

# Chemical Control of Faba Bean Chocolate Spot (*Botrytis fabae*) in Bale Highland, Ethiopia

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## Abstract

Chocolate spot is a serious disease that cause yield reduction on faba bean crop, thus, effective management is essential. The objective of this study was to manage chocolate spot of faba bean with host resistance and fungicides in Bale highlands. Field experiments were conducted at Madda Walabu University (MWU) and Harewa research sites during 2017. Treatments were designed in RCBD factorial with three replications. Four fungicides such as mancozeb, fungozeb, nativo and diprocon were evaluated at the rate of 2.5kg/ha, 2.5kg/ha, 0.75L/ha and 0.45L/ha respectively against four varieties. Fungicide application significantly reduced chocolate spot severity, AUDPC and disease progress rate. The highest chocolate spot PSI, AUDPC and disease progress rate was recorded from local unsprayed plot at both sites; whereas the lowest chocolate spot PSI (3.7%) was recorded from walki treated with fungozeb. Maximum grain yield was recorded from plots treated with fungozeb fungicide at both locations. Further study should be conducted on the frequency of those fungicides to identify the optimum level of application and thereby increase production and productivity of faba bean in the region and elsewhere with similar agroecological settings.

**Keywords:** AUDPC; *Botrytis fabae*; fungicide; PSI

## 1. INTRODUCTION

Faba beans (*Vicia faba* L.) is assigned to the central Asian, Mediterranean, and South American centers of diversity and believe to be a native to North Africa and Southwest Asia (Zohary and Hopf, 2000). It is an important pulse crop and occupies nearly 30.09 million hectares of land worldwide. It is an annual legume with one or more rigid, hollow and erect stems. It is produced in the world as source of protein (Mousa and El-Sayed, 2016). The protein fraction could be used for animal feed and the carbohydrate-rich fraction for biofuel production, as proposed for other legumes (Lamb *et al.*, 2014; Amato *et al.*, 2016).

It is an excellent complement of crop rotations for fixing atmospheric nitrogen (Jensen *et al.*, 2010; Ali *et al.*, 2014) and as green manure (Salmoeron *et al.*, 2010). In addition, faba bean is used as a source of cash crop for farmers and foreign currency in Ethiopia (Keneni and Jarso, 2002; Agegnehu and Fessehaie, 2006; Abebe *et al.*, 2014). Ethiopia is the second largest producer of faba bean after China in the world (FAOSTAT, 2017). In Ethiopia, faba bean is grown in the highlands (1780–3000 m.a.s.l.) with 700–1000 mm annual rainfall (Yohannes 2000). The area of faba bean production in Ethiopia has increased by 18.21% from 2010 cropping season to 2016; even though it has been increased there is no significant change in production due to biotic and abiotic factors (Agegnehu *et al.*, 2006; Sahile *et al.*, 2008; Shifa *et al.*, 2011; Ademe *et al.*, 2018).

As result of climate change different fungal pathogen evolved in new ways and faba bean production is seriously affected (Khan *et al.*, 2010; Sahile *et al.*, 2012). Thus, climate change and associated changes in disease scenarios will demand changes in crop and disease management strategies. Faba bean chocolate spot caused by *Botrytis fabae* is the most important disease throughout Europe, South East Asia, South America, Korea, India, Canada, Norway, the Middle east, north east and southern Africa, Australia, and Ethiopia (Akem and Bellar, 1999; Bouhassan *et al.*, 2004; Tivoli *et al.*, 2006; Sahile *et al.*, 2008). Yield losses as high as 90% and total crop failure in severe epidemics of *Botrytis fabae* have been reported from areas where extended periods of wet weather conditions prevail (Singh *et al.*, 2013). Chocolate spot is a major limiting factor in the main faba bean growing regions of Ethiopia and yield losses vary from 34 to 61 % (Dereje and Yaynu, 2001; Sahile *et al.*, 2012).

