tocha which is followed by Turi (966.2 kg/ha), while Mari (834.5 kg/ha) was poor yielding (Table 10). This is due to soil physico - chemical property differences at the experimental sites (Appendix Table 1B). According to Richardson and Horsham (2008) soil pH had shown a strong relationship with disease severity. Acidic soil (below pH 5.8) had significantly greater disease incidence caused by *B. fabae* than plants in basic soil (above pH 6.7). High value of HSW was recorded for the varieties Hachalu (92.5g), Moti (86.3g), Gebelcho (78.2g), Tumsa (74.8g) and Walki (69.4g) which had large seed size. Kuse had low (34.3g) HSW. This may be attributed to the long growing period of the crop at Tocha and Mari while lower rain shower especially late in the growing season at Turi (Appendix Table 2).

Correlations were found between all epidemiological measurements and yield and yield related traits used to quantify the effect of chocolate spot epidemics in the field (Appendix table 12). For this purpose, chocolate spot severity indices of the growing season and AUDPC values considered the simplest method. These represent the summation of host – environment interactions over the course of a growing season (Vander plank, 1984).

Chocolate spot severity was negatively related to seed yield of faba bean starting from the first date of disease assessment (35 DAP) until the last day of the assessment (98 DAP) except for Turi where no disease appeared at early time of assessments. Disease severity from 35 to 56 DAP at Tocha experimental site and from 35 to 98 DAP in Mari experimental site were negatively correlated and highly significant with seed yield ($p < 0.01$) having correlation values ranging from -0.592 to -0.361. But in Turi disease severity was negatively correlated and not significant ($p < 0.05$) except at 84 DAP with correlation value -0.335 (Table 13).

Also the AUDPC value showed varied negative correlation with pod number per plant and seed yield , at the Mari and Tocha experimental plots, and it is more in Mari (r = -0.847 to -0.593, 0.05) than in Tocha (r = -0.761, P $\leq$ 0.01) while at Turi negative and non significant correlation value was showed for seed yield (Figure 11A and B).

The linear regression of AUDPC better described the relationships between faba bean pod number per plant and seed yield with disease severity in Tocha and Mari compared to severity values of each assessment dates . The relationship described by the model accounted 22.4 to 64.8% for pod number per plant and 21.7 - 60.8 % for seed yield of the variance. The estimated slope of the regression line obtained were -0.805 and -2.1298, -0.6402 and -0.3267, and 1.0809 and -1.3279, for pod number per plant and seed yield at Tocha, Mari, and Turi, respectively. The estimates showed that for each unit increase in percent of chocolate spot AUDPC, there was a seed yield reduction 2.13, 0.33, and 1.32 kg/ha in Tocha, Mari and Turi, respectively (Figure 11B). This indicates that chocolate spot affected faba bean yield by flower abortion and plant damage. When compared to Tocha and Mari, chocolate spot epidemics were sufficiently delayed in Turi to allow the plants to set more productive pods. In fact, in Turi the mean seed weight was less for tested varieties than in other test locations, a reversal of the ranking of varieties for pod number per plant where chocolate spot was rated higher. These Turi data support the view that chocolate spot is mainly responsible for the yield loss at Tocha and Mari by early defoliation of flowers consequently low pod set. This kind of disease relation has also been seen in chocolate spot disease (Sahile *et al*., 2010) where Severity and AUDPC were inversely correlated with faba bean grain yield and some other foliar diseases such as leaf anthracnose (*Colletotrichum graminicola*) on alfalfa crop (Thomas *et al.* 1996), and spot blotch (*Bipolaris sorokiniana*) (Joshi *et al.* 2002).

