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Effect of Varieties and Fungicide Rate on Chocolate Spot (*Botrytis fabae*) Disease of Faba Bean (*Vicia faba* L.) at Tach Gayint District in South Gondar Zone, Amhara Region, Ethiopia

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Abstract

Faba bean is suffered with many biotic and abiotic factors. Chocolate spot disease, caused by *Botrytis fabae* is one of the biotic factors limiting yields of this crop resulting in yield losses up to 68% in Ethiopia. The experiment was conducted during 2020/2021 cropping season at Tach Gayint district, Ethiopia to determine the integration of faba bean varieties and fungicide rates on reducing chocolate spot disease. The experiment consisted of 12 treatments, viz. three faba bean varieties and four rate of Mancozeb fungicide in factorial arrangement. The experiment was laid out as randomized complete block design (RCBD) with three replications. Data was collected and analyzed. Results indicated that, disease incidence was reached at maximum percentage in all treatments on the last dates of assessment. But treatments were significantly difference in severity level. The least disease severity was recorded from varieties treated by 3.5 kg/ha of Mancozeb 80% WP with mean values Walki (12.7%), and (18.1% and 20.8%) on Gora and local variety respectively at the final dates of disease assessment. Similarly, the reduced AUDPC was also recorded from varieties treated by 3.5 kg/ha of Mancozeb 80% WP with mean values Walki (371.8% unit/day) and (539% and 686.4% days) on varieties Gora, and Local respectively. Whereas the maximum disease severity and AUDPC were obtained from unsprayed plots. Based on the results obtained, variety Walki treated with Mancozeb 80% WP at rate of 2.5 kg/ha and 3.5 kg/ha were effective to reduce the effect of chocolate spot disease for the study area.

Keywords

AUDPC, *Botrytis fabae*, Mancozeb, Disease Severity, *Vicia faba*, Variety

1. Introduction

Background and Justification

Faba bean (*Vicia faba*) is multi-purpose crop that plays an important role in the socio-economic life of farming communities in Ethiopia [1]. The crop is grown in the highlands (1800 - 3000 m.a.s.l) of the country which receiving an annual rainfall of 700 - 1000 mm where the need for cold temperature is met [2] [3]. The crop occupies the largest area in Ethiopia among other pulses [4] [5].

Despite the wide cultivation of the crop, its average yield is quite low in Ethiopia and the productivity is far below the potential because of several biotic and abiotic factors [6] [7] [8]. The production of faba bean is about 2.1 ton/ha compared with the production potential ranging from 2.3 to 3.9 tons/ha in Ethiopia [9].

Diseases are the most important factors limiting the production of faba bean. Chocolate spot disease, caused by *Botrytis fabae* is one of the yields limiting factor of this crop. It is the most important disease of faba bean worldwide and it can devastate the yield of unprotected crops up to 67% [10]. The disease is highly prevalent and destructive, causing yield loss up to 61% on a susceptible and 34% on tolerant faba bean varieties in the central highlands of Ethiopia [11]. [12] also reported even higher losses of 68% in the unsprayed faba bean plots in northwest Ethiopia. The disease can be occurred across all the agro-ecological zones but it is more serious in areas of high rainfall (>900 mm) and high elevation (>2000 m.a.s.l) [7].

Various management options have been developed to reduce the yield losses of faba bean due to chocolate spot worldwide including Ethiopia. These include the use of chemical fungicides such as Mancozeb, Fungozeb, Nativo and Diprocon [3], resistant/tolerant varieties [13], use of certain cultural practices such as crop residue management and altering planting date [14] and biological control like *Trichoderma harzianum*; *T. viride*; *Bacillus subtilis*, *Pseudomonas putida*, *Pseudomonas fluorescens* and *Ampelomyces qusisqualis* [15]. As [16] reported, Integration of faba bean varieties with foliar sprays protected high chocolate spot epidemics, increased yield, yield components and maximized marginal benefit compared to a single control approach. Although, several improved faba bean varieties are released and important fungicides are recommended [17]. Farmers still depend on local varieties and raise the lack of improved seed as major problem for faba bean production [18]. There is also a gap on proper usage of the fungicide including its rate, specificity and time of application. With increasing faba bean diseases particularly chocolate spot disease farmers become obligated to shift their land from faba bean to cereal crop production. Currently, there is an urgent need to improve faba bean yield since the crop remains an important crop in the study area. One way of confronting this challenge is with sound crop protection programs that increase the productivity of the crop by refinement of integrated disease management strategies. The objectives of this study were, to evaluate the integration of faba bean varieties and fungicide application rates for

reduction of faba bean chocolate spot disease intensity.

