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Epidemiology of chocolate spot (*Botrytis fabae* Sard.) on faba bean (*Vicia faba* L.) in the Highlands of Bale, Sinana district, Southeastern Ethiopia

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Field experiment was conducted at Sinana Agricultural Research Center in the 2014 main cropping season with the objectives of quantifying the severity of chocolate spot and to assess the effects of the chocolate spot epidemics on faba bean varieties. The treatments included four faba bean varieties (Local, Shalo, Mosisa and Walki) with four fungicide spray schedules (no spray, spray every 7 days, every 14 days and every 21 days) of the contact fungicide Mancozeb at the rate of 2.5 kg ha^{-}.^{1}The treatments were arranged in 4 x 4 factorial combinations in randomized complete block design (RCBD) with three replications. Interaction of varieties with fungicides showed significant (P≤0.05) difference in reducing chocolate spot percent severity index. The terminal chocolate spot severity did not exceed 61 % on the susceptible variety (local).The percent severity index of 54, 46 and 44% were recorded on the varities Walki, Shalo and Mosisa, respectively. The maximum AUDPC was calculated on the unsprayed plots of local variety and Walki, which were 1817 %-days and 1716.42 %- days respectively. The chocolate spot was increasing at a rate of 0.03 units per day in unsprayed plots of the local variety and at 0.019 units per day on the variety Walki. There was a possibility of chocolate spot disease management by short spray intervals using Mancozeb fungicide combined with moderately resistant cultivars under southeastern conditions of Ethiopia.

Key words: AUDPC, *Botrytis fabae*, disease progress curve, disease severity index, faba bean fungicide

INTRODUCTION

Faba bean is botanically known as *Vicia faba* L.; with the common names including broad bean, horse bean, tic bean and field bean. It is one of the earliest domesticated food legumes in the world, probably in the late Neolithic period (Metayer, 2004). In Ethiopia, faba bean is grown in the highlands (1800-3000 m.a.s.l) where the need for cold temperature is met (Yohannes, 2000). The crop occupies the largest area in Ethiopia among other pulses. Currently, the total area under cultivation is estimated to be about 521,000 ha from which 6,886,670

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quintals are produced (MoARD, 2008). The crop is grown in several regions of the country receiving annual rainfall of 700-1000 mm (ICARDA, 2006). Amhara and Oromia are the two major pulse-producing regions in Ethiopia. The Amhara Region has the largest pulse area (43.7%) and contributes to the highest production (47%) in the country followed by Oromia Region that has 38% of the area and contributes 39% to national production (CSA, 2007). Even though Ethiopia is the world's second largest producer of faba bean next to China, its share is only 6.96 % of world production and 40.5 % within Africa (Chopra *et al*., 1989). In Ethiopia, the average yield of faba bean under small-holder farmers is not more than 1.8 t ha^{-1} (CSA, 2014), despite the availability of high yielding varieties (> 2 t/ha) (MoA, 2011). The low productivity of the crop is attributed to susceptibility to biotic and abiotic stresses (Sahile *et al*., 2008 and Mussa *et al*., 2008). Of the biotic category, diseases are important factors limiting the production of food-legume crops as a whole and faba bean specifically in Ethiopia (Nigussie *et al*., 2008 and Berhanu *et al*., 2003). The most important yield limiting diseases are chocolate spot (*Botrytis fabae*), rust (*Uromyces vicia-fabae*), black rot (*Fusarium solani*), Parasitic weeds (*Orobanche* and *Phelipanche spp*) and Aschochyta blight (*Ascochyta fabae*) (Mussa *et al*., 2008; Ahmed *et al*., 2010). In recent years, in additional to the previous common diseases, the crop is threatening by new gall forming disease (*Olpidium spp*) with typical symptoms of green and sunken on the upper side of the leaf and bulged to the back side of the leaf, and finally develops light brownish color lesion, chlorotic galls and progressively broaden to become circular or elliptical uneven spots (Hailu *et al*., 2014).