Atotal of fourty four *Botrytis* isolates were isolated and identified from spotted leaves, pods, and stems and of faba bean plants, a result that was consistent with others reports (Mazen, 2004; Eisa *et al.,* 2006; Elwakil *et al.,* 2009). All were found to be *B. fabae* and none fitted the morphological description of *B. cinerea.* In pathogenicity tests, representative isolates caused typical chocolate spot symptoms and were re-isolated from infected leaves, indicating that *B. fabae* is the causal agent of chocolate spot in Dawuro faba bean production areas. This finding supports (Sahile *et al.,* 2012) the situation of the disease in Northern Ethiopia. Also there were significant differences in the frequency of isolates. This was the case with colony color and colony growth rate (Table 15), where 48 % of the isolates were grayish white, 27 % gray and 25 % white while 45 % of isolates were fast-growing, 39 % moderate growth rate, and 16 % had a slow growth rate compared among all isolates collected from faba bean fields. Similarly, Dereje (1996) reported that there was morphological variability among isolates of *B. fabae*. On the other hand, variations in the size of conidia from different isolates of *Botrytis* sp. were not uncommon (Mirzaei *et al*, 2007; Pandel *et al.*, 2010). Although colonies of *B. cinerea* and *B. fabae* on artificial media can be difficult to distinguish, possibly accounting for the apparent confusion between the species, the two species can always be distinguished by measuring their conidia (Harrison, 1988).

Also there was a wide variation of *B. fabae* isolates collected from Dawuro zone in their level of aggressiveness. The most virulent isolates, 27 % of the total, produced a greater leaf necrosis and with a shorter incubation period than less virulent isolate (Table 16). This indicates that more virulent isolate was able to invade and colonize host tissue more rapidly and resulted in more leaf damage. Similarly, Setti *et al*. (2009) and Muiru *et al*. (2010) reported that the most virulent isolates had a shorter incubation period with a larger lesion size and disease severity. Sakr *et al*. (2009) also showed that isolates having shorter latent periods and greater spore production are considered to be more virulent than isolates having longer latent periods and lesser spore production.

6. SUMMARY AND CONCLUSIONS

Faba bean is one of the major pulse crops produced in Ethiopia that serve as a multi-purpose crop in the socioeconomic life of farming communities mainly grown as a valuable source of protein and energy for both human food and animal feed . The yield of the crop is affected by fungal diseases like chocolate spot (*B. fabae*), cultural practices, their genetic make-up and the growing environmental conditions existing in the study area.

Chocolate spot is a damaging disease of faba bean throughout production areas and it was found widely distributed in both surveyed farmers fields and experimental plots of faba bean with variation in their severity. The mean chocolate spot severity ranged from 24.1% at Turi experimental plots to 69.4 % at Waka on farmers field. The higher mean chocolate spot severity frequently observed at high altitude ranges (6.4 – 34.6 %) than mid altitude ranges.

The association of independent variables such as peasant association, crop variety, planting time, altitude, and crop history was significantly associated with chocolate spot severity when entered into the logistic regression model as a single variable. However, when all variables entered last into the regression model, peasant association, altitude and crop history were the most important variable in its association with severity. Also it had been observed that higher severity of infected plants with chocolate spot in densely populated and unweeded fields than in sparsely populated and weeded fields.

The earliness of chocolate spot onset under field conditions could be explained by lower daily average temperature associated with prolonged precipitation at high altitude ranges (>2200m). At this suituations disease progresses up the canopy, with the spots rapidly expanding into large patches, which blight the leaves. Plants defoliate and lose flowers and pods. Flowers and pods may also develop lesions. Stems can become reddish-brown and weakened, aggressive development of stem infection late in the season can cause the crop to lodge. Whereas chocolate spot is likely to be yield limiting in later growth stages especially after canopy closure and after flowering in mid altitude ranges.

Disease progress curves indicated that epidemics on tested varieties progressed slowly at early growth stage, and then increased rapidly mostly at Tocha and Mari 56 DAP onwards until late assessment date, after which epidemics slowed as fewer healthy plants remained to be infected.

