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Severity of Chocolate Spot Disease on Faba Bean (Vicia faba L) and Characterization of Botrytis fabae Isolates in Southwest Ethiopia

Mesele Haile
Wondogenet Research Center, Ethiopian institute of Agricultural Research , P.O.Box 2003, Addis Ababa, Ethiopia

Abstract
Chocolate spot caused by the fungus Botytis fabae Sard. is the major disease threatening faba bean (Vicia faba L.) production in Ethiopia. However, the intensity and importance of this disease is not well studied in southwest Ethiopia. The present study was conducted to determine the severity and to characterize B. fabae isolates in major agro-ecologies of South west Ethiopia. A total of 44 faba bean fields were surveyed in 11peasant associations (PAs) of south west Ethiopia 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% to 69.4 % in 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 (>2200m.a.s.l). 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 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.
Keywords: Faba bean, chocolate spot, *Botrytis fabae,* severity, aggressiveness

1. Introduction

In Ethiopia, faba bean (*Vicia faba* L.) is grown in the highland and mid highland areas with an altitude ranging from 1800 to 3000 m.a.s.l. 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 because of many biotic and abiotic constraints (Agegnehu *et al.*, 2006; Sahile *et al*., 2008b).

Among biotic constraints, chocolate spot disease is one of the most threatening factors for the production of faba bean (Stoddard *et al.,* 2010). *Botrytis 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).

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).

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).

The producers are lack of information about the disease intensity and the fungus population in the study area. The objectives of this study therefore, to determine severity of chocolate spot and its association with cultural practices, and also to characterize isolates of *Botrytis fabae* collected in major agro-ecologies of faba bean production areas in southwest Ethiopia.

1.1 Description of the Study Areas

Field surveys were conducted in major areas of faba bean production areas of eleven peasant associations (PA) in southwest Ethiopia (Bale, Dali, Sengetsi, Waka, Mari, Gozoshasho, Medhanalem, Botori, Kechi, Gessachare and Tulema) during 2015 main growing season (Figure 1). These focused administrative units were selected based on their faba bean production and agro-ecology diversity. 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). From each PAs four faba bean fields, a total of 44 fields were surveyed from September 15 to October 5, 2015. 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).