Botrytis fabae is believed to be the only causal agent of chocolate spot in Ethiopia (Sahile, 2008). The disease affects the foliage, limits photosynthetic activity, and reduces faba bean production globally (Torres *et al*., 2004). In the Maghreb region (Libya, Tunisia, Algeria, Morocco), yield losses due to chocolate spot diseases can reach 60–80 % on susceptible cultivars (Bouhassan *et al*., 2004). Chocolate spot is a major limiting factor in the main faba bean growing regions of Ethiopia, and yield losses vary from 34.1 to 61.2% (Dereje and Yaynu, 2001). According to a survey conducted, this disease was prevalent in all the faba bean growing areas with a range of 1950-2400 m.a.s.l, including Sinana (Dereje *et al*., 1988). Overall yield loss of 45% on the local and 42.4% on *Shallo* (SARC, 2004). Therefore, the objective of this research was to quantifying the severity of chocolate spot and to assess the effects of the chocolate spot epidemics on faba bean varieties.

Table 1: Backgrounds of faba bean varities used in the experiment

Variety name	Year released	Breeder/Maintainer	Days to maturity	Yield (t ha^{-1})
Local	NA	NA	115	1.8
Shalo	1999	SARC/OARI	118	3.7
Mosisa	2012	SARC/OARI	151	4.8
Walki	2008	HARC/EIAR	146	5.2

NA= not available

MATERIAL AND METHODS

Description of Experimental Site

The experiment was conducted at research field of SARC. The location represents the high lands of major faba bean production area of Bale with high rainfall and is expected to be the suitable environment (hot spot) for the disease. Bale is characterized by bimodal pattern annual rain fall. The first rainy season occurs from March to June and the second from August to December. The two seasons are locally termed after the time of crop harvest as *Ganna (Belg) and Bona (Meher),* respectively. SARC is located at $7^{o}7'$ N (latitude) and $40^{o}10'$ E (longitude) at about 2400 meters above sea level (m.a.s.l) and receives 750 – 1000 mm mean annual rain fall and a mean annual temperature of 9 – 21 ºC (Nefo *et al*., 2008). The dominant soil type is pellic vertisol and slightly acidic.

Experimental Materials and Treatments

The experiment was conducted using four faba bean varieties (Local, Shalo, Mosisa and Walki). Based on their average disease score for the last three years conducted by SARC, Local variety was susceptible and Shallo was moderately susceptible, Mosisa and Walkias moderately resistant to chocolate spot. Planting was done on August 13, 2014. Different levels of chocolate spot epidemics were created by application of Mancozeb 80% WP at different time intervals. The fungicide was applied at a rate of 2.5 a.i kg/ha in three different spray schedules viz., every 7, 14 and 21 days and unsprayed plot was included. The seven day spray treatment started on the first day of chocolate spot symptom appearances. The 14 and 21 day interval spray treatments started at two and three weeks after onset of disease, respectively. Spraying then continue at the specified intervals until the crop attained its physiological maturity. Unsprayed plots were included for each variety to allow maximum chocolate spot development for comparison of the effect of disease levels on different parameters. During fungicide sprays, plastic sheet was used to separate the plot being sprayed from the adjacent plots and to prevent inter-plot interference due to spray drift.

Experimental Design and Field Plots

The experiment was laid out using randomized complete block design (RCBD) in factorial arrangement with three replications. There were a total of 16 treatments; which contained the combination of four faba bean varieties with four levels of fungicide spraying interval (including unsprayed checks). The plot size was 3m x 2.4m (7.2 m^2) having 6 seeding rows with 4 harvestable central rows while spacing of 1m, 0.5m and 0.4m, respectively were applied between block, plots and rows.

Data Collection

Disease severity assessment

Chocolate spot severity was assessed on 20 randomly selected and pre-tagged plants per plot at weekly interval from the time disease first appeared until the crop attained its physiological maturity. The average severity from the 20 plants per plot was used for analysis.