Many methods of control are possible such as the use of resistant genotypes, chemicals (fungicides), and biological, induced resistant and modified cultural practices. In fact, the amount of losses in seed yield due to a disease determines the importance of that disease. A chemical is recommended for use when the cost of its application equals to or is less than the returns gained. Management options for chocolate spot disease in Ethiopia include the use of resistant cultivars, chemical control (Mancozeb) and late planting, but these options were neither widely disseminated nor adopted by end users (farmers) (Agegnehu *et al.*, 2006). Sahile *et al.* (2008) reported that the cereal mixed cropping and fungicide application consistently reduced chocolate spot severity and increased the yield correspondingly. El-Sayed *et al.* (2011) also reported that Dithane M45, Galben manozeb and copper oxychloride chemicals reduced the growth of *B. fabae* under *in vitro* conditions. Generally, faba bean chocolate spot is serious problems in our country including Bale zone, therefore, integrating compatible disease control measures was needed to complement one another and minimize yield loss, the

objective of the present study was to evaluate the performance of fungicides and host resistance against faba bean chocolate spot in Bale highlands, Ethiopia.

## 2. MATERIALS AND METHODS

### 2.1. Experimental sites

Field experiments were conducted at Madda Walabu University (MWU) and Harewa Research sites during the main cropping in 2017. The two sites are characterized by weather conditions conducive for chocolate spot epidemic development but differ mainly in their altitude, temperature, and annual total rainfall (Table 1).

**Table 1. Characteristic features of the chocolate spot experimental sites in Bale highlands, Ethiopia**

| Experimental Site | Location                  | Altitude (m.a.s.l.) | Annual rain fall (mm) | Mean Minimum Temperature (°C) | Mean Maximum Temperature (°C) |
|-------------------|---------------------------|---------------------|-----------------------|-------------------------------|-------------------------------|
| MWU               | 6° 5'55" N - 39° 56'57" E | 2415                | 823                   | 9.4                           | 24.2                          |
| Harewa            | 6° 40'23" N- 40°15'24" E  | 2089                | 925                   | 15                            | 29                            |

### 2.2. Experimental design and treatments

A randomized complete block design was arranged in a factorial combination of four fungicides (Mancozeb, Fungozeb, Diprocon and Nativo) and four varieties (Local, Hachalu, Shallo and Walki) in three replications. The fungicides were sprayed at doses and schedules indicated in Table 2. Spraying of the fungicides was started 58 days after planting (DAP) at MWU and 82 DAP at Harewa. Control plots were sprayed with pure water. During fungicide sprays, plastic sheet was used to separate the plot being sprayed from the adjacent plots to prevent inter-plot interference of spray drift. The spacing was 1m between blocks, 0.5m between plots, 0.4m between rows and 0.1m between plants. Faba bean grain yield was harvested from middle rows of each plot, leaving two outer rows on both sides to avoid the border effect and adjusted at 9% seed moisture content. The yield data of the plots were converted to Kilogram per hectare. The plots were fertilized with diammonium phosphate at the rate of 100 kg ha<sup>-1</sup>. Unsprayed check plots was left in every replication as control. Weeding was done manually as needed. Disease development was entirely based on natural inoculum.

**TABLE 2. LIST OF FUNGICIDES, THEIR RESPECTIVE DOSES AND SPRAY SCHEDULES**

| Fungicide      | Dosage     | Fungicide spray schedules                          |
|----------------|------------|--|
| Mancozeb 80WP  | 2.50 kg/ha | Every 7 days (5 sprays at MWU, 3 sprays at Harewa) |
| Fungozeb 80WP  | 2.50 kg/ha | Every 7 days (5 sprays at MWU, 3sprays at Harewa)  |
| Diprocon 30 EC | 0.75 L/ha  | Every 14 days (3sprays at MWU, 2 sprays at Harewa) |
| Nativo SC 300  | 0.45 L/ha  | Every 14 days(3 sprays at MWU, 2 sprays at Harewa) |
| Control        | No spray   |  |