The tested varieties were significantly different for their reaction to chocolate spot without expressing the extreme reactions; they rated either moderate resistant (MR) or moderately susceptible (MS). It also showed the highly significant difference in terms of AUDPC. Moreover, AUDPC values were greater in MS than in MR varieties. This reflects the incompetent of MS varieties to slow down the disease at different phases of its progression. Five varieties such as Bulga-70, CS20Dk, Degaga, Kasa and Tesfa rated low AUDPC and less affected by chocolate spot and rated MR under all locations.These varieties reduced the apparent infection rate of chocolate spot by 13 - 50 % compared to others and also provided a substantial benefit in terms of faba bean yield.

Over all tested locations varieties such as CS20DK, Nc-58 and Moti were the top yielding varieties and best substitutes the exsisting local cultivar which can be preferable in cultivation and seed production.

Chocolate spot severity for all assessment dates was negatively related to seed yield of faba bean. But highly significant correlation were obtained 35 to 56 DAP at Tocha and for all assessment dates at Mari experimental sites having correlation values ranging from -0.592 to -0.361. But at Turi significant correlation was obtained later at 83 DAP with correlation value -0.335. Also the AUDPC value showed varied negative correlations at the Mari and Tocha experimental plots, and it is more in Mari (r =-0.847 & -0.593) than in Tocha (r = -0.761 & -0.555) for pod number per plant and seed yield, respectively. At Turi negative and non significant correlation value was showed only for seed yield.

The linear regression of AUDPC between faba bean pod number per plant and seed yield accounted 22.4 to 64.8% and 21.7 - 60.8 %, respectively. The estimated slope of the regression line obtained were -0.805 to 1.0809 for pod number per plant and -0.3267 to

1.3279 for seed yield. When compared to Tocha and Mari, chocolate spot epidemics were sufficiently delayed in Turi.

Based on morphological characteristics of conidia, all isolates examined were found to be *B. fabae* and none fitted the morphological description of *B. cinerea.* A significant difference was observed in the color and growth rate of the colony. There was also a wide variation among of *B. fabae* isolates in their level of aggressiveness and incubation period.

In conclusion, chocolate spot is important disease in faba bean production areas of southwest Ethiopia, and can influenced by cultural practices, growing situations and genetic makeup of the crop. However, field evaluations at existing agro-ecologies and disease tolerance of released varieties were best meets growers interest which helps for seed production and cultivation. The insight of this finding also help to design research objectives to overcome rejection of varieties developed by researchers alone, enhances the acceptance of varieties and reduces costs associated with variety development. In most cases improved varieties will perform better if accompanied by recommended cultural practices.

Even though, the tested varieties were not immune to the chocolate spot; but have much higher levels of tolerance than local cultivar. Therefore, an integrated approach is the key to successful management of chocolate spot in faba bean but attention to other management practices can reduce disease pressure and yield loss:

- Use the varieties with the highest level of tolerance to chocolate spot in respective production area like CS20DK, Degaga, Tesfa, Bulga-70 and Kasa.
- Farm hygiene and crop separation – preferably, the current crop should be sown more than 500 m from faba bean fields in the previous year. This will isolate it from sources of infection for fungal diseases. Volunteer faba bean plants appearing in late season can help to carry over diseases and should be eradicated.
- Seed should be sourced from the ‘cleanest’ crops. Old, frosted or damaged seed may have reduced germination and reduced vigor.
- Make faba bean part of a cropping system with cereals; crop rotation not more than 1 faba bean crop in 4 years.

- Fallow management - Soils should be well structured, clay loam or heavier in texture, and pH 5.2 to 8; treating the acidic soils with lime is also important.
- Intercropping with cereals to reducing chocolate spot severity by suppressive effects of a combination of host biomass reduction, altering microclimate and physical barriers to spore dispersal.
- Delayed sowing and wide row spacing to keep the canopy open longer- preferably for varieties having higher vegetative growth characters and wider canopy like Hachalu, Gebelcho, Walki, Tumsa, and Moti.
- Plan to harvest as early as possible to minimize disease infection on seed.
- Use of foliar fungicides like Chlorothalonil or mancozeb could be preferable as a foliar spray with added benefit of controlling other foliar diseases, when significant infection could be occurred at early growth stages. To achieve the cheapest fungicide management program, faba bean growers must also observe other aspects of the crop establishment package. That is, selecting a farm that is remote from where faba bean were grown in the previous year, both on their own and their neighbours' properties.

