

Evaluation of Faba bean Cultivars, Fungicides and Bio-control Agents for the Management of Chocolate Spot (*Botrytis fabae* Sard.) Disease in Kellem Wollega, Western Oromiya, Ethiopia

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Abstract

Faba bean (*Vicia faba* L.) is an important legume crop in the daily diet, good source of food nutrients, cash to the farmers and also foreign currency in Ethiopia. The chocolate spot disease caused by *Botrytis fabae* Sard is one of the most devastating soil-borne diseases and also the main constraints contributing to the low productivity of faba bean resulting in yield losses of up to 68% in Ethiopia. Therefore, an experiment was conducted at Haro Sabu Agricultural Research Center, Kellem Wollega, Western Oromiya, Ethiopia during the main cropping season of 2016, to evaluate three faba bean cultivars (Gora (EK01024-1-2), NC-58 and one local) combination with three fungicides (Mancozeb 80% WP, Folpan 80% WDG, and Mancolaxyl 72 WP (Mancozeb 64% + Metalaxyl 8%)) and two bio agents (*Trichoderma harzianum* and *T. viride*) under field conditions for the management of chocolate spot disease and also to assess the yield losses. A total of eighteen treatments were arranged in a randomized complete block design with three replications for the field experiment and non-spray fungicides and non-inoculated bio-agents treatments were used as control. There was a significant difference in the chocolate spot disease incidence, severity, and infected pods per plant, infected seeds and Area under Disease Progressive Curve (AUDPC) among treatments. Chocolate spot incidence, severity, infected pods per plant and AUDPC were highest in the control plots of NC-58 and Local cultivars when compared to the fungicides and bio agents treated seed plots. On the final date of disease assessment, among the cultivars, Gora cultivar was recorded the lowest disease incidence (42.33%) and the severity (18.63%), whereas the highest disease incidence of 100 and 90% and severity of 72.16 and 64.96 % were recorded on the NC-58 and Local cultivars on control plots, respectively. The highest reduction on disease severity (71.69%) was recorded on three times sprayed plots of Gora cultivar treated plots. Faba bean cultivars showed significant differences in their grain yields and hundred seeds weight but the fungicides sprayed plots were recorded significantly different from bio-agents treated and control plots. The highest grain yield of 3496 and 3340 kg /ha were also recorded from plots sprayed with Mancozeb 80% WP and Folpan 80 WDG on local cultivar. Relative yield losses of 2.57-53.17% on NC-58 cultivar, 4.46-40.96% on Local cultivar and 5.92-33.65% on Gora cultivar were recorded. The highest relative yield losses occurred on unsprayed (control) plots of NC-58 cultivar (53.17%) followed by Local (40.96%) and Gora cultivars (33.65%), respectively. The PDI, PDS and AUDPC were negatively correlated with PP, grain yield and HSW and positively correlated with each other. Mancozeb 80% WP fungicide applied on three cultivars exhibited maximum partial cost net benefit from all plots that means the highest (56135.00 ETB ha⁻¹) was recorded on cultivar Gora followed by on Local (52415.00 ETB ha⁻¹) and on NC-58 cultivar (45285.00 ETB ha⁻¹). Also the highest cost net benefit was recorded on Folpan 80 WDG fungicide treated plots on Gora (50637.00 ETB ha⁻¹) cultivar followed by Local (47692.00 ETB ha⁻¹), respectively. The results of the present study revealed that the novel possibility of using Mancozeb foliar spray with three times and *T. harzianum* seed treatments which were found to be an effective in decreasing chocolate spot disease symptoms on faba bean cultivars in Kellem Wollega, Western Oromiya and increased yield. This study could be provided some evidences on the response of faba bean cultivars, effect of bio-control agents and efficacies of fungicides and its application frequencies for the disease development and substantial increase on yield. Further, the effective and feasible integrated management options need to be developed on faba bean chocolate spot disease in the country.

Keywords: Faba bean, Chocolate spot disease, Cultivars, Fungicides, Bio-control agents, Yield and Yield loss, Cost benefit.

INTRODUCTION

Faba bean (*Vicia faba* L.) is an important diploid (2n = 12 chromosomes) Fabaceous pulse crops with commonly called as broad bean, horse bean, tic bean and field bean. It is one of the most important food legumes due to its high nutritive value both in terms of energy and protein contents (24-30%) and also an excellent nitrogen fixer (Metayer, 2004; Dagne *et al.*, 2016). Ethiopia is considered as the secondary center of diversity faba bean for widely grown in the mid-altitude and highland areas (1800-3000 m. a. s. l.) and serves as a multi-purpose crop leading the pulse category in area and production and it is a source of cash to the farmers and foreign currency to the country. It is the first among pulse crops cultivated in Ethiopia and leading protein source for the rural people

and used to make various traditional dishes. Faba bean is grown on 443,087.9 hectares in Ethiopia with an annual production of about 838,943.9 tons (CSA, 2016). The average yield of this crop under small-holder farmers was below 1.8 tons ha⁻¹ (CSA, 2016). This crop is grown in several regions of the country and production obtained from faba bean was 3.94% of the grain productions (CSA, 2016). Amhara and Oromia are the two major faba bean producing regions in Ethiopia. The Oromia region has the largest faba bean area (42.998%) and contributes to the highest production (48.27%) in the country followed by Amhara region that has 39.06% of the area and contributes 36.34% to national production (CSA, 2016). It is mainly produced in Tigray, Gondar, Gojjam, Wollo, Wollega, Shoa and Gamo-Gofa regions of Ethiopia.

Despite its wide cultivation, the average yield of faba bean is quite low in Ethiopia and the productivity of the crop is far below the potential because of several limiting biotic and abiotic constraints (Sahile *et al.*, 2008). Many diseases are affecting faba bean production and productivity, but only a few of them have economic significance. According to Shaile *et al.* (2008), diseases are the most important biotic factors limiting the production of faba bean in Ethiopia. Chocolate spot of faba bean, caused by *Botrytis fabae* is the most widespread and destructive faba bean disease in Ethiopia, with estimated yield reductions of up to 68% on susceptible cultivars (Shaile *et al.*, 2010). This disease exists across all the agro-ecological zones but it is more serious in areas of high-rainfall (>900 mm) and high elevation of >2000 m. a. s. l (Sahile *et al.*, 2008).

Although chocolate spot is serious problem of faba bean production in Dambi-Dolo and Western Oromiya region, its economic impact has not been evaluated. Different management options have been developed to reduce the yield losses in faba bean due to chocolate spot disease worldwide and also in Ethiopia; the use of chemical fungicides, resistant/ tolerant varieties, use of certain cultural practices such as crop residue management and altering planting date (Dereje and Tesfaye, 1993; Sahile *et al.*, 2008). In addition to the assessment of various management methods like chemical, cultural and use of biological methods are urgently needed in order to provide an alternative to other control methods (Shaile *et al.*, 2011). Currently, there is an urgent need to improve faba bean yield, since this crop remains an important part of Ethiopian diet. Recent innovation showed that biological control of crop disease is getting increased attention as an environmentally sound approach. But in Ethiopia, this method has received comparatively little attention (Sahile *et al.*, 2011). The use of *Pseudomonas fluorescens* and *Trichoderma* species are becoming increasingly common as an effective, economic and environment friendly approach and also effectively controlling many seed and soil borne pathogens including chocolate spot disease (Sahile *et al.*, 2008a). However, the management of faba bean chocolate spot disease through the effect of fungicides and biological control agents has not been studied so far in West and Kellem Wollega, Western Oromiya region, Ethiopia. Therefore, this study was carried out to evaluate the faba bean cultivars, fungicides and bio-control agents for the management of chocolate spot disease and to assess the yield and yield components under field conditions and also to assess the economic benefit of fungicides, bio-control agents and cultivars for the management of chocolate spot.

MATERIALS AND METHODS

Description of the study area

The field experiment was conducted in Haro Sabu Agricultural Research Center (HSARC) Mata Sub-site in Sayo District, Western Oromia, Ethiopia during the main cropping season of 2016. Sayo District is located at 652 km West of Addis Ababa and its geographic location is 8.5333°N latitude and 34.80117°E longitude with an elevation of 1754 - 2200 m. a. s. l. The area receives high rainfall with minimum and maximum temperature of the site is 13°C and 27°C, respectively and characterized by wet and humid climatic conditions where the chocolate spot is known to be consistently prevalent and severe on local cultivars (MoARD, 2011). The soil of the experimental study site is vertisol with light black in color and sandy loam soil type with pH value of 6. **Experimental materials used**

Three faba bean cultivars viz. Gora (EK01024-1-2), NC-58 and one Local were used in this experimental study. The two improved cultivars were selected based on their differential reaction to chocolate spot. Gora (EK01024-1-2) was moderately resistant and NC-58 was susceptible to chocolate spot (MoARD, 2011). Seeds of all the three cultivars were obtained from Holleta and Kulmusa Agricultural Research Centers, Ethiopian Agricultural Research Institute (MoARD, 2011) and all the cultivars have wide-range of environmental adaptations in Ethiopia. Three fungicides (Mancozeb 80% WP, Folpan 80% WDG, and Mancoyaxyl 72 WP (Mancozeb 64% + Metalaxyl 8%) and two bio-control agents (*Trichoderma harzianum* and *T. viride*) were used in this study. All the fungicides were obtained from local market and bio-control agents, *T. harzianum* and *T. viride* were obtained from Department of Plant Sciences, Ambo University and APPRC, Ambo, Ethiopia.