Disease severity on leaves was rated using 1-9 rating scale (Bernier *et al*.,1993),where 1= no disease symptoms or very small specks; 3= few small discrete lesions; 5= some coalesced lesions with some defoliation; 7= large coalesced sporulating lesions, 50% defoliation and some dead plant; and 9= Extensive lesions on leaves, stems and pods, severe defoliation, heavy sporulation, stem girdling, blackening and death of more than 80% of plants and according to Bernier *et al*.(1984), the disease severity scores was converted in to Percentage severity index (PSI) for analysis using the following formula.

$$\text{PSI} = \frac{\text{Snr}}{\text{Nps x Msc}} \text{ X } 100$$

Where:
Snr = sum of numerical ratings
Nps = number plants scored
Msc = maximum score on the scale

Weather parameters

Daily temperature (minimum and maximum) and rainfall (number of rainy day with in growing season) and, relative humidity were obtained from SARC Weather Station (Table 2).

Yield and its components

Plant heights, pods per plant, seeds per pod, nods bearing pods and biomass on 20 randomly taken pre-tagged plants in each plot were recorded. The 100 seed-weight of randomly taken seeds from each plot at 10% moisture content and the yield of faba bean were recorded. Data on days to 50% flowering, and days physiological maturities were also recorded from each plot.

Data Analysis

Independent variables for field experiment data under different treatments was analyzed using logistic model, ln[y/ (1-y)] (Campbell and Madden, 1990) with the SAS Procedure (SAS Institute, 1998). The slop of the regression line estimated the disease progress rate in different treatments. ANOVA was performed for disease severity index (Wheeler, 1969), AUDPC (Campbell and Madden, 1990), and rate of disease progress (r) were analyzed. Mean separation was made based on the LSD at the 5% probability level. AUDPC values were calculated for each plot using the following formula (Campbell and Madden 1990).

$$\text{AUDPC} = \sum_{i-1}^{n-1} 0.5(x_{i+1} + x_i)(t_{i+1} - t_i)$$

Where, X_i= the PSI of disease at the i^{th} assesment
t_i= is the time of the i^{th} assessment in days from the first assessment date
n= total number of disease assessments
Logistic, [ln [(Y/1-Y)], (Vander Plank 1963) models was used for estimation of disease parameters from each treatment. The goodness of fit of the models was tested using coefficient of determination (R^2). These parameters were used in analysis of variance to compare the disease progress among the treatments. .

RESULTS AND DISCUSSION

Chocolate Spot Epidemics (Development)

Disease progress curves

Chocolate spot was first seen in Sinana 76 days after planting (DAP) on October 28, 2014 and then increased through time that was recorded on the experimental plot on all varieties at the flowering stage of the crop. However, significant variation in the disease severity was between 7-days spray interval plots and the unsprayed plots started from 83 DAP on all varieties (Figure 1, 2, 3 and 4). At the time disease on set, chocolate spot was not significantly different among the varieties. The symptom appeared on all varieties at the same time. Similarly the work of Bouhassan *et al*.(2004) reports that the disease progress profiles of the resistant and susceptible checks were similar up to the 3rd time of scoring. Although slight difference in disease onset might have passed during inspection before the disease was first observed. The experiments revealed that growth curves were almost similar for all during cropping season and moderately resistant though moderately susceptible show less significant differences.

Percentage severity index

The two-way interaction effects of fungicide treatment by varieties showed significant difference (*P≤0.05*) in percent severity index of chocolate spot starting from 104 DAP (Table 3). The highest mean disease severity index at (104 DAP) was 45.18% recorded on unsprayed local variety. At the last date of assessment (118DAP) up to 61% chocolate spot severity was recorded on unsprayed plot of susceptible local variety, and reduced to 11.11% by weekly fungicide application. However, higher final

Table 2: Number of rainy days, total rain fall, relative humidity percent and maximum and minimum temperature at Sinana, from July – December 2014 during faba bean growing period.