Critical issues for future research are tracking changes in population structure of key pathogens and also validation and up-scaling of proven integrated disease management strategies, working with different varieties released for the same agro-ecologies in Ethiopia. Climatic/season variability will affect the importance of diseases; therefore model-based decision support system research needs special attention. The current faba bean varieties are rated moderately resistant to moderately susceptible to chocolate spot and not immune. Therefore, development of new varieties that can be the substitute for them should be considered.

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8. APPENDIXES

Appendix Table 1A.Characteristic features of study areas in four districts of Dawuro zone.

District	PA[1]	Altitude (m.a.s.l)[2]	Latitude [2]	Longitude[2]
Essera	Bale	2407-2543	$7^0$62'151"N	$37^0$91'733"E
	Dali	2125-2132	$7^0$17'240"N	$37^0$20'365"E
	Sengetsi	1982-2106	$7^0$08'940"N	$37^0$03'551"E
Mareka	Waka	2304-2474	$7^0$05'140"N	$37^0$16'096"E
	Mari	2445-2489	$7^0$02'185"N	$37^0$05'870"E
	Gozoshasho	1805-1912	$7^0$05'528"N	$37^0$01'113"E
Tocha	Medhanalem	2666-2782	$7^0$05'528"N	$37^0$02'158"E
	Botori	2354-2411	$7^0$52'940"N	$37^0$06'421"E
	Kechi	1995 -2135	$7^0$06'147"N	$37^0$11'696"E
Loma	Gessachere	2015-2104	$7^0$12'055"N	$37^0$14'713"E
	Tulema	2243-2478	$7^0$17'940"N	$37^0$09'521"E

[1]PA=peasant association; [2] geographic locations =Measured using GPS

Appendix Table 1B. The results of soil analysis at the experimental sites.

Site	Altitude (m)	Latitude	Longitude	Depth cm	pH (1:1 H_2O)	EC m3	% C	% OM	% T.N	AvP ppm	Exch Acidity Meq/100g	Exchangeable bases				Texture			Textural class
												Ca	Mg	Na	K	% sand	% clay	% slit	
Tocha	2658	$7^0$05'528"N	$37^0$02'158"E	20	5.4	0.1	1.37	2.35	74.2	0.12	1.37	6.22	2.61	0.22	1.55	28	18	54	Slity loam
Mari	2444	$7^0$02'185"N	$37^0$05'870"E	20	5.1	0.06	0.78	1.34	52.8	0.07	0.64	7.38	3.78	0.28	1.05	36	22	42	loam
Turi	1805	$7^0$05'528"N	$37^0$01'113"E	20	5.7	0.06	1.13	1.95	46.2	0.1	0.88	6.28	3.67	0.89	1.09	24	26	50	Slity loam

*Soil samples collected from top 20cm depth & analyzed at Hawasa soil testing center.

Appendix Table 2. Monthly average minimum and maximum temperature (^{0}C) and total rainfall (mm) of the experimental period, 2011 growing season.

Month	[1]Tocha and Mari					[2]Turi				
	Air temperature (^{0}c)			TRF	RD	Air temperature (^{0}c)			TRF	RD
	Min.	Max.	Av.			Min.	Max.	Av.		
July	8.3	18.5	11.4	158	30	14	22.4	17.95	150	21
August	8.1	17.8	12. 5	279	28	14	23.9	19.8	218	18
September	15.5	21.5	16.5	174	30	15	23.4	19.3	104	21
October	17.2	22.3	17.75	108	27	18	24.2	20.2	109	16
November	19.1	24.8	20.5	129	24	19	25.6	22.4	72.8	17
December	21.2	24.9	21	116	21	22	24.7	23.4	106	12
Mean	14.9	21.6	17.4	160	27	17	24	20.5	127	18

TRF= monthly total rain fall (mm), RD = number of rainy days per month,
[1 and 2] Recorded at Loma[1] and Tercha meteorological stations.