Experimental design, treatments and applications

A total of 18 treatments were arranged in a randomized complete block design with three replications and unsprayed control and Mancozeb 80% WP sprayed plots were used as a standard check (Table 1; Figures 1 & 2). Plot size was consisted of 2m x 2m and an inter-row and intra-row spacing of 40 cm and 10 cm, respectively, which having 5 seedling rows with three rows per plot harvested and each row accommodating 20 plants. Disease

assessments were carried out from 13 plants which were tagged from the three central rows. The fungicides were applied as per recommendation of the manufacturers using a manually-pumped knapsack sprayer of 15 liter capacity (Table 2). The spraying was started soon after the first chocolate spot disease lesion was observed on the foliage and continued depending on the needs of the variety. Folpan was applied for four consecutive weeks at seven days interval whereas Mancozeb and Mancolaxyl were applied three times at fourteen days interval. The bio-control agents were treated in seeds at sowing time as suspension of *T. harzianum* 2×10^7 spores / ml and *T. viride* 2×10^9 spores / ml concentration with 10 g / kg of seeds. Di-ammonium phosphate (DAP) and urea were applied at the time of planting and during weeding at the rate of 46 kg/ha and 18 kg/ha, respectively (Tamene and Tadese, 2013). Agronomic practices were carried out in all the field plots as per recommendations.

Table 1: Experimental design and treatment combinations under field conditions

Sl.No.	Treatments
1	Local + Mancolaxyl
2	Local + Folpan
3	Local + Mancozeb -Standard check
4	Local + <i>Trichoderma harzianum</i>
5	Local + <i>Trichoderma viride</i>
6	Local + un sprayed, control check
7	Gora (EK01024-1-2) + Mancolaxyl
8	Gora (EK01024-1-2) + Folpan
9	Gora (EK01024-1-2) + Mancozeb -Standard check
10	Gora (EK01024-1-2) + <i>Trichoderma harzianum</i>
11	Gora (EK01024-1-2) + <i>Trichoderma viride</i>
12	Gora (EK01024-1-2) + un sprayed, control check
13	NC -58 + Mancolaxyl
14	NC -58 + Folpan
15	NC -58 + Mancozeb -Standard check
16	NC -58 + <i>Trichoderma harzianum</i>
17	NC -58 + <i>Trichoderma viride</i>
18	NC -58 + un sprayed, control check

Table 2: Rates and frequencies of application of fungicides and bio-control agents in the treatments

Fungicides /Bio-control agents	Trade name	Common name	Rate (kg/ha)	Interval of sprayed	Friquency
Mancozeb 80% WP	Mancozeb	Mancozeb	2	14	3 times
Folban 80 WDG	Folpet	Folpet	2.6	7	4 times
Mancolaxyl 72% WP	Mancozeb + Metalaxyl WP	Mancozeb + Metalaxyl WP	2.5	14	3 times
T-22	<i>Trichoderma harzianum</i>	<i>Trichoderma harzianum</i>	10g / kg of seeds	once	As seed treatment
--	<i>Trchoderma viride</i>	<i>Trchoderma viride</i>	10 g/kg of seeds	once	As seed treatment



Figure 1 A and B. Seedling of Faba bean cultivars grown on experimental field

Disease assessment

Disease incidence and severity

Disease assessment were made on 13 pre-tagged plants from the three central rows of each plot starting from the onset of the disease and continued every ten days till crop maturity. Both diseased and healthy plants were counted from the pre-tagged plants and the percentage of disease incidence (PDI) was calculated according to the formula used by Wheeler (1969):

$$PDI(\%) = \frac{\text{Number of diseased plants}}{\text{Total number of plants inspected}} \times 100$$

Disease severity was assessed as the percentage of the total leaf surface covered with chocolate spot lesions on each expanded leaflet separately at regular intervals using a 0–9 scale (Table 3) (Ding *et al.*, 1993; Wulita, 2015). The severity grades were converted into percentage severity index (PSI) according to the formula by Wheeler (1969).

$$PSI(\%) = \frac{\sum \text{Individual numerical ratings}}{(\text{Total number of plants assessed} \times \text{Maximum score in the scale})} \times 100$$

Table 3: Percent of infection and scale for faba bean chocolate spot

Scale	Description
0	no visible infection on leaves
1	a few dot-like accounting for less than 5% of total leaf area
3	discrete spots less than 2 mm in diameter (6–25% of leaf area)
5	numerous scattered spots with a few linkages, diameter 3–5 mm (26–50% of leaf area) with a little defoliation
7	confluent spot lesions (51–75% of leaf area), mild sporulation, half the leaves dead or defoliated
9	complete destruction of the larger leaves (covering more than 76% of leaf area), abundant sporulation, heavy defoliation and plants darkened and dead

Area under Disease Progress Curve (AUDPC)

The progress of chocolate spot was plotted over time using to mean percentage severity index for each faba bean variety at each plot, and the DSI values were also used calculate apparent infection rate (*r*). The AUDPC values (%-day) were calculated for each variety according to the mid-point rule formula (Campbell and Madden, 1990).

$$AUDPC = \sum_{i=1}^{n-1} 0.5(X_{i+1} + X_i)(t_{i+1} - t_i)$$

Where X_i is the disease severity of chocolate spot at i th assessment date, T_i 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 in percentage and time in days, AUDPC was express in proportion days.

Disease progress rate

The apparent infection rate, expressed in disease units per day, was calculated from disease severity data transformed to logistic model ($\ln [(Y/1-Y)]$) (Van der Plank, 1963) and Gompertz, $-\ln[-\ln(Y)]$ where Y and $1-Y$ represent the proportion of infected plants and the proportion of healthy plants remaining in the plot, respectively. The transformed values (y) were regressed.

Growth parameters

Days from planting to the emergence of 50% plants per plot were recorded. Days to flowering were recorded for each plot when 50% of the plants in a plot flowered. Days to 90 % maturity of the crop when 90% of the pods in the plot reached physiological maturity. The height of plants from the ground to the tip of the plants was measured five randomly selected plants per plot at maturity.

Yield and yield components

The number of pods per plant was counted on five randomly taken plants from 13 tagged plants from three central rows and the means were recorded as number of pods/plant. The total number of capsules from five randomly taken plants from 13 tagged plants from three central rows which were threshed and number of seeds were counted and the total number of seeds was divided by total number of capsules to compute average number of seeds per pod. The grain yield per plot from the three central rows was recorded.

$$\text{Adjusted yield per plot} = (\text{Fw} (100 - \text{Amc}) \times \text{RDW})$$

Where: Fw = Field weight; Amc = Actual moisture content; RDW = Recommended dry weight

The grain yield in gram per plot was then calculated per hectare basis. The weight of 100 randomly taken seeds from the yield of each plot was recorded.

Relative yield loss (%)

The relative loss in yield of each treatment was determined as percentage of that of protected plots of the

experiment. Losses were calculated separately for each of the treatment and yield component of faba bean was determined as a percentage of that of the protected plots and the yield loss was calculated based on the formulas:

$$\text{RYL (\%)} = \frac{(Y_p - Y_t)}{Y_p} \times 100$$

Where, RYL = relative yield loss in percent, Y_p = yield from the maximum protected plots and Y_t = yield from other plots.

Percent yield recovery was calculated to compare the yield differences among fungicides and cultivars and other treatments using the formula:

Correlation between yield and disease parameters

The correlations among the disease parameters and with the all yield components were tested at 5% probability level. The reliable yield loss was estimated on the basis of the severity level by employing regression equations.

Cost benefit analysis

The prices of faba bean seeds (birr/kg) were assessed from the local market and the total price of the commodity obtained from each treatment was computed on hectare basis. Input costs like fungicide, cultivars, bio-control agents and labor were converted into hectare basis according to their frequencies used. The prices for each cultivar per kg were assessed. Fungicides and the bio-control agents cost was estimated based on the price of company. Cost of the labor was in Birr per man-days; cost of spray and spray equipment to spray one week, two weeks and three weeks up to nine weeks per hectare were also calculated. Cost of spray equipment (knapsack sprayer) was in Birr per day assessed. Based on the obtained data from the above mentioned parameters, cost benefit analysis was performed using partial budget analysis. Partial budget analysis is a method of organizing data and information about the cost and benefit of various agricultural alternatives (CIMMYT, 1988).

Partial budgeting is employed to assess profitability of any new technologies (practice) to be imposed to the agricultural business. Marginal analysis is concerned with the process of making choice between alternative factor-product combinations considering small changes. Marginal rate of return is a criterion which measures the effect of additional capital invested on net returns using new managements compared with the previous one (CIMMYT, 1988). It provides the value of benefit obtained per the amount of additional cost incurred percentage. The formula is as follows:

$$\text{MRR} = \frac{\text{DNI}}{\text{DIC}}$$

Where, MRR is marginal rate of returns, DNI, difference in net income compared with control, DIC, difference in input cost compared with control.

Data Analysis

The analysis of variance (ANOVA) was performed for the disease parameters (incidence, severity, AUDPC) and yields parameters (seed yield per pod/plant and yield loss) using GenStat software. Least significant difference (LSD) values were used to separate treatment means ($P < 0.05$) among the treatments. Correlation coefficient (r) between yield and severity as well as were determined through yield components correlation analysis using GenStat 15th edition software, following analysis using the standard procedure (Gomez and Gomez, 1984).