### 2.3. Disease assessment

Severity of faba bean chocolate spot was assessed eight times at MWU and four times at Harewa at weekly intervals starting from the first appearance of the disease symptoms in the experimental plots. Disease severity was recorded from 10 randomly selected and pre-tagged plants in the central rows of each plot separately, for the three layers of the canopy (top, middle and bottom). Severity was rated using a 1–9 scale, where 1 indicates no visible symptom and 9 represents disease covering greater than 80% of leaf area (ICARDA, 1986). Disease severity scores were converted into percentage severity index (PSI) for analysis (Wheeler, 1969).

$$PSI = \frac{\text{Sum of numerical ratings} \times 100}{\text{Number of plants scored} \times \text{Maximum score on scale}}$$

Means of canopy layers were determined per plant and then mean per plot was determined for data analysis. Area under diseases progressive curve (AUPDC) was calculated by using the formula

$$AUDPC = \sum_i^{n-1} \left( \frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

Where  $y_i$  is the disease severity expressed in percentage at the  $i^{\text{th}}$  observation,  $t_i$  is the time of  $i^{\text{th}}$  assessment in days and  $n$  is the total number of days disease was assessed. Because severity was expressed in percentage and time in days, AUDPC was expressed in % days. Since the epidemic period of the two locations varied, AUDPC were standardized by dividing the values to the epidemic period of the respective locations (Campbell and Madden, 1990). The epidemic periods were 56 and 28 days at MWU and Harewa, respectively and AUDPC values were standardized accordingly.

## 2.4. Data Analysis

Disease progression of chocolate spot from each treatment was calculated by transforming the percent disease severity values to the Gompertz model  $\ln[-\ln(Y)]$ , where 'y' is disease severity scores in proportion (Van der Plank 1963). The transformed data were then regressed over time (as DAS) so as to get the disease progress rate, which is the coefficient of the regression line. The Gompertz model was chosen because it had the best fit to the data based on coefficients of determination and standard errors for y. AUDPC values were used in the analysis of variance to compare amount of disease among plots with different treatments. For each response, the validity of model assumptions (normal distribution and constant variance of the error terms) was verified by examining the residuals as described in (Montgomery 2013; Shifa *et al.* 2018). Independence of the error terms assumption was validated through randomization of the treatments within each block. When treatment effect was significant means were separated using Fisher's protected least significance difference (LSD) test at 0.05 level of probability. The data of the two locations were treated separately because in most cases there was a significant difference between the two locations. The data were analyzed using Statistical Analysis System (SAS) software version 9.2 (SAS Institute 2008).