Month	Number of rainy days	Total rain fall (mm)	Relative humidity (%)	Daily temperature Maximum (^{0}C)	Daily temperature Minimum (^{0}C)
July	10	81	81.5	20.9	12.06
August	18	212.5	80.6	20.88	11.73
September	22	165.5	85.6	19.85	11.78
October	23	110	86.5	18.9	11.5
November	15	58	84	19.3	10.9
December	1	0.5	71.03	20.43	9.7
Mean	**14.83**	**104.58**	**81.53**	**20.04**	**11.27**

disease severity index (54%) was recorded on moderately resistant variety Walki as compared to the moderately susceptible variety Shalo (Table3). The reason for this might be due to resistance of the varieties loosed with time or it could be due to varying levels of virulence of pathogen, and this may indicate the appearance of new races of *B. fabae* in the area.

Mean chocolate spot severity index up to 47% and 44% were recorded on unsprayed plots of the variety Shalo and Mosisa at 118 DAP respectively (Table3).This finding was similar with Sahile *et al*. (2008c) who reported that mean disease severity index at 107 DAP ranged from 33.9 to 38.9% in sprayed plots in comparison with 47.9% in unsprayed plots, and from 25 to 46.6% in sprayed plots, in comparison with 56.7% in unsprayed plots.
Similarly the work of El-Sayed *et al* .(2011) reported that on Variety Giza 3 Mohassen and Sakha1 severity of infection at the fifth score were 43.67% and 46.670%.Under naturally infected plots, were 39% and 35.33%, while under protected plots, were 2.0% and 1.443%, respectively.

At 104 DAP, there were significant differences ($P \leq 0.05$) in percent severity index among fungicides, varieties and their interaction (Table3). On plot sprayed weekly interval; severity of chocolate spot was reduced to trace level. The weekly fungicide for all faba bean varieties reduced the severity of chocolate spot to the minimum level (11.11%) starting the fifth scoring dates (104DAP).
On the other hand spraying at 21 days interval has markedly reduced chocolate spot severity compared to unsprayed treatment, even though the effect was not statistically significant in any of the varieties. This was due to the lower severity of chocolate spot in Sinana during 2014 main cropping season as compared to the other season. The experiment indicates that higher frequency of fungicide application was needed on susceptible varieties, particularly when the conditions are favorable for the disease development. If the weather condition was not favorable only 2 or 3 times spray was also enough whether the varieties are susceptible or moderately resistant.

The chocolate spot progress curves attained typical sigmoid shape for all spray intervals except for the weekly sprayed plots of each variety (Figure 1, 2, 3 and 4). Such disease progress curves are characteristics of polycyclic diseases (Vander Plank, 1963). The experiments revealed that growth curves almost similar for all during cropping season and moderately resistant and moderately susceptible show less significant differences. This study showed that different faba bean varieties and Mancozeb spray intervals influenced chocolate spot epidemics, slowing the disease progress rate and increasing faba bean grain yield (Sahile *et al.,* 2008a).

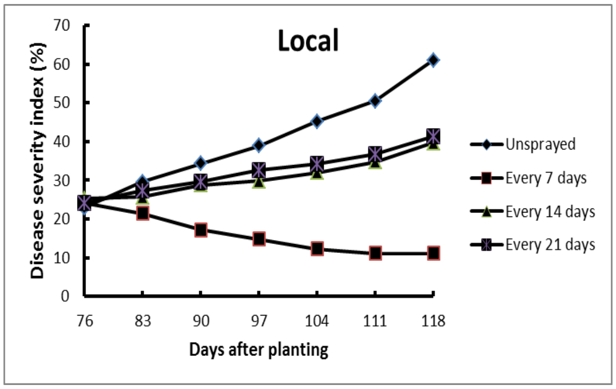

Figure 1: Chocolate spot progress curve as affected by different spray interval on the faba bean varieties Local at Sinana, in 2014 main season (chocolate spot was assessed every seven days starting from 76 DAP).

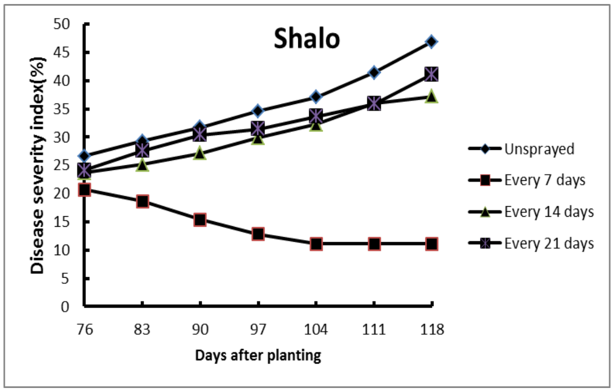

Figure 2: Chocolate spot progress curve as affected by different spray interval on faba bean varieties Shalo at Sinana in 2014 main season (chocolate spot was assessed every seven days starting from 76 DAP).