Results and discussion

Disease incidence

Chocolate spot of faba bean was first observed on spreader rows (62 DAS after sowing) on experimental fields at the end of October 2016 and it was recorded on the leaves of faba bean cultivars tested 2-3 weeks later. On the experimental plots, chocolate spot was first appeared on the cultivars, NC-58 and Local but three days later was observed on Gora cultivar. There was a significant difference ($P < 0.05$) between cultivars for chocolate spot incidence recorded as well as on all assessment dates from 72 -112 DAS (Table 4; Figures 2-4). Variation in the disease might be due to the difference in resistance levels of the cultivars (Table 4). The disease was more rapidly on the cultivar NC-58, which showed higher level of final disease incidence (100%) followed by Local cultivar (90%). Lower disease incidence (80.67%) was recorded on cultivar Gora, which was reported as a moderately resistance cultivar (Table 4). This observation was agreed with the earlier reports by Bond and Pope, (1980); Sahile *et al.*, (2008) and Tamene and Tadese (2013) found that the disease development rate that was affected by the resistant level of the crop which is high on susceptible and low on resistant ones. Even though, the highest final disease incidence of 100% was recorded from cultivar of NC-58, this value was not significantly different from the other two cultivars. In addition, there were no significant differences among the Local and Gora cultivars for the first two assessments and significant differences ($P < 0.05$) from third assessment (92 DAS) to final assessment (112 DAS) (Table 4). This observation was agreed with earlier report by Tamene and Tadese (2013) found that the Gora cultivar was moderately resistance to faba bean chocolate spot disease and also no significant differences among the Local and Gora cultivars for the first two assessments.

Regardless of the cultivars, maximum disease incidences were recorded from the untreated control, 23% at

the initial (72 DAS) and 100% at the final (112 DAS) date of disease assessment, whereas, the least disease incidences were recorded from plots treated with Mancozeb foliar spray fungicide, 10% and 42.33%, at the initial (72 DAS) and final (112 DAS) dates of disease assessment, respectively (Table 5). From all treatments, Mancozeb, exhibited less than 50% disease incidence up to final assessment followed by Folpan and Mancolaxyl sprayed plots showed less than 55% until last assessment (Table 5). There was no significance differences between fungicide sprayed and unsprayed plots of the local cultivar. Variation on chocolate spot disease incidence was exhibited among the three fungicides on the three cultivars. Chemical management recommended in Ethiopia for this disease is on application of Chlorothalonil or Mancozeb and late planting (Dereje and Tesfaye, 1993; Sahile *et al.*, 2008b). Foliar application of protective fungicides like Chlorothalonil (Bravo 500 or Daconil 2787) at the rate of 2.5 kg active ingredient per hectare every 10 days, when infection reached 30% but Mancozeb at the rate of 3 kg active ingredient per hectare every week at threshold level was recommended in Ethiopia.

In this study, on Gora cultivar, there was no significant differences between the three fungicides up to third assessments (92 DAS), but there were differences between Mancozeb 80% WP and the other two fungicides, Folpan 80 WDG and Mancolaxyl 72 WP from third assessments to final (112 DAS) date of assessment. There were no significant differences between Folpan 80 WDG and Mancolaxyl 72 WP on Gora cultivar for all assessments. There were significant differences between Mancozeb and other fungicide sprayed plots only at the final (112 DAS) assessment on cultivar NC-58 cultivar and non-significant differences on Folpan 80WDG and Mancolaxyl 72 WP treated plots on NC-58 cultivar (Table 5). These results were supported by EARO (2001), mancozeb gave good control of chocolate spot in faba bean and increased seed yield. Similarly, Sahile *et al.* (2008) reported that mancozeb spraying at a rate of 2.5 kg ha⁻¹ reduced incidence and severity of chocolate spot. Dereje and Tesfaye (1993) and Sahile *et al.*, (2008b) reported that the Mancozeb at the rate of 3 kg active ingredient per hectare every week at threshold level was effective agent chocolate spot and accordingly recommended for use in Ethiopia. Similarly in this study, Mancozeb at the rate of 2 kg active ingredient per hectare applied every week at threshold level reduced incidence and severity of this disease. Cultivars which were treated with bio-agents, *T. harzianum* and *T. viride* were relatively showed significant differences ($P \leq 0.05$) on disease incidence among unsprayed cultivars during all assessment days (72, 82, 92, 102 and 112 DAS). This observation was agreed with earlier report by Tamane and Tadese (2013) found that the cultivars of faba bean treated with bio-agents under *in vivo* pot culture conditions showed significant differences on disease incidence among unsprayed cultivars. Chocolate spot incidence, generally, increased throughout the assessment days which showed the spread of the disease in field. Although the chocolate spot symptoms was started to appear from 72 DAS onwards without heavy seedling infection. Sahile *et al.* (2008) predicted the possible occurrence of a marked susceptibility phase at the early stage of pod formation when no heavy seedling infection is observed. Faba bean chocolate spot disease is highly favored by cool and wet weather (Sahile *et al.*, 2008). The main cropping season of 2016 was accompanied by frequent rainfall and moderate temperature as usual. According to weather information from the meteorological station in Dambi Dolo, a mean minimum and maximum temperature of 13 and 25.1°C and mean of daily rainfall 1415.2 mm were recorded in August and September respectively. The prevailing weather conditions encountered at the pod development stage might have increased the susceptibility of the crop and resulted in the observed higher disease incidences in the field. Sahile *et al.* (2008) indicated that the pod setting/development stage of the crop is the most susceptible to attack by chocolate spot. In this study, the disease incidence reached its maximum (100%) on the control plots of NC-58 cultivar and 90% on Local cultivar at this time of the pod setting/development stage of the crop growing season.

Table 4: Incidence of chocolate spot disease on different faba bean cultivars

Cultivars	Disease incidence				
	72 DAS	82 DAS	92 DAS	102 DAS	112 DAS
Local	16.67 ^b	38.67 ^{ab}	60.33 ^b	85.0 ^b	90.67 ^b
Gora	15.0 ^b	27.67 ^b	44.0 ^c	69.33 ^c	80.67 ^c
NC-58	23.0 ^a	47.33 ^a	70.33 ^a	98.67 ^a	100.0 ^a
Mean	18.22	37.9	58.2	84.3	90.44
LSD (0.05)	3.998*	14.24*	9.0**	10.47**	5.289**
CV (%)	9.7	16.6	6.8	5.5	2.6

Das=days after sowing, ns=non-significant, LSD= Least significant difference, CV= coefficient of variations, **= highly significant difference at $P < 0.01$, *= significant difference at $P < 0.05$.

Disease severity

The Percentage Severity index (PSI) data revealed that the severity of chocolate spot on the control plot was higher than the treated plots. Highly significant differences ($P < 0.05$) among treatments were recorded at all dates of assessment. There was a significant differences ($P < 0.05$) on chocolate spot disease PSI among three cultivars starting from second assessment (82 DAS) up to final day of assessment (112 DAS) (Table 6). There were no significant differences between NC-58 and Local cultivars through all the assessment period beginning from 72 DAS (Table 6). Variation in the disease severity of the chocolate spot disease of faba bean of Gora cultivar (42.09%)

and the other two cultivars, NC-58 (72.16%) and Local (64.96%) was exhibited due to the difference in resistance levels of the cultivars. Gora cultivar showed moderate level of final disease percentage severity index of 42.09% (Table 6). This observation is agreement with an earlier report by Tamene and Tadesse (2013) found that the Gora cultivar was moderately resistant to faba bean chocolate spot disease.



Figure 2: Chocolate spot incidence on faba bean cultivar NC-58.

A. before disease on set, B. 92 DAS on control plot, C. 82 DAS on Mancozeb sprayed plot and D. 102 DAS on Folpan sprayed plot under experimental field conditions.



Figure 3: A. Local cultivar sprayed Mancozeb fungicides under experimental fields.

B. Chocolate spot incidence recorded on faba bean cultivar Local (control) 102 DAS under experimental fields.



Figure 4: Gora cultivar (A) 72 DAS and (B) 92 DAS (control) at 102 DAS under experimental fields.

Table 5 Incidence of chocolate spot disease on faba bean cultivars treated with fungicides and bio-control agents under field conditions

Cultivars	Fungicides and Bio-agents Treatments	Days of disease incidence assessment				
		72 DAS	82 DAS	92 DAS	102 DAS	112 DAS
Local	Mancozeb 80% WP	9.33 ^c	18.33 ^c	27.0 ^c	39.33 ^c	48.67 ^c
	Folpan 80 WDG	11.33 ^{bc}	19.67 ^c	29.67 ^c	43.33 ^c	52.0 ^c
	Mancolaxyl 72 WP	12.33 ^b	21.67 ^c	30.0 ^c	45.33 ^c	53.33 ^c
	<i>T. harzianum</i>	15.67 ^a	30.0 ^b	50.67 ^b	69.33 ^b	81.33 ^b
	<i>T. viride</i>	15.67 ^a	30.67 ^b	49.67 ^b	73.33 ^b	81.67 ^b
	Unsprayed(control)	16.67 ^a	38.67 ^a	60.33 ^a	85.0 ^a	90.67 ^a
LSD (0.05)		2.277**	7.508**	6.523**	6.973**	4.867**
CV (%)		9.3	15.6	8.7	6.5	3.9
Gora	Mancozeb 80 %WP	10.0 ^b	15.67 ^c	22.0 ^d	34.33 ^c	42.33 ^c
	Folpan 80 WDG	9.0 ^b	18.67 ^{bc}	31.67 ^c	41.33 ^{bc}	52.0 ^b
	Mancolaxyl 72 WP	10.0 ^b	22.67 ^b	38.33 ^{bc}	48.67 ^b	57.67 ^b
	<i>T. harzianum</i>	14.33 ^a	29.0 ^a	45.0 ^{ab}	65.33 ^a	77.33 ^a
	<i>T. viride</i>	15.0 ^a	29.33 ^a	48.67 ^a	69.33 ^a	77.67 ^a
	Unsprayed(control)	15.0 ^a	29.67 ^a	44.0 ^{ab}	69.33 ^a	80.67 ^a
LSD (0.05)		2.387**	5.782**	7.96**	7.97**	6.885**
CV (%)		10.7	13.2	11.4	8	5.9
NC-58	Mancozeb 80% WP	11.33 ^c	21.33 ^c	29.0 ^c	41.33 ^c	52.33 ^d
	Folpan 80 WDG	14.0 ^{bc}	24.0 ^c	32.33 ^c	44.33 ^c	57.67 ^c
	Mancolaxyl 72 WP	12.33 ^{bc}	21.33 ^c	31.33 ^c	45.33 ^c	57.33 ^c
	<i>T. harzianum</i>	15.67 ^b	33.0 ^b	51.67 ^b	70.67 ^b	83.67 ^b
	<i>T. viride</i>	15.33 ^b	32.33 ^b	53.33 ^b	73.0 ^b	82.67 ^b
	Unsprayed(control)	23.0 ^a	47.33 ^a	70.33 ^a	98.67 ^a	100.0 ^a
LSD (0.05)		3.494**	5.056**	5.763**	5.75**	2.851**
CV (%)		12.6	9.3	7.1	5.1	2.2