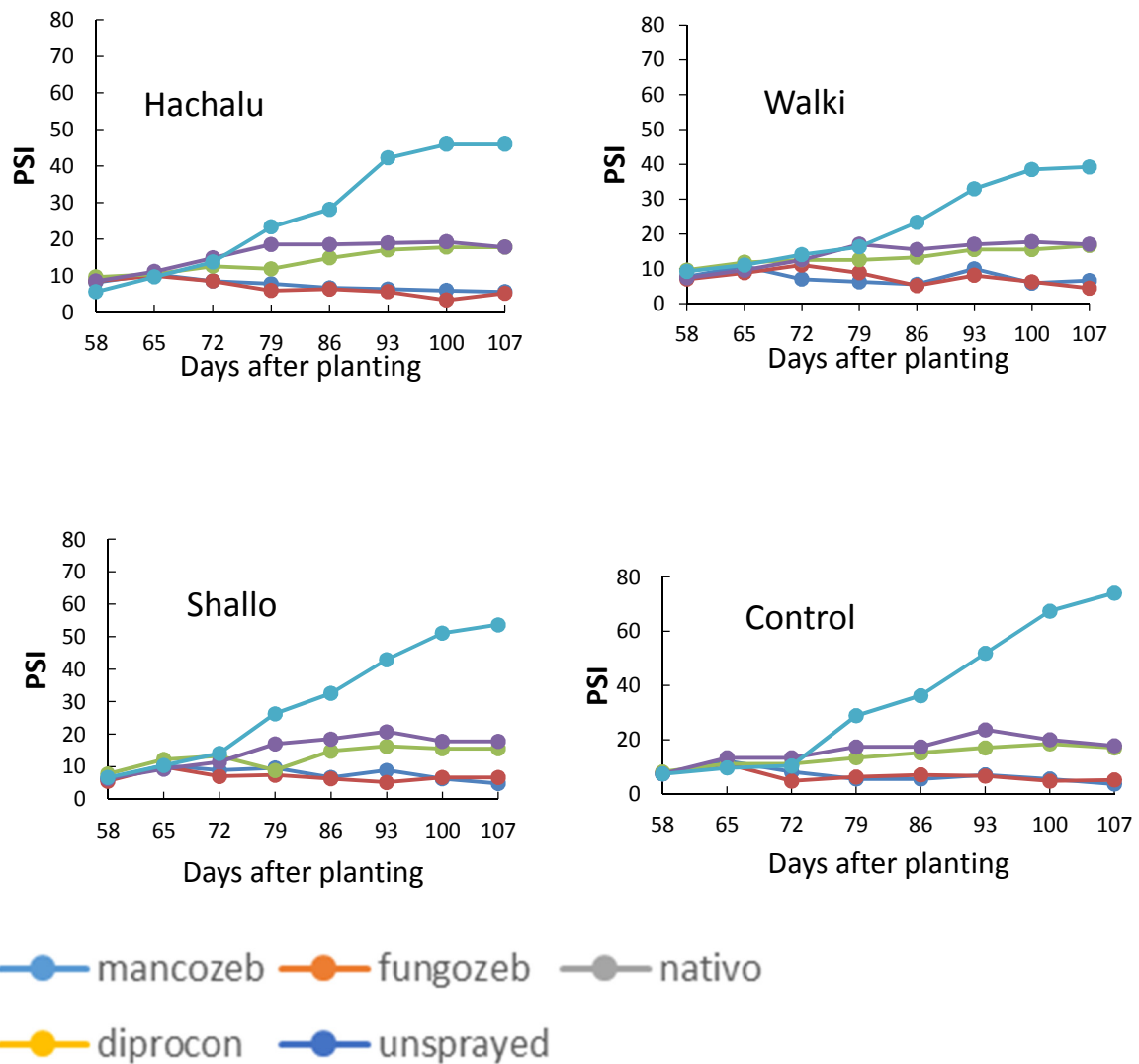
## 3. Results

### 3.1. Percentage severity index (PSI)

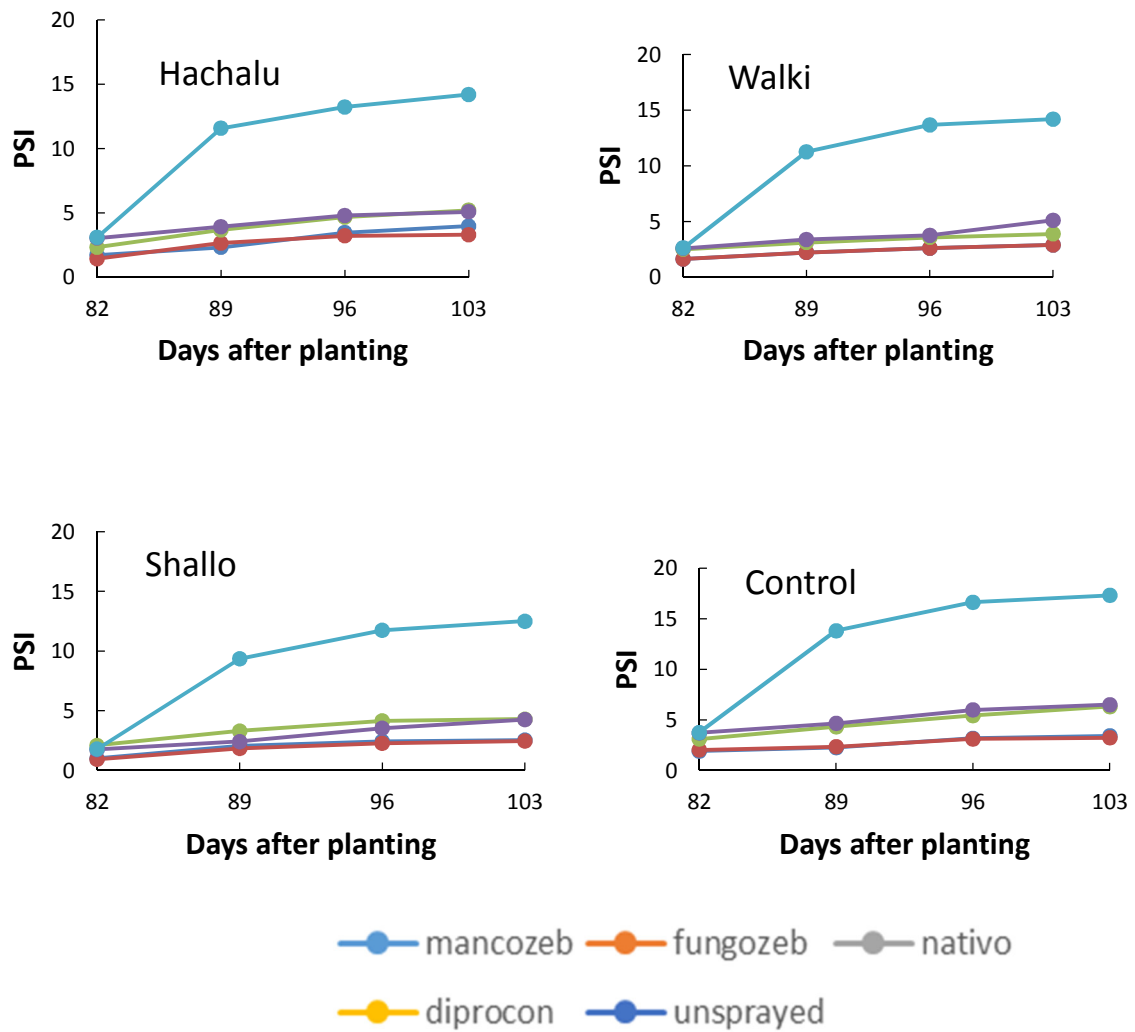
The disease severity of chocolate spot on all varieties treated with four fungicides progression at both locations presented in Figures 1 and 2. Chocolate spot PSI showed significant different at MWU on all varieties at different days of recordings except first and second days of records. Similarly, fungicides showed highly significant difference ( $P < 0.0001$ ) on chocolate spot PSI at MWU in all days of recordings except first and second days of records. This indicated that contribution of fungicides in controlling the diseases was after two successive foliar sprays. At Harewa varieties and fungicides showed significant different at all days of records on chocolate spot PSI. The mean PSI of chocolate spot was different on plots treated with four fungicides at both locations (Figures 1 and 2). The highest mean chocolate spot on hachalu variety was recorded from unsprayed plot (53.70%) and the lowest was recorded from plot treated with mancozeb (4.80%). The lowest chocolate spot PSI (3.70%) was recorded from walki treated with fungozebe as compared to the rest varieties. Mean chocolate spot PSI of local variety ranged from (4.40% to 74.10%) (Table 3). At Harewa there was similar trend that the highest chocolate spot PSI recorded from naturally infected plots, while lowest was from fungicide treated plots.