2. Materials and Methods

2.1. Description of the Experimental Site

The experiment was conducted at Agat farmer training center site in Tach Gayint district. Geographic location of the experimental site was 11˚34'53.9"N latitude and 38˚29'41.7"E longitude with an elevation of 2671 m.a.s.l [19].

2.2. Experimental Materials

In the experiment three faba bean varieties viz. *Gora* (EK01024-1-2), *Walki* (improved variety) and one Local variety were used. Varieties were selected based on their resistance level to chocolate spot. *Walki* is highly resistance, *Gora* (EK010241-2) moderately resistant [20] and local susceptible to chocolate spot was taken. [7] reported that in most cases local varieties are low yielding and highly susceptible to both biotic and abiotic stresses.

Seeds of the two varieties (*Gora* and *Walki*) were obtained from Adet and Gondar Agricultural Research Centers and local cultivar from farmer saved seeds. Fungicide (Mancozeb 80% WP) was used in this study and it was obtained from legal and authorized local market, since it is available in the area. As [17] Pulse crop manual, it is indicated, Mancozeb 80% WP is the best fungicide to manage chocolate spot of faba bean and also based on the research of [16] on management of chocolate spot the highest grain yield was recorded from plots sprayed with Mancozeb 80% WP.

2.2.1. Experimental Design and Procedures

The treatments were arranged in a randomized complete block design (RCBD) with factorial combination of four application rates of Mancozeb fungicide (unsprayed, 1.5 kg/ha, 2.5 kg/ha and 3.5 kg/ha) and three varieties (*Local*, *Gora and Walki*) in three replications (**Table 1**). The recommended rate of Mancozeb 80% WP for the management of chocolate spot disease 2.5 kg/ha were used as a base line [3] [16] [17]. 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 plots were 2.5 m (length) × 1.6 m (width) with four seedling rows. Spacing between blocks was 1 m and spacing's between plots, rows, and plants was 0.5 m, 0.4 m, and 10 cm, respectively. Faba bean grain yield was harvested from middle rows of each plot, leaving two outer rows on both sides to avoid the border effect. The yield data of the plots was converted to ton per hectare. The plots were fertilized with Diammonium phosphate (DAP) at the rate of 100 kg/ha^{-1} and Weeding was performed three times. Disease development was entirely based on natural inoculums in which the site is hot spot area for the disease.

2.2.2. Data Collection

All disease data were recorded since disease on set observed on the field and

Table 1. Experimental design and treatment combinations under field conditions.

No	Treatments
1	Local unsprayed
2	Local + *Mancozeb* 1.5 kg/ha
3	Local + *Mancozeb* 2.5 kg/ha
4	Local + *Mancozeb* 3.5 kg/ha
5	*Walki* unsprayed
6	*Walki* + *Mancozeb* 1.5 kg/ha
7	*Walki* + *Mancozeb* 2.5 kg/ha
8	*Walki* + *Mancozeb* 3.5 kg/ha
9	*Gora* unsprayed
10	*Gora* + *Mancozeb* 1.5 kg/ha
11	*Gora* + *Mancozeb* 2.5 kg/ha
12	*Gora* + *Mancozeb* 3.5 kg/ha

continued every ten days until the crop maturity.

1) Disease Incidence

Disease incidence is the percentage of plants which show symptoms of infection from the total plants considered. Both diseased and healthy plants were counted from the quadrate for disease incidence. The percentage of disease incidence (PDI) was calculated according to the formula indicated below [21].

$$\text{Disease incidence} = \frac{\text{No. of diseased plants}}{\text{total plants observed}} \times 100$$

2) Disease Severity

The disease severity was recorded from ten pre-tagged plants in each plot. It was recorded 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 2**).