Appendix Table 3. ANOVA table for the disease severity index (DSI) of each assessment dates under field conditions.

Location	Source	df	Disease Assessment days after planting									
			35	42	49	56	63	70	77	84	91	98
Tocha	Variety	13	17.9**	25.9**	34.5**	37.1**	27.7**	60.2**	72**	100.5**	128.4**	128.8**
	Block	2	0.8^{ns}	1.1^{ns}	7.6^{ns}	3.7^{ns}	0.2^{ns}	3.0^{ns}	4.5^{ns}	0.75^{ns}	0.5^{ns}	2.31^{ns}
	Residue	26	1.2	2.2	3.2	2.7	1.2	1.8	2.28	1.15	0.3	1.12
Mari	Variety	13	6.6*	9.4**	5.9*	8.6**	33.8**	57.6**	113.9**	153.2**	182.4**	189.9**
	Block	2	1.7^{ns}	0.6^{ns}	3.5^{ns}	2.2^{ns}	6.7^{ns}	6.7^{ns}	1.7^{ns}	0.3^{ns}	2.1^{ns}	0.21^{ns}
	Residue	26	1.9	1.2	1.9	1.9	4.1	3.12	2.3	1.3	2.4	1.15
Turi	Variety	13	0	0.9*	5.8**	7.4**	10.7**	12.9**	8.9**	6.7**	9.3**	6.2**
	Block	2	0	0.6^{ns}	3.9*	1.7^{ns}	2.9^{ns}	2.3^{ns}	1.3^{ns}	0.2^{ns}	0.7^{ns}	1.0^{ns}
	Residue	26	0	1.2	0.9	1.2	1.6	1.56	1.5	0.9	1.1	1.3

a Values are mean squares. * Significant at $P \leq 0.05$; ** significant at $P<0.01$; ns, not significant at 5%

Appendix Table 4. ANOVA table for AUDPC of fourteen faba bean varieties, 2011 cropping season.

Location	Source	DF	SS	MS	F Value	Pr > F
Tocha	Model	15	3590403	239360	114.59	<.0001
	Variety	13	3580186	275399	131.85	<.0001
	Block	2	10216	5108	2.45	0.1064
	Error	26	54308	2089		
	Total	41	3644711			
		R^2=0.99	CV=2.4			
Mari	Model	15	3682229	245482	54.39	<.0001
	Variety	13	3671312	282409	62.57	<.0001
	Block	2	10917	5458	1.21	0.3146
	Error	26	117345	4513		
	Total	41	3799574			
		R^2= 0.97	CV=3.65			
Turi	Model	15	423674.2	28244.9	22.94	<.0001
	Variety	13	417689	32129.9	26.09	<.0001
	Block	2	5985.2	2992.6	2.43	0.1078
	Error	26	32016.8	1231.4		
	Total	41	455691			
		R^2= 0.93	CV=2.66			

Appendix Table 5. ANOVA table for infection rate of fourteen faba bean varieties, 2011 cropping season.

Location	Source	DF	SS	MS	F Value	Pr > F
Tocha	Model	15	0.016	0.0011	3.13	0.0053
	Variety	13	0.0156	0.0012	3.52	0.0031
	Block	2	0.0004	0.0002	0.58	0.5661
	Error	26	0.0089	0.0003		
	Total	41	0.0249			
		R^2= 0.64	CV= 10.66			
Mari	Model	15	0.04829	0.00322	5	0.0002
	Variety	13	0.04772	0.00367	5.7	<.0001
	Block	2	0.00057	0.00028	0.44	0.6493
	Error	26	0.01673	0.00064		
	Total	41	0.06502			
		R^2= 0.74	CV=15.28			
Turi	Model	15	0.0021	0.00014	2.57	0.0168
	Variety	13	0.002	0.00016	2.84	0.0113
	Block	2	0.0001	0.00004	0.81	0.4568
	Error	26	0.0014	0.00006		
	Total	41	0.0035			
		R^2=0.60	CV= 5.8			

Appendix Table 6. ANOVA table for pod number per plant of fourteen faba bean varieties, 2011 cropping season.