### 3.2. Area under disease progress curves

The AUDPC on all varieties treated with different fungicides were different in all successive diseases assessments. At MWU, the highest chocolate spot AUDPC values of 42.34% days was recorded from local unsprayed plot followed by shallo unsprayed, whereas the minimum AUDPC values of 11.79 % days was recorded from hachalu treated with fungozebe and walki treated with diprocon respectively. The AUDPC value on shallo variety varied from (14.13% to 37.77 % days). In the same way the AUDPC value of chocolate spot on local variety was varied from (13.03% to 42.34% days) at MWU (Table 3). Similarly, at Harewa the AUDPC value of chocolate spot on hachalu variety ranged from (1.17 % to 11.68 % days). On the other hand on local variety AUDPC was highest as compared to the rest of cultivars (Table 3).



**FIGURE 1. FABA BEAN CHOCOLATE SPOT PERCENTAGE SEVERITY INDEX (PSI) AT DIFFERENT DAYS OF RECORDING ON FOUR VARIETIES SPRAYED WITH FOUR FUNGICIDES AT MWU IN 2017.**



**FIGURE 2. FAB BEAN CHOCOLATE SPOT PERCENTAGE SEVERITY INDEX (PSI) AT DIFFERENT DAYS OF RECORDING ON FOUR VARIETIES SPRAYED WITH FOUR FUNGICIDES AT HAREWA IN 2017.**

**Table 3. Percentage severity index (PSI), Standardized AUDPC and disease progress rate on four fungicides treated varieties at MWU and Harewa in 2017 main cropping season.**

| Variety | Fungicide  | MWU   |        |        | Harewa |        |        |
|---------|------------|-------|--------|--------|--------|--------|--------|
|         |            | PSI   | rAUDPC | Rate   | PSI    | rAUDPC | Rate   |
| Hachalu | Mancozeb   | 6.70  | 13.130 | -0.013 | 2.50   | 2.500  | 0.003  |
|         | Fungozeb   | 4.80  | 11.790 | -0.012 | 2.50   | 1.170  | 0.002  |
|         | Native     | 15.60 | 18.530 | 0.016  | 4.30   | 2.980  | 0.012  |
|         | Diprocon   | 17.80 | 22.490 | 0.018  | 4.30   | 3.480  | 0.012  |
|         | No spray   | 53.70 | 32.780 | 0.035  | 12.50  | 11.680 | 0.014  |
|         | Mean       | 19.70 | 19.740 | 0.017  | 5.20   | 4.370  | 0.009  |
|         | LSD (0.05) | 5.80  | 0.896  | 0.002  | 1.50   | 0.068  | 0.002  |
|         | CV (%)     | 15.60 | 2.400  | 5.230  | 14.90  | 0.830  | 10.100 |
| Shallo  | Mancozeb   | 5.60  | 14.130 | -0.033 | 4.00   | 4.080  | 0.005  |
|         | Fungozeb   | 5.20  | 12.460 | -0.024 | 3.30   | 3.570  | 0.003  |
|         | Native     | 17.70 | 18.250 | 0.025  | 5.20   | 5.480  | 0.008  |
|         | Diprocon   | 17.80 | 20.580 | 0.028  | 5.10   | 5.940  | 0.010  |
|         | No spray   | 45.90 | 37.770 | 0.070  | 14.20  | 13.920 | 0.018  |
|         | Mean       | 18.40 | 20.640 | 0.036  | 6.40   | 6.590  | 0.008  |
|         | LSD (0.05) | 3.80  | 0.902  | 0.002  | 1.40   | 1.820  | 0.001  |
|         | CV (%)     | 11.10 | 2.320  | 2.800  | 11.90  | 14.650 | 8.260  |
| Walki   | Mancozeb   | 4.40  | 12.600 | -0.008 | 2.90   | 4.650  | 0.004  |
|         | Fungozeb   | 3.70  | 12.170 | -0.003 | 2.70   | 4.210  | 0.003  |
|         | Native     | 16.70 | 18.410 | 0.012  | 3.90   | 6.500  | 0.010  |
|         | Diprocon   | 17.00 | 19.610 | 0.013  | 4.10   | 7.030  | 0.012  |
|         | No spray   | 39.30 | 28.500 | 0.026  | 14.20  | 14.300 | 0.015  |
|         | Mean       | 16.80 | 18.260 | 0.014  | 5.60   | 7.340  | 0.011  |
|         | LSD(0.05)  | 4.30  | 0.034  | 0.003  | 1.00   | 0.120  | 0.002  |
|         | CV (%)     | 13.50 | 0.098  | 9.320  | 9.80   | 0.850  | 9.200  |
| Local   | Mancozeb   | 6.70  | 13.330 | -0.019 | 3.40   | 9.220  | 0.013  |
|         | Fungozeb   | 5.20  | 13.030 | -0.017 | 3.20   | 4.750  | 0.013  |
|         | Native     | 17.00 | 20.630 | 0.023  | 6.30   | 9.650  | 0.016  |
|         | Diprocon   | 17.80 | 22.470 | 0.031  | 6.50   | 10.830 | 0.018  |
|         | No spray   | 74.10 | 42.340 | 0.093  | 17.30  | 20.350 | 0.029  |
|         | Mean       | 23.60 | 22.370 | 0.030  | 7.40   | 10.960 | 0.018  |
|         | LSD(0.05)  | 6.00  | 0.270  | 0.002  | 1.60   | 0.079  | 0.002  |
|         | CV (%)     | 13.50 | 0.065  | 2.230  | 11.80  | 0.383  | 5.290  |