Das=days after sowing, ns=non-significant, LSD= Least significant difference, CV= Coefficient of Variations, **= highly significant difference at P<0.01, *= Significant difference at P<0.05

Maximum disease severity, 44.44% at the second period of assessment (82 DAS) and 72.6 % at the final (112 DAS) dates of disease assessment were recorded from unsprayed control plots of NC-58 cultivar. The least disease severity (18.63%) was recorded on Gora cultivar plots treated with Mancozeb foliar spray fungicide followed by plots treated with Folpan foliar spray fungicide (28.2%) (Table 7). Among the bio agents, the least disease severities (values) were recorded from Gora cultivar plots treated with *T. harzianum* followed by plots treated with *T. viride* (Table 7). The observed high severity grades scored in the cultivar NC-58 could be due to the accumulation of secondary inoculum due to cultivar susceptibility. There was significantly differences (P<0.01) for chocolate spot disease percentage severity index between bio-agents treated, chemicals applied and unsprayed

(control) plots. Cultivars which treated with bio-agents, *T. harzianum* and *T. viride* were relatively significant differences ($P \leq 0.05$) of chocolate spot disease severity from unsprayed Local and NC-58 cultivars starting from second assessment to final assessment (82 to 112 DAS) (Table 7). However, the lowest severity (40.17%) was recorded on the cultivar Gora and the highest disease severity (50.0%) was assessed on NC-58 cultivar (Table 7). The analysis of variance showed that there was non-significant differences ($P < 0.05$) on disease percentage severity among the three fungicide sprayed plots. On Local and NC-58 cultivars, there were no significant differences from first assessment to final assessment between the three fungicides, but in cultivar Gora, there was a significant difference of chocolate spot disease severity at final assessment (112 DAS). The lowest severity was recorded in Mancozeb 80%WP fungicide sprayed treatment that was statistically not significantly different from other two fungicides sprayed and highly significant different the treatments which treated with bio-agents and fungicides unsprayed treatment plots (Table 7).

Table 6 Percentage severity index of chocolate spot disease on different faba bean cultivars

Cultivars	Percentage severity index (%)				
	72 DAS	82 DAS	92 DAS	102 DAS	112 DAS
Local	23.07 ^a	42.09 ^a	50.64 ^a	55.17 ^{ab}	64.96 ^a
Gora	17.94 ^a	31.54 ^b	37.6 ^b	38.5 ^b	42.09 ^b
NC-58	25.64 ^a	44.44 ^a	55.13 ^a	63.5 ^a	72.16 ^a
Mean	22.2	39.4	47.8	52.4	59.7
LSD (0.05)	11.63 ^{ns}	8.27 [*]	12.99 [*]	17.18 [*]	11.87 ^{**}
CV (%)	23.1	9.3	12	14.5	8.8

DAS=days after sowing, ns=non-significant, LSD= least significant difference, CV= coefficient variations, **= highly significant difference at ($p < 0.01$), *= significant difference at ($p < 0.05$)

Table 7 Percentage severity index of chocolate spot on faba bean cultivars treated with fungicides and bio-control agents under field conditions

Varieties	Fungicides and Bio-agents	Percentage disease severity index				
		72 DAS	82 DAS	92 DAS	102 DAS	112 DAS
Local	Mancozeb 80% WP	12.82 ^a	17.94 ^d	23.07 ^d	28.2 ^c	25.64 ^c
	Folpan 80 WDP	15.38 ^a	24.82 ^{cd}	28.2 ^{cd}	35.9 ^{bc}	33.33 ^{bc}
	Mancolaxyl 72 WP	12.82 ^a	25.64 ^{cd}	30.77 ^{bcd}	35.9 ^{bc}	33.33 ^{bc}
	<i>T. harzianum</i>	12.82 ^a	39.54 ^{ab}	39.31 ^b	46.79 ^{ab}	44.23 ^b
	<i>T. viride</i>	14.38 ^a	30.47 ^{bc}	32.77 ^{bc}	39.95 ^{bc}	43.59 ^b
	Unsprayed(control)	23.07 ^a	42.09 ^a	50.64 ^a	55.17 ^a	64.96 ^a
LSD (0.05)		10.73 ^{ns}	9.07 ^{**}	9.6 ^{**}	12.12 ^{**}	13.29 ^{**}
CV (%)		38.8	16.6	15.5	16.5	17.9
Gora	Mancozeb 80 %WP	9.57 ^b	12.13 ^b	15.38 ^c	16.06 ^c	18.63 ^c
	Folpan 80 WDP	12.82 ^{ab}	17.92 ^{ab}	22.3 ^{bc}	20.51 ^{bc}	28.2 ^b
	Mancolaxyl 72 WP	10.25 ^b	25.64 ^a	25.64 ^{abc}	25.64 ^b	28.2 ^b
	<i>T. harzianum</i>	15.38 ^{ab}	25.64 ^a	19.2 ^c	36.75 ^a	40.17 ^a
	<i>T. viride</i>	12.82 ^{ab}	26.39 ^a	33.33 ^{ab}	38.46 ^a	41.02 ^a
	Unsprayed(control)	17.94 ^a	28.2 ^a	37.6 ^a	40.17 ^a	42.09 ^a
LSD (0.05)		7.619 ^{ns}	10.44 [*]	13.36 [*]	6.795 ^{**}	8.88 ^{**}
CV (%)		31.9	26.3	28.7	12.6	14.8
NC-58	Mancozeb 80% WP	10.25 ^c	20.51 ^c	28.2 ^b	31.62 ^c	35.9 ^c
	Folpan 80 WDG	10.25 ^c	21.26 ^c	28.2 ^b	35.9 ^{bc}	36.75 ^c
	Mancolaxyl 72 WP	12.82 ^{bc}	17.94 ^c	31.72 ^b	34.19 ^c	36.75 ^c
	<i>T. harzianum</i>	20.51 ^{ab}	35.9 ^b	40.38 ^{ab}	44.44 ^b	49.38 ^b
	<i>T. viride</i>	15.38 ^{bc}	33.33 ^b	35.62 ^b	44.51 ^b	50.0 ^b
	Unsprayed(control)	25.64 ^a	44.44 ^a	53.46 ^a	63.5 ^a	72.16 ^a
LSD (0.05)		8.73 [*]	7.315 ^{**}	15.94 [*]	9.83 ^{**}	7.323 ^{**}
CV (%)		30.3	13.9	24.2	12.8	8.6

Das=days after sowing, ns=non-significant, LSD= Least significant difference, CV= Coefficient of variations, **= highly significant difference ($P < 0.01$), *= significant difference ($P < 0.05$).

Area under Disease Progress Curve (AUDPC)

There were highly significant differences ($P < 0.01$) on AUDPC of faba bean cultivars. The analysis of variance exhibited Gora cultivar was significantly different on chocolate spot AUDPC from other two cultivars (NC-58 and Local). There were no significant differences between NC-58 and Local cultivar AUDPC. The maximum AUDPC was calculated on the plots of NC-58 and Local cultivars which were 2103 and 1919 (%-day), respectively (Table

8). AUDPC values varied among the faba bean cultivars depending on the resistance levels of the cultivars and it is known that AUDPC is directly related to the yield loss (Singh and Rao, 1989). There was a significant differences ($P < 0.05$) on chocolate spot AUDPC between bio-agents treated, chemicals applied and unsprayed (control) plots. Cultivars which treated with bio-agents, *T. harzianum* and *T. viride* were relatively significant differences on chocolate spot AUDPC from unsprayed NC-58 cultivar and *T. harzianum* treated Gora cultivar plot was not significantly different from Gora cultivar sprayed with Mancofaxyl 72 WP fungicide (Table 9). Generally, there were no significant differences on AUDPC between the two bio-agents on all the three cultivars (Table 9).