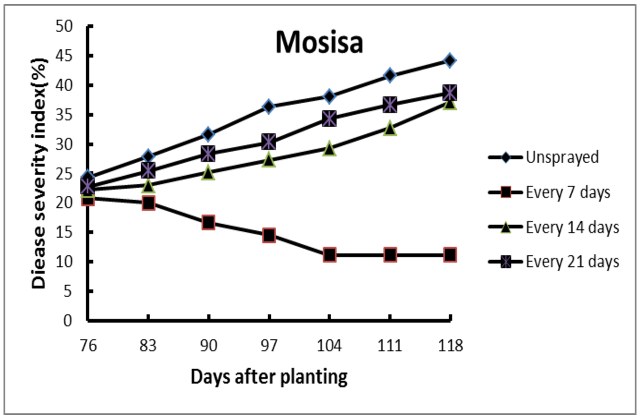

Figure 3: Chocolate spot progress curve as affected by different spray interval on faba bean varieties Mosisa at Sinana in 2014 main season (chocolate spot was assessed every seven days starting from 76 DAP).

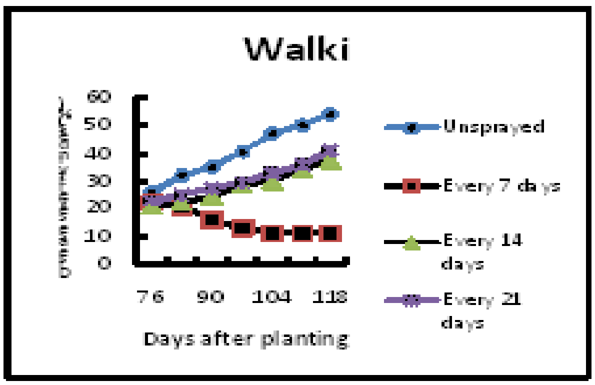

Figure 4: Chocolate spot progress curve as affected by different spray interval on faba bean variety Walki at Sinana in 2014 main season.(chocolate spot was assessed every seven days starting from 76 DAP).

Area under Disease Progress Curve (AUDPC)

Area under disease progress curve exhibited highly significant difference ($P \leq 0.01$) among main effect of varieties and fungicide sprayed treatments at Sinana (Table 4). The highest AUDPC (1619%-days) was recorded on the untreated and the lowest AUDPC (628 %-days) in weekly Mancozeb-treated plots (Table 4).For the fungicide treated every 14 and 21 days interval the AUDPC of 1227%-day and 1320%-day respectively. The Highest areas under disease progress curve was recorded on Local (1282%-days) and followed by the AUDPC (1202.12%-days.) of the variety Walki (Table 4). With this agreement the previous findings at the northern Ethiopia by Samuel *et al.* (2008a) indicated that short fungicide spray intervals (7 days) reduced the disease severity, AUDPC, and disease progress rate and increased the yield than the other spray intervals of Mancozeb and the AUDPC was higher on the local cultivar.

Disease progress rate

Logistic model explain better than Gompertiz model in describing the rate of chocolate spot infection. Logistic model has been reported to be most appropriate for temporal analysis of disease development because of wide application and goodness of fit for describing many epidemics (Campbell and Madden, 1989).The coefficient of determination (R^2) was higher for logistic model than Gompertiz. This indicates that chocolate spot infection rate was related to the logarithm of the ratio of the amount of diseased and health tissues present as described by the Campbell and Madden (1989).