CV, coefficient of variation; LSD least significant difference; rAUDPC, standardized AUDPC; rate, disease progress rate; PSI, percentage severity index; MWU, Madaa Walabu University

### 3.3. Diseases progress rate

The disease progress rate of chocolate spot showed highly significant difference on varieties ( $p < 0.0001$ ), fungicides and the interaction effects. At MWU on unsprayed plot apparent infection rate was 0.093 units per day on local variety. This rate was retarded about five and six times by the application of mancozeb and fungozeb respectively. Hachalu treated with mancozeb and fungozeb reduced about two times than unsprayed plot of 0.026 units per day. Walki treated with fungozeb have less apparent infection rate than the other varieties treated with fungicides. At Harewa the apparent infection rate on unsprayed plots of local, shallo, hachalu and walki varieties were 0.029, 0.015, 0.014 and 0.018 units per day (Table 3) respectively. Apparent infection rate ranged from (0.002-0.014) and (0.003-0.018) units per day on hachalu and shallo respectively.

### 3.4. Grain Yield

The maximum grain yield was recorded from walki treated with fungozeb (4000 kg/ha) followed by walki treated with mancozeb (3892 kg/ha). The second highest grain yield recorded from hachalu treated with fungozeb 3860 kg/ha which had no significant difference with fungozeb treated plot (Table 4). Similar trend was seen at Harewa. Minimum grain yield was recorded from unsprayed plots of all varieties at both locations.

TABLE 4. MEAN GRAIN YIELD OF FOUR FABA BEAN VARIETIES TREATED WITH FOUR FUNGICIDES AT MWU AND HAREWA IN 2017 MAIN CROPPING SEASON.