There were significant differences of AUDPC fungicides sprayed treatments on all three cultivars and significantly reduced the AUDPC value when compared to all other bio-agents treat and the unsprayed cultivar (control) for each cultivar. On Gora cultivar, there were significant difference AUDPC treatments sprayed fungicides of Mancozeb 80% WP and Folban 72 WDG. The lowest 577 and 812 (%-days) AUDPC value was recorded from the plots sprayed with Mancozeb 80% WP and Folpan 80% WDG on Gora cultivar, respectively (Table 9) followed by plots sprayed with Mancozeb 80% WP on Local cultivar 884 (%-day) AUDPC was recorded. AUDPC is generally used to make comparison between treatments (Xu, 2006) and to evaluate the resistance of plant species to the pathogen (Mikulova *et al.*, 2008; Irfaq *et al.*, 2009). Moreover, Jerger (2004) indicated that comparisons of disease progress curves and AUDPC between treatments are the most commonly used tools for evaluating practical disease management strategies.

Disease progress rate

Both logistic and Gompertz models were tested to choose the best fitted one in describing the rate of the disease development. The coefficient of determination (R^2) was larger for Gompertz model than for logistic model for all severity assessments. Hence, based on their coefficient of determination values (R^2), Gompertz model was found to be better than the logistic model for the chocolate spot disease and was used to determine the disease progress rate parameters. 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 (1989). Based on Gompertz model, the regression equation used to describe the rate of chocolate spot progress was not significant for all three cultivars apparently because of no difference disease development between the cultivars (Table 8). The apparent infection (r) between the cultivars related each other, regression equations with (r) ranging from 0.0828-0.0988 were produced when chocolate spot severity was regressed over time in days after planting (Table 8).

Table 8 Area under disease progress curve and apparent infection rate(r) of chocolate spot on different faba bean cultivars

Varieties	AUDPC	apparent infection rate(r)
Local	1919 ^a	0.09738
Gora	1360 ^b	0.08285
NC-58	2103 ^a	0.0988
Mean	1794	0.09301
LSD (0.05)	322.9**	
CV %	7.9	

AUDPC= area under disease progress curve, ns=non-significant, LSD= least significant difference, CV= coefficient variations, **= highly significant difference ($P < 0.01$), *= significant difference ($P < 0.05$)

According to regression equation used to describe the rate of chocolate spot progress, there was no significant difference on apparent infection rate for all fungicide sprayed and bio-agents treated plots. Similarly, there was no significant differences between fungicides sprayed plots on all cultivars for the same parameter. On NC-58 cultivar the highest progress rates of 0.0968 and 0.0937 Gompit per day were calculated from Mancozeb 80% WP and the Folpan 80 WDG fungicides sprayed plots, respectively and the lowest was 0.0672 recorded on fungicide Mancozeb 80% WP sprayed plot. On Local cultivar, the highest progress rates of 0.0958 and 0.0944 Gompit per day were calculated from Folpan 80 WDG and the Mancofaxyl 72 WP fungicides sprayed plots, respectively and the lowest was 0.0575 fungicide of Mancozeb 80% WP sprayed plot (Table 9). On Gora cultivar also the highest result were 0.0689 and 0.0652 infection rate per a day calculated Mancofaxyl 72 WP and Mancozeb 80% WP sprayed fungicides, respectively and the lowest was 0.0367 fungicide of Folpan 80 WDG sprayed plots (Table 9). Based on the model, the regression equation used to describe the rate of chocolate spot progress was not significant different between the two Bio-agents (*T. harzianum* and *T. viride*).

Table 9 AUDPC and apparent infection rate(r) of chocolate spot on faba bean cultivars treated with fungicides and bio-control agents under field conditions

Varieties	Fungicides and Bio-agents - Treatments	AUDPC% -days	apparent infection rate(r)
Local	Mancozeb 80% WP	884 ^d	0.0575
	Folpan 80 WDG	1133 ^{cd}	0.0958
	Mancolaxyl 72 WP	1154 ^{cd}	0.0944
	<i>T. harzianum</i>	1542 ^b	0.0982
	<i>T. viride</i>	1322 ^{bc}	0.0665
	Unsprayed(control)	1919 ^a	0.0974
LSD (0.05)		326.0**	
CV (%)		13.5	
Gora	Mancozeb 80% WP	577 ^c	0.0352
	Folpan 80 WDG	812 ^{de}	0.0467
	Mancolaxyl 72 WP	910 ^{cd}	0.0689
	<i>T. harzianum</i>	1094 ^{bc}	0.0737
	<i>T. viride</i>	1251 ^{ab}	0.0769
	Unsprayed(control)	1360 ^a	0.0828
LSD (0.05)		239**	
CV (%)		13.1	
NC-58	Mancozeb 80% WP	1034 ^c	0.0672
	Folpan 80 WDG	1089 ^c	0.0937
	Mancolaxyl 72 WP	1086 ^c	0.0968
	<i>T. harzianum</i>	1557 ^b	0.0982
	<i>T. viride</i>	1462 ^b	0.0667
	Unsprayed(control)	2103 ^a	0.0988
LSD (0.05)		253.2**	
CV (%)		10	

AUDPC= area under disease progress curve, ns=non-significant, LSD= least significant difference, CV= coefficient variations, **= highly significant difference (P<0.01), *= significant difference (P<0.05).

Crop growth parameters

The analysis of variance (ANOVA) on days to 50% emergence revealed that cultivars were not-significant differences (P < 0.05). All the three cultivars not different on emerged days and seeds planted in all plots emerged almost uniformly within 12-14 days of after sowing (Table 10).

The analysis of variance exhibited that there was no significant (P ≤ 0.05) differences on day to 50% flowering among all cultivars (Table 10). The longest (51 and 50 days) period to flowering was recorded on the cultivar Gora and Local cultivars, respectively while the shortest (47 days) period to flowering was taken by the cultivar NC-58. However, the cultivars did not showed any significant variations between them, in days to 50% flowering date (Table 10). Analysis of variance showed that there was significant differences (P ≤ 0.05) day to 90 % maturity of faba bean cultivars. When compared to three cultivars, NC-58 cultivar showed significantly different from Gora and local cultivars (Table 10). But there were no significant differences on Gora and Local cultivars. There were no significant differences (P ≤ 0.05) day to 90% maturity regardless of bio-agents treated, fungicide applied and cultivars (Table 11). The longest durations of 128.3 and 127.3 days to 90% maturity were recorded for the cultivars of Local and Gora, respectively. These results are also relatively confirmed by the findings of Tamene *et al.* (2013) who reported that the faba bean cultivar Gora to attained such its maturity. On the other hand, the cultivar NC-58 reached to 90% maturity in relatively a shorter period of 121.4 days after sowing; even though statistically not significant (Table 11). Thus the variability in attaining the maturity for the cultivars might be attributed to their inherent genetic variability, environmental conditions and the effect of the disease.

Table 10 Crop growth parameters of faba bean cultivars as affected chocolate spot

Varieties	Plant growth parameters				
	ED	FD	PSD	MD	PH
Local	13.67 ^a	50.67 ^a	94.0 ^a	126.0 ^a	134.6 ^a
Gora	13.0 ^{ab}	51.0 ^a	91.67 ^a	127.7 ^a	136.3 ^a
NC-58	12.67 ^b	47.0 ^a	91.0 ^a	121.3 ^b	132.4 ^a
Mean	13.11	49.56	92.22	125	134.4
LCD (0.05)	0.76ns	5.527ns	5.604ns	4.341*	31.72ns
CV (%)	2.5	4.9	2.7	1.5	10.4

ED= emergency day, FD= flowering day, PSD=pod setting day, MD= maturity day, PH= plant height, ns=non-significant, LSD= least significant difference, CV= coefficient of variations, **= highly significant difference at (P<0.01), *= significant difference at (P<0.05)

Table 11 Crop growth parameters of faba bean cultivars treated with fungicides and bio-control agents against chocolate spot under field conditions

Varieties	Fungicides and Bio-agents	Plant growth parameters			
		FD	PSD	MD	PH
Local	Mancozeb 80% WP	51.0 ^a	91.67 ^c	128.3 ^a	135.6 ^a
	Folpan 80 WDG	50.67 ^a	92.67 ^b	128.0 ^a	142.5 ^a
	Mancolaxyl 72 WP	50.67 ^a	92.67 ^b	127.0 ^a	139.1 ^a
	<i>T. harzianum</i>	50.67 ^a	91.0 ^{ed}	124.30 ^b	140.0 ^a
	<i>T. viride</i>	50.33 ^a	90.67 ^d	126.0 ^{ab}	142.8 ^a
	Unsprayed(control)	50.33 ^a	94.0 ^a	126.0 ^{ab}	140.1 ^a
	LSD (0.05)	1.033ns	0.920**	2.433*	17.39ns
CV	1.1	0.5	1.1	6.8	
Gora	Mancozeb 80% WP	50.33 ^a	91.33 ^a	127.3 ^a	134.0 ^a
	Folpan 80 WDG	49.0 ^b	91.0 ^a	127.3 ^a	133.5 ^a
	Mancolaxyl 72 WP	50.0 ^{ab}	93.0 ^a	127.0 ^a	136.5 ^a
	<i>T. harzianum</i>	50.33 ^a	91.0 ^a	126.3 ^a	125.2 ^a
	<i>T. viride</i>	50.0 ^{ab}	92.0 ^a	127.0 ^a	133.5 ^a
	Unsprayed(control)	50.0 ^{ab}	91.67 ^a	126.7 ^a	140.7 ^a
	LSD (0.05)	1.315ns	3.098ns	2.692ns	18.54ns
CV (%)	1.4	1.9	1.2	7.6	
NC-58	Mancozeb 80% WP	47.33 ^a	90.67 ^{ab}	121.3 ^b	133.6 ^a
	Folpan 80 WDG	47.33 ^a	88.67 ^b	121.3 ^b	130.1 ^a
	Mancolaxyl 72 WP	47.0 ^a	91.0 ^{ab}	122.0 ^{ab}	138.0 ^a
	<i>T. harzianum</i>	47.0 ^a	89.0 ^b	122.7 ^a	140.3 ^a
	<i>T. viride</i>	48.0 ^a	88.67 ^b	121.3 ^{ab}	134.0 ^a
	Unsprayed(control)	47.0 ^a	92.33 ^a	121.3 ^{ab}	132.4 ^a
	LSD (0.05)	1.51ns	2.478*	1.473ns	16.7ns
CV (%)	1.8	1.5	0.7	6.8	

FD= flowering day, PSD= pod setting day, MD= maturity day, PH= plant height, ns=non-significant, LSD= least significant difference, CV= coefficient of variations, **= highly significant difference (P<0.01), *= significant difference (P<0.05).