Location	Source	DF	SS	MS	F Value	Pr > F
Tocha	Model	15	496.81	33.12	31.78	<0.0001
	Variety	13	483.9	37.22	35.72	<0.0001
	Block	2	12.9	6.45	6.19	0.0063
	Error	26	27.1	1.04		
	Total	41	523.9			
		R^2=0.95	CV=8.79			
Mari	Model	15	218.36	14.56	11.21	<0.0001
	Variety	13	215.45	16.57	12.76	<0.0001
	Block	2	2.9	1.45	1.12	0.342
	Error	26	33.76	1.3		
	Total	41	252.12			
		R^2= 0.87	CV=9.29			
Turi	Model	15	190.12	12.67	8.74	<0.0001
	Variety	13	186.5	14.35	9.89	<0.0001
	Block	2	3.62	1.81	1.25	0.3039
	Error	26	37.71	1.45		
	Total	41	227.83			
		R^2=0.83	CV=9.9			

Appendix Table 7. ANOVA table for seed number per pod of fourteen faba bean varieties, 2011 cropping season.

Location	Source	DF	SS	MS	F Value	Pr > F
Tocha	Model	15	4.6	0.308	1.6	0.1418
	Variety	13	4.3	0.33	1.71	0.1174
	Block	2	0.3	0.167	0.87	0.4321
	Error	26	5	0.192		
	Total	41	9.62			
		R^2= 0.48	CV=13.54			
Mari	Model	15	4	0.27	1.2	0.33
	Variety	13	3.8	0.29	1.32	0.26
	Block	2	0.2	0.1	0.43	0.66
	Error	26	5.8	0.22		
	Total	41	9.8			
		R^2= 0.41	CV=14.93			
Turi	Model	15	8.167	0.544	3.58	0.0021
	Variety	13	8.119	0.625	4.11	0.0011
	Block	2	0.048	0.024	0.16	0.8558
	Error	26	3.952	0.152		
	Total	41	12.119			
		R^2= 0.67	CV=11.95			

Appendix Table 8. ANOVA table for seed yield (kg/ha) of fourteen faba bean varieties, 2011 cropping season.

Location	Source	DF	SS	MS	F Value	Pr > F
Tocha	Model	15	1983892.3	132259	36.58	<0.0001
	Variety	13	1982126.8	152471	42.16	<0.0001
	Block	2	1765.5	883	0.24	0.7852
	Error	26	94017.9	3616		
	Total	41	2077910.1			
		R^2= 0.95.5	CV=3.4			
Mari	Model	15	1072940.5	71529.4	39.82	<0.0001
	Variety	13	1068907.1	82223.6	45.78	<0.0001
	Block	2	4033.3	2016.7	1.12	0.3406
	Error	26	46700	1796.2		
	Total	41	1119640.5			
		R^2= 0.96	CV=5.08			
Turi	Model	15	549946	36663.1	10.45	<0.0001
	Variety	13	545540	41964.6	11.97	<0.0001
	Block	2	4406	2203	0.63	0.5415
	Error	26	91183	3507.1		
	Total	41	641130			

Appendix Table 9. ANOVA table for hundred seed weight (g) of fourteen faba bean varieties, 2011 cropping season.

Location	Source	DF	SS	MS	F Value	Pr > F
Tocha	Model	15	15895.8	1059.7	195.12	<0.0001
	Variety	13	15867.7	1220.6	224.75	<0.0001
	Block	2	28	14	2.58	0.0949
	Error	26	141.2	5.4		
	Total	41	16037			
		R^2=0.99	CV=3.68			
Mari	Model	15	13264.9	884.3	212.12	<0.0001
	Variety	13	13241.2	1018.5	244.32	<0.0001
	Block	2	23.7	11.8	2.84	0.0766
	Error	26	108.4	4.17		
	Total	41	13373.3			
		R^2=0.99	CV=3.53			
Turi	Model	15	12602.6	840.2	823.7	<0.0001
	Variety	13	12538.9	964.5	945.62	<0.0001
	Block	2	63.6	31.8	31.2	<0.0001
	Error	26	26.5	1		
	Total	41	12629.1			
		R^2= 0.99	CV=1.77			

Appendix Table10. ANOVA table for combined yield and related parameters, 2011 cropping season.