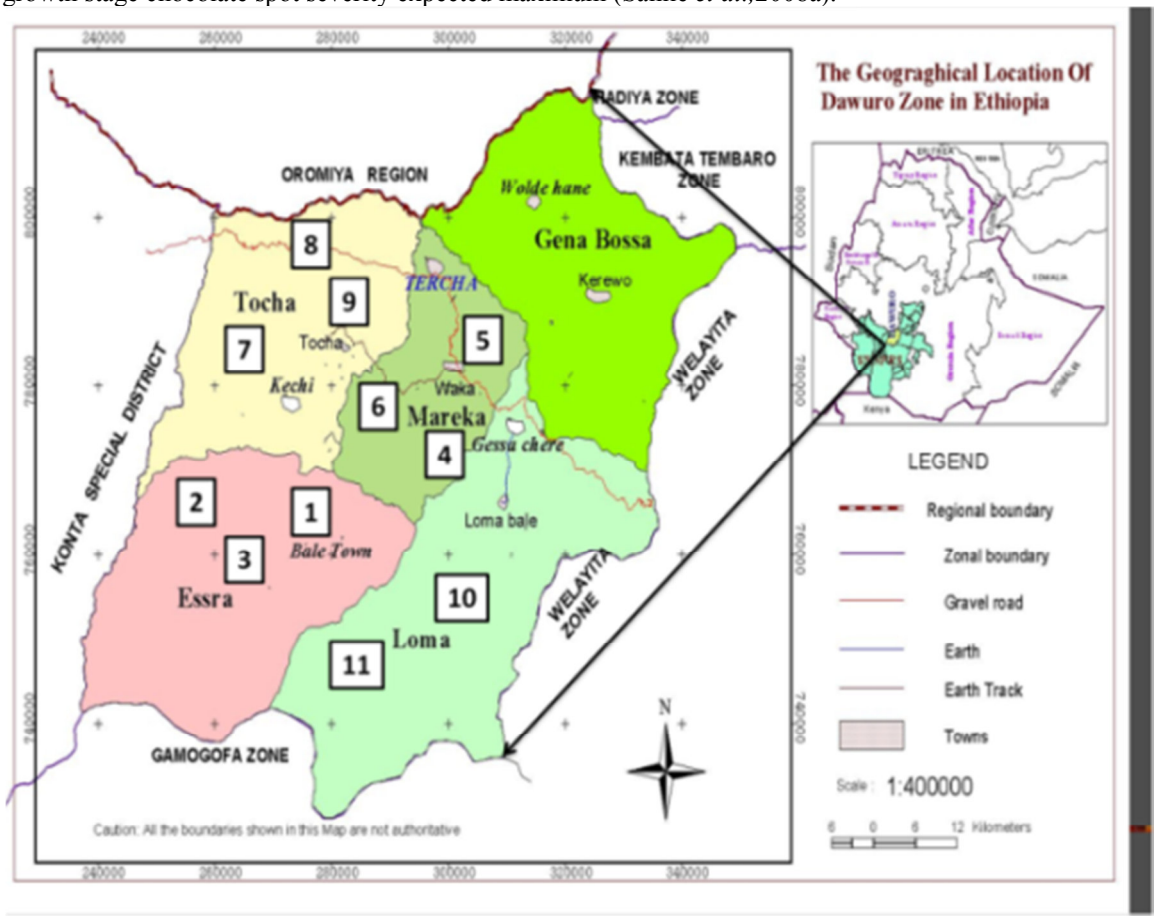


Figure 1. **The map of the study area in southwest Ethiopia and the sampling sites**
Note: 1=Bale, 2=Dali, 3=Sengetsi, 4 = Gozoshasho, 5=Waka, 6=Mari, 7=Kechi,
8= Medanalem, 9=Botori, 10=Gessachare and 11=Tulema

2. Materials and Methods

2.1 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; 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.

2.2 Disease Sample Collections

Representative samples of leaves showing symptoms of chocolate spot were collected from all the surveyed farms. 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 and kept at 4 ^{0}C.

2.3 Studies in the Laboratory

2.3.1 Media Preparation

Botrytis fabae pathogen 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).

2.3.2 Isolation of the pathogen

Samples of naturally infected faba bean leaves with symptoms of chocolate spot disease were cut into small pieces, each with a single lesion. 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.

2.3.3 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 and identification keys (Marthin and Panela, 1985).

2.3.4. 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).

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).

2.3.5 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 × l0^5 spores /ml) was determined using haeymacytometer from sporulating media. After the spore concentration was standardized, inoculation test were carried out.

2.4 Study in green house

2.4.1 Aggressiveness of B. fabae isolates

Seeds from tolerant variety CS20DK 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 per isolate). Plants sprayed with distilled water were used as a control. The inoculated and uninoculated plants were maintained under greenhouse conditions.

2.4.2 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).

2.5 Re-Isolation of the pathogen

The pathogen was re-isolated 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.