Analysis variance exhibited the plant height was not significantly different among all treatments interval. There was no significant difference between all the cultivars, fungicides sprayed and bio-agents treated plots (Tables 11 and 12). Data on yield parameters showed highly significant differences (P<0.01) among treatments in the number of pods per plant, seeds per pod and seed yield, and 100 seeds weight. Plots treated with Mancozeb foliar spray fungicide gave the highest number of pods per plant followed by Folpan and Mancolaxyl foliar spray fungicides treated plots. Among the bio agents, *T. harzianum* gave the highest number of pods per plant (Tables 12 and 13).

Yield and yield components

Data on yield parameters showed highly significant differences (P<0.01) among treatments in the number of pods per plant, seeds per pod and seed yield, whereas, no significant differences were observed in 100 seeds weight. Plots treated with Mancozeb foliar spray fungicide gave the highest number of pods per plant followed by Folpan and Mancolaxyl foliar spray fungicides. Among the bio agents, *Trichoderma harzianum* gave the highest number of pods per plant (Tables 12 & 13). There was no significant difference (P<0.05) on number of pod per plant between all cultivars (Table 12). The results showed that the recorded levels of chocolate spot incidence and severity did not influence faba bean number of pods per plant between cultivars. These results are also relatively confirmed by the findings of Tamene *et al.* (2013) who reported that the faba bean cultivar Gora to attained chocolate spot incidence and severity did not influence faba bean number of pods per plant between cultivars and its maturity. Analysis of pods per plant indicated that there was a significant difference on number of pods per plant (P < 0.05) among fungicides sprayed cultivars of Gora and NC-58 and bio-agents (*T. harzianum* and *T. viride*) treated plots and unsprayed (control) plots of these two cultivars (Table 12). Mancozeb 80% WP treated plots of Gora cultivar showed significant differences from other the two fungicides (Folpan 80 WDG and Mancolaxyl 72 WP). Also Mancozeb 80% WP sprayed NC-58 cultivar plots showed significant different from Mancolaxyl 72 WP sprayed plots of NC-58 cultivar (Table 12). But non-significant different on Local cultivar in all fungicide sprayed plots. There was no significant difference (P<0.05) on number seeds per pod among treatments (regardless of the cultivars, fungicides, and bio-agents). The number seeds per pod were not significant differences among the three fungicides sprayed, as well as between bio-agents treated and the three cultivars (Table 12). Number of seeds per pod on faba bean is dependent on the amount of fertile florets. Chocolate spot attack on pods did not reduce the number of seeds per pod rather than, reduce size and weight of seeds (Table 12). There were highly significant differences (P<0.01) on hundred seeds weight (HSW) among the cultivars (Table 12). Gora and Local cultivars were significant different (P < 0.05) from NC-58 cultivar. This was due to Gora and Local cultivars were large

seed size than cultivar of NC-58. Because of their seed size difference impossible to compared hundred seeds weight of NC-58 with hundred seeds weight of Gora and Local cultivars. The weight of hundred seeds of cultivars directly related to its seeds size (Table 12).

In all three cultivars there were highly significant differences ($P < 0.01$) on hundred seeds weight between fungicides sprayed, bio-agents treated and unsprayed (control) plots within the same cultivars (Table 13). Mancozeb 80% WP and Folban 80 WDG fungicides spray treated plots of Gora cultivar was significantly different for hundred seeds weight (Table 13). Bio-agents treated Gora cultivar was significantly different for hundred seeds weight of unsprayed control. All three fungicides sprayed Local cultivar plots were significantly different for hundred seeds weight from bio-agents treated and unsprayed control of Local cultivar. Mancozeb 80% WP Fungicide sprayed Local cultivar was significantly different on hundred seeds weight from Mancozeb 72 WP fungicide sprayed plots of this cultivar (Table 13). These results are also relatively confirmed by the findings of Tamene *et al.* (2013) who reported that the faba bean cultivars to attained chocolate spot incidence and severity was influenced faba bean yield and yield components particularly number of seeds/ pod and hundred seeds weight between the cultivars and its maturity. There were highly significant difference ($P \leq 0.01$) on grain yield in Kg/ha among the cultivars (Table 12). Gora and Local cultivars were significantly different ($p \leq 0.05$) from NC-58 cultivar but no significant different between the two cultivars. However, higher yield was obtained from Gora cultivar as compared to Local cultivar (Tables 12 and 13). In all the three cultivars, there were highly significant differences ($P \leq 0.01$) on grain yield between the fungicides sprayed and bio-agents treated plots when compared to unsprayed (control) plots within the same cultivars (Table 13). There were significant differences (<0.05) grain yield kg/ha between bio-agents treated and untreated (control) plots of NC-58 and Local cultivars. But there were no significant differences between the two bio-agents treated plots of NC-58 and Local cultivars. Similarly there was a significant different grain yield recorded on Gora cultivar between bio-agents treated plots when compared to untreated (control) plots (Table 13).

Generally, the highest yield (3731 kg/ha) was recorded on Gora cultivar which was sprayed with Mancozeb 80% WP fungicide followed by Folban 80 WDG fungicide sprayed plots (3510 kg/ha) and the third highest was 3496 kg/ha from Local cultivar which was sprayed with Mancozeb 80% WP fungicide. The lowest grain yield (1420 kg/ha) was assessed from fungicide sprayed and bio-agents treated cultivar of NC-58 plots (Table 13). Furthermore, the highest grain yield 3731 kg/ha was recorded on Gora cultivar that was sprayed with Mancozeb 80% WP and the lowest yield of 2513 kg/ha was recorded from unsprayed (control) plot of this cultivar. The highest 3510 kg/ha and the lowest grain yield on Local cultivar was recorded from Folban 80 WDG sprayed and unsprayed (control) plots, respectively. The highest grain yield on NC-58 cultivar was 2954 kg/ha which sprayed with Mancozeb 80% WP whereas the lowest of this cultivar was 1420 kg/ha from unsprayed (control) plots. These results are also relatively confirmed by the findings of Tamene *et al.* (2013) who reported that the faba bean cultivars to attained chocolate spot incidence and severity which was influenced faba bean yield and yield components between the cultivars and its maturity.

Table 12 Yield and Yield components of faba bean cultivars as affected by chocolate spot

Varieties	Yield and Yield components			
	PPP	SPP	HSW (gm)	Yield (kg/ha)
Local	7.5 ^a	2.797 ^a	63.4 ^a	2064 ^a
Gora	6.3 ^a	2.75 ^a	74.37 ^a	2513 ^a
NC-58	5.943 ^a	2.603 ^a	42.13 ^b	1420 ^b
Mean	6.58	2.72	60	1999
LSD (0.05)	2.615ns	0.717ns	11.08**	487.1**
CV (%)	17.5	11.6	8.2	10.8

PPP= pod per plant, SPP= seed per pod, HSW= hundred seed weight, kg/ha= kilo gram per hectare, ns=non-significant, LSD= least significant difference, CV= coefficient of variations, **= highly significant difference at ($P < 0.01$), *= significant difference at ($P < 0.05$)

Table 13 Yield and yield components of faba bean cultivars treated with fungicides and bio-control agents against chocolate spot under field conditions

Cultivars	Fungicides and Bio-agents- Treatments	Yield and yield components			
		PPP	SPP	HSW (gm)	Yield (kg/ha)
Local	Mancozeb 80% WP	9.01 ^a	2.893 ^a	82.33 ^a	3496 ^a
	Folpan 80 WDG	7.61 ^a	3.09 ^a	79.4 ^{ab}	3340 ^{ab}
	Mancolaxyl 72 WP	8.557 ^a	2.817 ^a	78.37 ^b	3319 ^{ab}
	<i>T. harzianum</i>	8.0 ^a	2.923 ^a	70.3 ^c	2800 ^{bc}
	<i>T. viride</i>	8.277 ^a	2.713 ^a	68.2 ^c	2744 ^c
	Unsprayed(control)	7.5 ^a	2.797 ^a	63.4 ^d	2064 ^d
LSD (0.05)		1.693ns	0.5594ns	2.972**	540.4**
CV		11.4	10.7	2.2	10
Gora	Mancozeb 80% WP	10.433 ^a	2.65 ^a	89.17 ^a	3731 ^a
	Folpan 80 WDG	9.01 ^{ab}	2.717 ^a	87.2 ^a	3510 ^a
	Mancolaxyl 72 WP	8.41 ^{bc}	2.663 ^a	82.6 ^b	3438 ^a
	<i>T. harzianum</i>	6.61 ^{cd}	2.737 ^a	80.27 ^b	2902 ^b
	<i>T. viride</i>	6.2 ^d	2.37 ^a	79.8 ^b	2837 ^b
	Unsprayed(control)	5.943 ^d	2.75 ^a	74.37 ^c	2513 ^b
LSD (0.05)		2.018**	0.5024ns	3.142**	494.3**
CV (%)		14.3	10.4	2.1	8.6
NC-58	Mancozeb 80% WP	9.353 ^a	2.477 ^a	68.47 ^a	3032 ^a
	Folpan 80 WDG	9.033 ^{ab}	2.13 ^a	65.43 ^a	2954 ^a
	Mancolaxyl 72 WP	8.233 ^{bc}	2.613 ^a	65.43 ^a	2896 ^a
	<i>T. harzianum</i>	7.967 ^c	2.603 ^a	56.1 ^b	2279 ^b
	<i>T. viride</i>	7.333 ^c	2.417 ^a	55.37 ^b	2236 ^b
	Unsprayed(control)	6.3 ^d	2.603 ^a	42.13 ^c	1420 ^c
LSD (0.05)		0.912**	0.4923ns	4.91**	604.1**
CV (%)		6.2	10.9	4.6	13.4