Significant ($P \leq 0.05$) differences were observed on disease progress rate among fungicide spray schedules at initial dates of disease progress assessments. Disease progress rate of 0.012945, 0.004332, 0.002532 and 0.007366 units-day^{-1} were recorded on unsprayed, weekly sprayed, every 14 days sprayed and every 21 days sprayed respectively (Table 5). These results indicated that the disease has progressed at faster rate on the unsprayed plot four times than weekly sprayed plots.

There were also significant ($P \leq 0.05$) differences on disease progress rate among varieties at the final dates of disease progress assessments. Disease progress rate of varieties Local, Shalo, Mosisa and Walki were 0.010183, 0.008247, 0.005872 and 0.005697 units-day^{-1} respectively (Table 6). These results indicated that the disease has progressed on local variety which was three times faster than the disease progress rate on variety Mosisa and Walki.

Effect of chocolate spot on Grain Yield of faba bean

The main effects of varieties and fungicide application interval showed significant ($P \leq 0.05$) difference on yield of faba bean (Table 7). The analysis of variance (ANOVA) for grain yield showed significant ($P \leq 0.05$) difference among the faba bean varieties. The highest mean grain yield among the varieties was obtained from variety Walki (3846 kg ha^{-1}) and followed by variety Mosisa (3661kg ha^{-1}). The lowest mean grain yield was obtained from local variety (3265.8 kg ha^{-1}) (Table11). There were significant differences ($P \leq 0.05$) in grain yield among the main effects of Mancozeb spray intervals. Mancozeb increased the yield of faba bean varieties, compared to the respective unsprayed controls. The mean grain yield of (4040.3 kg ha^{-1}) across four faba bean varieties was recorded from every seven days spray intervals (Table 7). The unsprayed plots had significantly ($P \leq 0.05$) reduce the grain yields (3119.9 kg ha^{-1})

Table 2: Chocolate spot severity index on the four fababean varieties with different fungicide spray schedules at Sinana, 2014 main season.

Variety	Fungicide Spray interval (days)	Chocolate spot severity index		
		104DAP	111DAP	118DAP
Local	No spray	45.18[a]	50.51[a]	61.07[a]
	7	12.22[f]	11.11[b]	11.11[f]
	14	31.96[de]	34.7[c]	39.7d[e]
	21	34.18[bcd]	36.66[c]	41.25[cde]
Shalo	No spray	37.03[bc]	41.4[d]	46.85[c]
	7	11.11[f]	11.11[d]	11.11[f]
	14	32.25[de]	35.92[c]	37.22[e]
	21	33.66[bcde]	35.85[c]	44.14[cd]
Mosisa	No spray	38.07[b]	41.59[b]	44.14[cd]
	7	11.11[f]	11.11[d]	11.11[f]
	14	29.22[e]	32.74[c]	37.07[e]
	21	34.25[bcd]	36.66[c]	38.66[de]
Walki	No spray	46.92[a]	49.92[a]	53.74[b]
	7	11.11[f]	11.11[d]	11.11[f]
	14	30.25[de]	34.29[c]	37.70[e]
	21	33.07[cde]	36.11[c]	41.14[cde]
SE (N=4)		**2.81**	**2.83**	**3.5**
LSD (0.05)		**4.69**	**4.72**	**5.85**

Mean Values in the same letter within a column are not significantly different at 5% probability level; Ns = Non-significant; DAP = Days after planting

Table 3: Main effects of varieties and fungicide Spray schedules on the last chocolate spot severity index and AUDPC at Sinana during 2014 main cropping season

Factor	Final PSI	AUDPC
Variety		
Local	38.28a	1282a
Shalo	34.08bc	1169b
Mosisa	32.75c	1140.19b
Walki	35.92ab	1202.12ab
CV%	**9.95**	**8**
LSD(0.05)	**2.92**	**80.4**
Fungicide spray interval(days)		
No spray	51.45a	1619a
7	11.11c	628d
14	37.92b	1227c
21	40.55b	1320b
CV%	**9.95**	**8**
LSD (0.05)	**2.92**	**80.4**

Mean Values in the same letter within a column are not significantly different at described probability level; CV = coefficient of variation ; LSD = Least significant difference; AUDPC = Area under disease progress curve; PSI= Percent severity index assessed at 118 DAP.