| Variety | Fungicide | MWU           | Harewa        |
|---------|-----------|---------------|---------------|
|         |           | Yield (kg/ha) | Yield (kg/ha) |
| Hachalu | Mancozeb  | 3845.67       | 2414.03       |
|         | Fungozeb  | 3860.83       | 2491.74       |
|         | Nativo    | 3575.17       | 2224.11       |
|         | Diprocon  | 3319.14       | 2204.17       |
|         | No spray  | 2438.67       | 1626.23       |
|         | Mean      | 3407.94       | 2229.50       |
|         | LSD(0.05) | 2.51          | 334.40        |
| Shallo  | Mancozeb  | 3554.94       | 2365.63       |
|         | Fungozeb  | 3752.94       | 2406.24       |
|         | Nativo    | 3091.43       | 2120.83       |
|         | Diprocon  | 3116.73       | 2191.58       |
|         | No spray  | 1648.23       | 1452.00       |
|         | Mean      | 3046.00       | 2124.47       |
|         | LSD(0.05) | 249.70        | 126.40        |
| Walki   | Mancozeb  | 3892.06       | 2544.10       |
|         | Fungozeb  | 4000.86       | 2548.89       |
|         | Nativo    | 3819.00       | 2290.64       |
|         | Diprocon  | 3698.11       | 2063.54       |
|         | No spray  | 3480.30       | 1465.93       |
|         | Mean      | 3778.24       | 2095.65       |
|         | LSD(0.05) | 49.56         | 241.90        |
| Local   | Mancozeb  | 3466.70       | 2272.56       |
|         | Fungozeb  | 3585.36       | 2346.87       |
|         | Nativo    | 2593.57       | 1924.17       |
|         | Diprocon  | 2534.50       | 2126.04       |
|         | No spray  | 1275.39       | 1267.84       |
|         | Mean      | 2689.59       | 1989.80       |
|         | LSD(0.05) | 2.94          | 493.90        |
|         | CV (%)    | 0.06          | 13.19         |

CV coefficient variation, LSD least significant differences

## DISCUSSION

The trials conducted at two locations have demonstrated that foliar sprayed fungicides significantly reduced severity of faba bean chocolate spot, its progress rate, AUDPC, and thereby improved faba bean yield. The present work demonstrated that fungozeb and mancozeb reduced the symptom of chocolate spot as compared with nativo and diprocon fungicides. The result is in harmony with El-sayed *et al.* (2011) who reported application of Dithane M45 on the cultivars reduce the symptom of chocolate spot up to hundred percent as compared to naturally infected plots. Similarly, Sahile *et al.* (2008) reported that application of mancozeb at seven days interval significantly reduced chocolate spot severity and Kora *et al.* (2017) management of chocolate spot by mancozeb fungicide effectively control faba bean chocolate spot. Similarly, Galloway (2008) found that 3 applications of mancozeb were needed to provide effective control of soya bean rust.

Higher values of AUDPC and apparent infection rate were accompanied with lower yield. Accordingly, the maximum AUDPC values were recorded from plots treated with diprocon and the minimum AUDPC was recorded from plots treated with fungozeb and mancozeb fungicides. The apparent infection rate was high on naturally infected plots, but it was slow on fungicide sprayed plots. The application of contact fungicides minimized faba bean chocolate spot about four to six times from the unsprayed plots. This is in agreement with Kora *et al.* (2017) and Sahile *et al.* (2008) in which application of mancozeb retarded the apparent infection rate much smaller than unsprayed plots and increased the yield. Fungicide application increased yield and seed per plant about four times as compared to control (Teshome and Tegegne, 2013). Bitew and Tigabie, (2016) also reported similar results that among fungicides sprayed plots; better grain yield were recorded on bayleton

(2124.0 kg), mancozeb (1702.3 kg), Ridomil gold (1471.7 kg) and chlorothalonil (1470.9 kg) fungicides. Emeran *et al.* (2011) reported a maximum of 14% yield increase was obtained after a double fungicide treatment, third treatment increase up to a maximum of 27% in seed weight.

From the present study fungozebe with walki variety control chocolate spot and gave high grain yield. Further investigation should be necessary for best recommendation rate for the producers to manage faba bean chocolate spot, especially study on frequency of the fungicides is essential to recommend the effective rate for control of chocolate spot disease for the farmers. In addition studies on variability of *B. fabae* isolates from major faba bean growing areas in Ethiopia should be studied and a durable disease resistant faba bean should be developed.

### Competing Interests

The authors declare that they have no competing interests.

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