PPP= pod per plant, SPP= seed per pod, HSW= hundred seed weight, Kg/ha= kilo gram per hectare, ns=non-significant, LSD= least significant difference, CV= coefficient of variations, **= highly significant difference at (p<0.01), *= significant difference at (P<0.05)

Relative Yield loss

A relative reduction in yield (kg/ha) due to the disease occurred in all three cultivars. Yield losses were computed relative to the average yield from plots with the maximum protection against the disease (i.e. plots with highest yield and lowest disease severity in each cultivar). Nevertheless, the grain yield losses were reduced by all fungicides and bio-agents treated plots as compared with the unsprayed control plots (Table 14). The yield loss was significantly different (P < 0.05) in all three cultivars. The highest i.e. 53.17% relative yield losses occurred on the unsprayed control NC-58 susceptible cultivar. The second highest 40.96% relative yield losses occurred on the unsprayed control plot of Local cultivar. Relatively, lowest yield loss of 2.57% and 4.46% were recorded from plots sprayed with Folpan 80 WDG on NC-58 cultivar and Mancolaxyl 72 WP on Local cultivar, respectively. Relative yield losses of 2.57-53.17% on NC-58 cultivar, 4.46-40.96% on Local cultivar and 5.92-33.65% on Gora cultivar were recorded (Table 14). The highest 28.27 % relative yield loss occurred on the unsprayed NC-58 susceptible cultivar when compared to the bio-agent treated plots and the second highest yield loss was recorded (27.15%) in unsprayed (control) Local cultivar plots with *T. harzianum*. Relative yield losses of 26.25-53.17 % on NC-58 cultivar when compare with Bio-agents, 12.52-40.96 % on Local cultivar and 22.22-33.65 % on Gora cultivar were recorded compared with Bio-agents treated plots.

The hundred seeds weight (HSW) losses were computed relative to the average hundred seeds weight from plots with the maximum protection against the disease (i.e. plots with highest HSW and lowest disease severity in each cultivar). The highest 38.47% relatively hundred seeds weight loss occurred on unsprayed of NC-58 cultivar and followed 22.99% relatively hundred seeds weight loss occurred on Local cultivar (Table 14). Relatively less hundred seeds weight reduction occurred on cultivar Gora due to lower severity index. These results are also relatively confirmed Chocolate spot can cause up to 61% yield losses on susceptible genotypes and 34% on tolerant genotypes under Ethiopian conditions (Dereje and Yaynu, 2001). A yield loss of up to 68% was reported on CS20DK and local cultivars of faba bean under rain fed conditions of

Northern Ethiopia, who reported that the faba bean cultivars to attained chocolate spot disease incidence and severity which was influenced faba bean yield losses between the cultivars (Sahile *et al.*, 2010).

Table 14 Yield loss of faba bean cultivars due to chocolate spot

Cultivars	Fungicides and Bio-agents-Treatments	Yield and relative yield loss					
		Yield (kg ha ⁻¹)	RYL (Kg/ha)	RYL (%)	HSW (gm)	Loss (gm)	Loss (%)
Local	Mancozeb 80% WP	3496 ^a	0.0	0.0	82.33 ^a	0.0	0.0
	Folpan 80 WDG	3340 ^{ab}	177	5.063	79.4 ^{ab}	2.93	3.56
	Mancolaxyl 72 WP	3319 ^{ab}	156	4.462	78.37 ^b	3.96	4.81
	<i>T. harzianum</i>	2800 ^{bc}	696	12.908	70.3 ^c	12.03	14.61
	<i>T. viride</i>	2744 ^c	752	21.51	68.2 ^c	14.13	17.16
	Unsprayed (control)	2064 ^d	1432	40.96	63.4 ^d	18.93	22.99
Gora	Mancozeb 80% WP	3731 ^a	0.0	0.0	89.17 ^a	4.87	5.58
	Folpan 80 WDG	3510 ^a	221	5.923	87.2 ^a	0.0	0.0
	Mancolaxyl 72 WP	3438 ^a	293	7.853	82.6 ^b	4.6	5.28
	<i>T. harzianum</i>	2902 ^b	829	22.22	80.27 ^b	6.93	7.95
	<i>T. viride</i>	2837 ^b	894	23.96	79.8 ^b	7.4	8.49
	Unsprayed (control)	2513 ^b	1218	33.65	74.37 ^c	12.83	14.71
NC-58	Mancozeb 80% WP	3032 ^a	0.0	0.0	68.47 ^a	0.0	0.0
	Folpan 80 WDG	2954 ^a	78	2.573	65.43 ^a	3.04	4.44
	Mancolaxyl 72 WP	2896 ^a	136	4.485	65.43 ^a	3.04	4.44
	<i>T. harzianum</i>	2279 ^b	796	26.25	56.1 ^b	12.37	18.07
	<i>T. viride</i>	2236 ^b	753	24.901	55.37 ^b	13.1	19.13
	Unsprayed (control)	1420 ^c	1612	53.17	42.13 ^c	26.34	38.47
Mean		2862			71.57		
LSD (0. 05)		492.9(**)			4.193(**)		
CV (%)		10.4			3.5		

RYL=relative yield loss, kg ha⁻¹= kilogram per hectare, HSW= hundred seed weight, gm=gram, ns=non-significant, LSD= least significant difference, CV= coefficient of variations, **= highly significant difference at (P<0.01), *= significant difference at (P<0.05).

Correlation between yield and disease parameters

Correlation analysis of severity, AUDPC and yield exhibited highly significant (P<0.01) association with different fungicides and bio-agents treatments (Table 15). The severity and AUDPC values were negative but significant correlation was found between grain yield and hundred seeds weight (HSW). The values of the disease severity were correlated with coefficient ranged from -0.51 to -0.80 for hundred seeds weight and from -0.54 to -0.85 for grain yield (Table 15). Similarly, AUDPC was strong negative correlation with hundred seeds weight (-0.78) and grain yield (-0.85). AUDPC and severity themselves were even highly and positively (0.54 to 0.96) correlated with each other except 72 DAS disease severity non-significant. The number of pods per plant was positively correlated with grain yield and hundred seeds weight. However, there was highly negative correlated with disease severity and AUDPC. The number of seeds per pod was except 72 DAS and 92 DAS non-significant correlated with disease severity and highly negative correlated with AUDPC. Number of seeds per pod was non-significant correlated with number of pods per plant. Consequently, the result of this study indicated that the yield of the faba bean crop was significantly affected by disease severity that also influenced the AUDPC.

Table 15 Coefficients (r) linear correlation between of disease parameters and yield as well as yield components under field conditions

parameters	Disease severity days after sowing					AUDPC	PPP	SPP	HSW	Grain yield
	72	82	92	102	112					
AUDPC	0.07ns	0.93**	0.54**	0.96**	0.94**	1				
PPP	-0.35**	-0.50**	-0.41**	-0.49**	-0.46**	-0.50**	1			
SPP	0.79**	-0.04ns	0.52**	-0.01ns	-0.14ns	-0.37**	-0.09ns	1		
HSW	-0.51**	-0.66**	-0.70**	-0.76**	-0.80**	-0.78**	0.31*	0.24ns	1	
Grain yield	-0.54**	-0.77**	-0.76**	-0.79**	-0.85**	-0.85**	0.49**	0.23ns	0.84**	1

AUDPC= area under disease progress curve, PPP= pods per plant, SPP= seeds per pod, HSW= hundred seeds weight, **, *, Correlation is significant at the (p<0.01) and (P<0.05) significance level, respectively and ns= non-significant;

The regression analysis of the final severity (112 DAS) as predictor with yield (dependent variable) showed a significant (p ≤ 0.01) relationship. The regression equation of the grain yield (kg ha⁻¹) = - 423.5X + 4333.54 (r² = 92.6%, p = 0.000) demonstrated reduction of about 423.5 kg ha⁻¹ grain yield with the increase of 1% severity (Figure 5). Similarly, regression analysis of the final severity (112 DAS) as predictor with hundred seeds weight (dependent variable) showed a significant (P ≤ 0.01) relationship. The regression equation of the hundred seed

weight (g) = - 7.63X + 98.09, ($r^2 = 69\%$, $P = 0.000$) demonstrated reduction of about 7.63 gram in HSW with the increase of 1% severity (Figure 6). The values of coefficient of determination (R^2) explained that 92.6% of the losses in grain yield were due to the effect of the faba bean “chocolate spot” disease infection of faba bean estimated as the final severity on the yield loss (%). (The independent variable ‘x’ indicates the disease severity level in percentage).