NPP	Model	43	909.79	21.2	16.65	<0.0001
	VAR	13	684.63	52.7	41.5	<0.0001
	LOC	2	10.1	5.1	3.98	0.0224
	BLK	2	13.83	6.9	5.44	0.006
	VAR*LOC	26	201.22	7.74	6.09	<0.0001
	Error	82	104.17	1.27		
	Total	125	1013.97			
		R^2= 0.90	CV=9.4			
YLD (kg)	Model	43	25270348	587682	200.1	<0.0001
	VAR	13	2395025	184232.7	62.7	<0.0001
	LOC	2	21672517	10836259	3689.3	<0.0001
	BLK	2	1256.33	628	0.21	0.8079
	VAR *LOC	26	1201549	46213	15.7	<0.0001
	Error	82	240850	2937		
	Total	125	25511198			
		R^2=0.99	CV= 4.55			
HSW	Model	43	42716	993.4	283.4	<0.0001
	VAR	13	39809.2	3062.3	873.7	<0.0001
	LOC	2	963.8	481.9	137.5	<0.0001
	BLK	2	104.1	52	14.8	<0.0001
	VAR*LOC	26	1838.6	70.71	20.2	<0.0001
	Error	82	287	3.5		
	Total	125	43003			
		R^2= 0.99	CV=3.2			

Appendix Table 11. ANOVA table for Mean severity index of chocolate spot at eleven peasant asociations (PA) of farmers' field, 2011 cropping season.

Source	DF	SS	MS	F Value	Pr > F
Model	10	2705	271	8.43	<0.0001
PA	10	2705	271	8.43	<0.0001
Error	33	1059	32.1		
Total	43	3764			
	R^2=0.72	**CV=10.8**			

Appendix Table 12. Pearson correlation coefficients between epidemiological measurements and yield and yield components at three locations, 2011 growing season.

Location	Parameter	AUDPC	IR	NPP	NSP	HSW	YLD
Tocha	DSI	0.87**	0.37*	-0.51**	-0.01	0.23	-0.44**
	AUDPC		0.28	-0.46**	0.05	0.32	-0.26
	IR			-0.22	0.01	0.2	0.18
	NPP				0.03	-0.55	0.09
	NSP					0.22	0.23
	HSW						0.44**
Mari	DSI	0.97**	0.82**	-0.33*	-0.17	0.28	-0.53**
	AUDPC		0.78**	-0.29	-0.13	0.28	-0.55**
	IR			-0.23	-0.29	0.36*	-0.35*
	NPP				-0.04	-0.50**	0.22
	NSP					0.16	-0.45**
	HSW						0.15
Turi	DSI	0.61**	0.64**	0.29	-0.04	-0.03	-0.15
	AUDPC		0.24	0.46*	0.03	-0.45	-0.26
	IR			0.11	-0.04	-0.03	-0.03
	NPP				-0.36*	-0.42**	0.24
	NSP					0.08	-0.28
	HSW						0.05

* Significant at $P \leq 0.05$; ** highly significant at $P<0.01$

DSI=Disease severity index at last assessment date; AUDPC=Area under disease progress curve for growing season; IR=chocolate spot infection rate; NPP=Number of pods per plant; NSP=Number of seeds per plant; HSW=Hundred seed weight; SYD=Seed yield

Apendex plate1. Isolation and identification of *Botrytis fabae* isolates.

Appendix plate 2. Pathogenicity of *Botrytis fabae* isolates on var. CS20DK under green house conditions.