2.6. Data Analysis

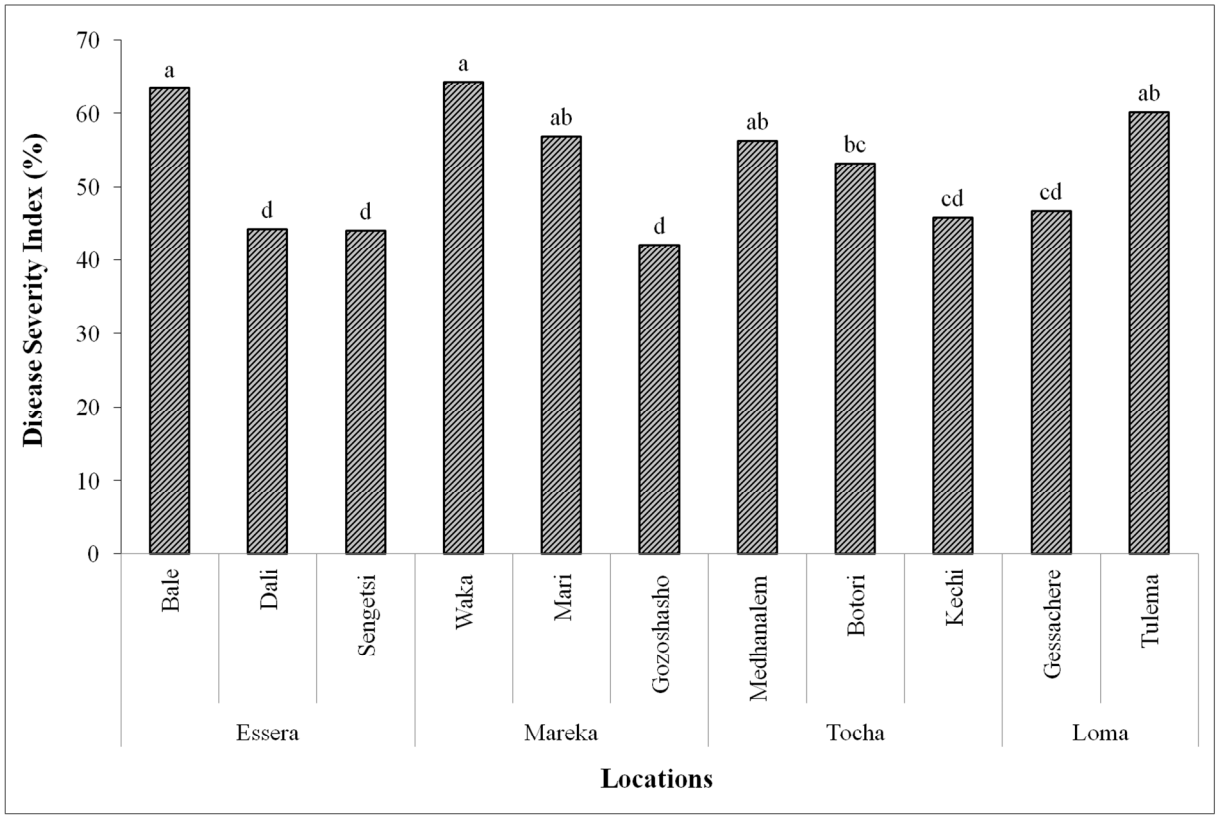
For disease survey, primarily descriptive **statistics** was performed on data collected from each field. In addition, analysis was conducted to describe the distribution and association of chocolate spot severity in relation to independent variables. Contingency X^2 tests were used to compare the frequencies 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 ≤ 0.05.

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. 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 (SAS Institute, 2008). Pearson's correlation coefficients were calculated to test the strength of relationships among the parameters. Chi Square for independence of frequencies in a variable was used to analyze the collected data for laboratory and greenhouse experiments.

3. Results

3.1 Severity of Chocolate Spot

The survey results indicated that, the total fields surveyed was 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, 2015 cropping season.

Table 1. Monthly average minimum and maximum temperature (^{0}C) and total rainfall (mm) of the experimental period, 2015 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

There was significant difference ($p < 0.05$) in the mean disease severity among the peasant associations. Among all PAs, the highest disease severity was observed at Waka and Bale with 64.4 and 63.3 %, respectively (Table 2).

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 2). It appeared that the status of weed infestation had an influence on chocolate spot severity (54.9 %) was recorded in not weeded fields.

Table 2. Mean severity index of faba bean chocolate spot for different independent variables in 2015 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)

3.2. Association of Faba Bean Chocolate Spot with Independent Variables

The association of variables is presented in Table 3. 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.

The results for analysis of deviation for variable and variable class are presented in Table 4. 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 4 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 3. 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.*

Table 4. 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]		Variable class	Estimate [c]	SE	Odds ratio [d]
			DC	PR>x^2				
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.*

3.3 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 5).

Table 5. Morphological characteristics *B. fabae* isolates from faba bean fields in Dawuro zone, southwest Ethiopia (n=44).

	Colony color			Colony shape			Colony texture			Growth rate		
PA	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.

3.4 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 6). 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 6. 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

4. Discussion

This study has provided information on the chocolate spot severity and *B. fabae* isolates characteristics in faba

bean production areas of southwestern Ethiopia. Chocolate spot is a damaging disease of faba bean throughout production areas; during 2015 growing season, it was found widely distributed in both surveyed faba bean fields 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). In the 2015 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. 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 (Table 1). 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).

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.

A total of forty four *Botrytis* isolates were isolated and identified from spotted leaves 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 also the causal agent of chocolate spot in southwestern Ethiopia. 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 south western Ethiopia 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 5). 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.

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.

5. Conclusion and Recommendation

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 released varieties in the study area 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 such as 1) Advice farmers to use the improved faba bean varieties with the highest level of tolerance to chocolate spot in respective production area like CS20DK, Degaga, Tesfa, Bulga-70 and Kasa. 2) 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. 3) Seed should be sourced from the 'cleanest' crops. Old, frosted or damaged seed may have reduced germination and reduced vigor. 4) Make faba bean part of a cropping system with cereals; crop rotation not more than one faba bean cropping in four years.

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