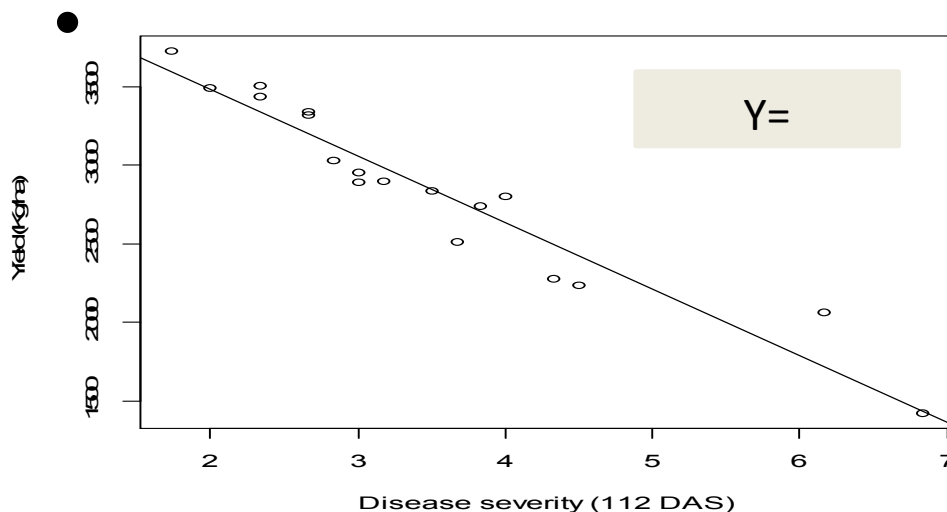


Figure 5 Linear regression of grain yield and disease severity,

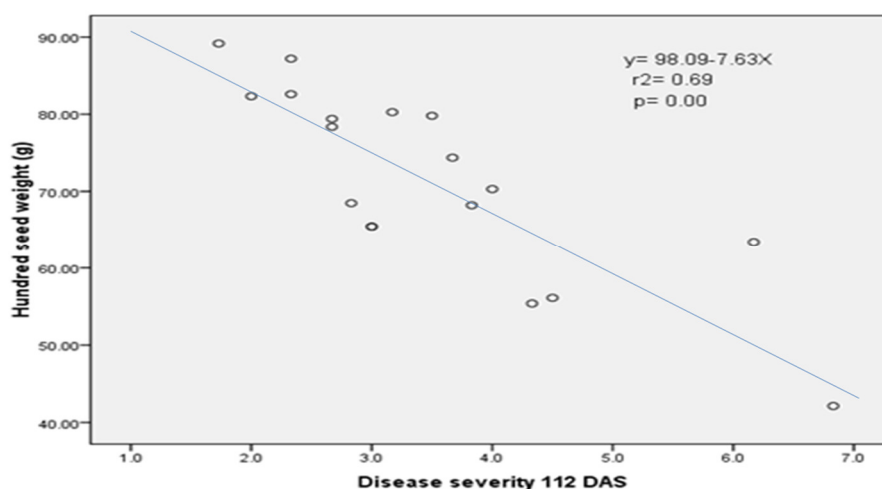


Figure 6. Linear regression of hundred seed weight and disease severity.

Cost benefit analysis

The net benefit exhibited variation among fungicides application and bio-agents treated plots. Partial budget analysis was calculated based on cost of variable inputs of the year 2016 cropping season and net benefit was estimated based on mean of local market price and farmers supplied produce to the market. Mancozeb 80% WP fungicide applied on three cultivars exhibited maximum partial cost benefit from all plots that means the highest (56135.00 ETB ha⁻¹) was recorded on cultivar Gora, followed by on Local cultivar (52415.00 ETB ha⁻¹). The Mancolaxyl 72 WP and Folpan 80 % WDG applied fungicides plots also showed good results. The highest cost net benefit was recorded on Folpan 80 WDG fungicide treated plots on Gora (50637.00 ETB ha⁻¹) cultivar followed by Local cultivar (47692.00 ETB ha⁻¹), respectively. The highest cost benefit (45285.00ETB ha⁻¹) was obtained from plots sprayed with Mancozeb 80% WP fungicide on NC-58 cultivar.

Table 16 Partial budget analysis for chocolate spot management in faba bean

Cultivars	Fungicides and Bio-agents Treatment	General cost benefit						
		(A) adj. yield(t.ha-1)	(B) price (ETBt-1)	(C) sale revenue (A*B)	(D) marginal cost (ETBha-1)	(E) net profit (ETB (C-D))	(F) marginal benefit (ETB)	MRR (F/D)(%)
Local	Mancozeb 80% WP	3.49	15500	54095	1680	52415	20485	1219.3
	Folpan 80 WDG	3.32	15500	51460	3768	47692	15762	418.3
	Mancolaxyl 72 WP	3.34	15500	51770	2130	49640	17710	831.4
	<i>T. harzianum</i>	2.8	15500	43400	950	42450	10520	1107.4
	<i>T. viride</i>	2.74	15500	42470	950	41520	9590	1009.5
	Unsprayed(control)	2.06	15500	31930	0.0	31930	0.0	0.0
Gora	Mancozeb 80% WP	3.73	15500	57815	1680	56135	17230	1025.6
	Folpan 80 WDG	3.51	15500	54405	3768	50637	11732	311.4
	Mancolaxyl 72 WP	3.44	15500	53320	2130	51190	12285	576.7
	<i>T. harzianum</i>	2.9	15500	44950	950	44000	5095	536.3
	<i>T. viride</i>	2.84	15500	44020	950	43070	4165	438.4
	Unsprayed(control)	2.51	15500	38905	0.0	38905	0.0	0.0
NC-58	Mancozeb 80% WP	3.03	15500	46965	1680	45285	23275	1385.4
	Folpan 80 WDG	2.95	15500	45725	3768	41957	19947	529.3
	Mancolaxyl 72 WP	2.89	15500	44795	2130	42665	20655	969.7
	<i>T. harzianum</i>	2.24	15500	34720	950	33770	11760	1237.9
	<i>T. viride</i>	2.28	15500	35340	950	34390	12380	1303.2
	Unsprayed(control)	1.42	15500	22010	0.0	22010	0.0	0.0

Adj. yield= adjusted yield, ETBt⁻¹= Ethiopian birr per tons, ETBha⁻¹= Ethiopian birr per hectare, MRR= marginal rate return.

Conclusions

This study results showed that faba bean cultivars had different responses to the chocolate spot disease under field conditions. The cultivar Gora showed lower levels of disease incidence and NC-58 and Local cultivars exhibited high level of disease incidence. Specially, NC-58 cultivar at 112 DAS assessment, the levels of disease incidence was 100% on control plots. The highest disease severities (72.16 and 64.96%) were recorded from the cultivar NC-58 (control) and Local (control), respectively, at the final assessment date (112 DAS). The highest disease severity revealed that the susceptibility of these cultivars to chocolate spot. At this assessment date, the cultivar Gora (control) showed lowest (42.96%) disease severity which moderately resistance to the disease cultivar. The lowest disease severities (18.63 and 28.2 %) were recorded on Gora cultivar plots sprays treated with Mancozeb 80% WP followed by Folpan 80 WDG, respectively. On NC-58 cultivar, the lowest disease severities (35.9 and 36.75%) were recorded from Mancozeb 80% WP followed by Folpan 80 WDG sprayed plots. The lowest grain yield 1420 Kg/ha from cultivar of NC-58 (control). The maximum grain yields from fungicides sprayed 3731 and 3510 Kg/ha were obtained from the cultivar Gora which sprayed Macozeb 80% WP and Folpan 80 WDG, respectively. The relative yield losses of 1618 and 1432 % were recorded from the unsprayed control plots of NC-58 and Local cultivar, respectively. Application of Mancozeb 80% WP and Folpan 80 WDG fungicides sprayed relatively grain yield increase of 32.65 and 30.72 % on Gora cultivar, 40 and 38.22 % on Local cultivar and 53.17 and 51.8 on NC-58 cultivar increases over the unsprayed control plots, respectively. The bio-agents *T. harzianum* and *T. viride* treated plots relatively grain yield increase 25.4 and 24.83 % on Gora cultivar, 32.04 and 31.4 % on Local cultivar and 40 and 39.2% on NC-58 cultivar increases over the unsprayed control plots, respectively. Mancozeb 80% WP fungicide applied on three cultivars exhibited maximum partial cost net benefit from all plots that means the highest (56135.00 ETB ha⁻¹) was recorded on cultivar Gora, followed by on Local cultivar (52415.00 ETB ha⁻¹) and on NC-58 cultivar (45285.00 ETB ha⁻¹). Also the highest cost net benefit was recorded on Folpan 80 WDG fungicide treated plots on Gora (50637.00 ETB ha⁻¹) cultivar followed by Local cultivar (47692.00 ETB ha⁻¹), respectively. The results of the present study revealed that the novel possibility of using Mancozeb foliar spray with three times at the rate of 2 kg active ingredient per hectare at threshold level was recommended in Ethiopia and *Trichoderma harzianum* seed treatments which were found to be an effective in decreasing chocolate spot disease symptoms on faba bean cultivars in Kellem Wollega, Western Oromiya and increased yield. This study could be provided some evidences on the response of faba bean cultivars, effect of bio-control agents and efficacies of fungicides and its application frequencies for the disease development and substantial increase on yield. Management options of chemical fungicides and bio-control agents could be recommended to the farmers in Ethiopia for this disease to control.

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