Table 5: Main effects of fungicide spray schedule and varieties on the initial rate (units-day) of chocolate spot at Sinana, 2014 main cropping season.

Factor	Chocolate spot infection rate (unit/day)	SE infection rate	R^{2c} (%)	Significance (P)
Variety				
Local	0.005312a	0.001547	48.42	0.0018
Shalo	0.003816a	0.001547	55.75	0.0196
Mosisa	0.004052a	0.001547	70.94	0.0137
Walki	0.005333a	0.001547	63.31	0.0017
CV %	**115**			
LSD(0.05)	NS			
Fungicide Spray interval				
No spray	0.012945a	0.001547	72.15	<.0001
7	-0.004332d	0.001547	47.43	0.0089
14	0.002532c	0.001547	66.20	0.1122
21	0.007366b	0.001547	52.64	<.0001
CV%	**115**			
LSD (0.05)	**0.004469**			

Mean Values in the same letter within a column are not significantly different at described probability level; CV = coefficient of variation; SE= standard error of main factor; R^2 = Coefficient of determination

Table 6: Main effects of fungicide spray schedule and varieties on the final rate (units-day) of chocolate spot at Sinana, 2014 main cropping season.

Factor	Chocolate spot infection rate (unit/day)	SE infection rate	R^{2c} (%)	Significance (P)
Variety				
Local	0.010183[a]	0.001128	48.42	<.0001
Shalo	0.008247[b]	0.001128	55.75	<.0001
Mosisa	0.005872[b]	0.001128	70.94	<.0001
Walki	0.005697[ab]	0.001128	63.31	<.0001
CV %	**52.1**			
LSD(0.05)	**0.003260**			
Fungicide Spray interval				
No spray	0.018163[a]	0.001128	72.15	<.0001
7	-0.004040[c]	0.001128	47.43	0.0012
14	0.007381[b]	0.001128	66.20	<.0001
21	0.008495[b]	0.001128	52.64	<.0001
CV%	**52.1**			
LSD (0.05)	**0.003260**			

Table 7: Main effects of faba bean varieties and fungicide spray schedules on yield and hundred seed weight at Sinana in 2014 main cropping season.

Factor	Yield(kg ha^{-1})	Hundred seed weight(g)	Yield RL%
Variety			
Local	3265.8[c]	47.8[c]	35.45
Shalo	3440.2b[c]	54.5[a]	13.96
Mosisa	3661.6[ab]	51.3[b]	17
Walki	3846.0[a]	53.8[a]	21.52
CV (%)	**12.3**	**4.8**	
LSD (0.05)	**363.0**	**2.087**	
Fungicide			
No spray	3119.9 [c]	50.7[b]	31.35
7	4040.3[a]	54.0[a]	11.46
14	3640.9 [b]	51.4[b]	20.13
21	3412.5[b]	51.2[b]	24.99
CV%	**12.3**	**4.8**	
LSD(0.05)	**363.0**	**2.087**	

Mean Values in the same letter within a column are not significantly different at described probability level; CV = coefficient of variations; LSD = Least significant difference probability.

CONCLUSION

In Bale highlands, chocolate spot epidemics occurs frequently and caused yield losses since farmers grow local susceptible landraces and do not apply fungicides to manage the disease. Results have revealed that the faba bean varieties and fungicide application consistently reduced chocolate spot severity and increased the yield correspondingly. Shorter fungicide spray intervals reduced the disease and increased the yield compared to the unsprayed plots. This disease is favored by warm temperature (15- 22^{0}C) and humid weather (above 90% RH) conditions.

Integration of faba bean varieties with different reaction to chocolate spot with foliar sprays protected the faba bean varieties from high chocolate spot epidemics, increased yield, yield components and maximized marginal benefit compared to a single control approach. Spraying Mancozeb minimized the chocolate spot progress and produced higher yield than unsprayed treatments. The faba bean varieties Shalo, Mosisa and Walki appeared to be moderately resistant to chocolate spot and these are currently promising for production through integration with fungicide during epidemic development of the chocolate spot diseases.

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