The severity grades were converted into percentage severity index (PSI) according to the formula by [22].

$$\text{PSI} = \frac{\text{Sum of numerical rating}}{\text{No. of plant scored} \times \text{max max score on scale}} \times 100$$

3) Area under Disease Progress Curve (AUDPC)

The area under the disease progress curve (AUDPC) was calculated for each plot from PSI according to [23].

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

where, x_i represents the cumulative disease severity expressed as a proportion at the ith observation, t_i the time of the ith assessment, and n the total number of observations. AUDPC values were expressed in %-days.

4) Disease progress rate

Table 2. Percent of infection and scale for recording severity of chocolate spot.

0 = No disease symptoms
3 = Few small disease lesions
5 = Some coalesced lesions with some defoliation
7 = Large coalesced lesion sporulation lesions, 50% defoliation and some dead plants
9 = Extensive, heavy sporulation, stem girdling, blackening and death of more than 80% of plants.

Source: [24].

Disease progress rate was calculated using the appropriate model for each treatment. The apparent infection rate, expressed in disease units per day, was calculated from disease severity data transformed to logistic model ($\ln[(Y/1 - Y)]$) [25] 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 over time (as DAS) [23].

2.2.3. Statistical Data Analysis

Data was subjected to analysis of variance (ANOVA) to determine the effects of varieties and fungicide rate combination. ANOVA in the significant of their effect, means will separated using Fisher's protected least significance difference (LSD) test at 0.05 level of probability [26]. The data was analyzed using Statistical Analysis System (SAS) software version 9.2 [27].

3. Results and Discussion

Evaluation of Chocolate Spot Disease Management Options

1) Disease incidence

On the experimental plots, chocolate spot was first appeared on the Local variety (51 Days after Sowing [DAS]) and four and seven days later was observed on *Gora* and *Walki* varieties respectively. The analysis of variance indicated that the main effect of varieties and fungicide rates significantly ($P \leq 0.05$) reduce the incidence of chocolate spot disease recorded on all assessment dates from 62 - 112 DAS. Variation in the disease might be due to the difference in levels of resistance of the varieties and fungicide application rates (**Table 3**).

The disease was more rapid on local variety, which reached at higher level of final disease incidence (100%) followed by *Gora* (96.7%) 112 DAS. Lower disease incidence (85%) was recorded on variety *Walki*, (**Table 3**) at the final date of assessment. This observation was agreed with the earlier reports by [7] [16] 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. Similarly, [28] reported that, *Walki* was highly resistance to chocolate spot disease than other evaluated eight faba bean varieties (*Tumsa*, *Walki*, *Shallo*, *Degaga*, *Gebelcho*, *Hachalu Moti and Mosisaa*).

Table 3. Main effect of faba bean varieties and fungicide rates on incidence of chocolate spot disease under field condition at Tach Gayint District during 2020/2021.

		62 DAS	72 DAS	82 DAS	92 DAS	102 DAS	112 DAS
Variety	Local	56.67[a]	69.167[a]	84.2[a]	91.7[a]	97.5[a]	100[a]
	Walki	20[c]	37.5[c]	56.7[c]	75.8[b]	80.8[b]	85[b]
	Gora	40[b]	58.3[b]	72.5[b]	89.2[a]	92.5[a]	96.7[a]
	LSD	5.8336	3.8291	5.2969	5.592	8.2	5.4485
Fungicide rate	Unsprayed	45.6[a]	71.1[a]	81[a]	96.7[a]	97.8[a]	97.8[a]
	1.5 kg/haM 80% WP	41.1[ba]	58.9[b]	74.4[b]	88.9[b]	91[ba]	96.7[ba]
	2.5 kg/haM 80% WP	35.6[bc]	46.7[c]	67.8[c]	82.2[c]	96.7[a]	91.1[c]
	3.5 kg/haM 80% WP	33[c]	43.3[c]	61[d]	76.67[c]	83[b]	90[c]
	LSD (0.05)	6.7361	4.4215	6.1164	6.3487	9.4627	6.2914
	CV (%)	17.7	8.2	8.79	7.6	10.7	6.8

Means within the same column followed by the same letter(s) are not significantly different; DAS = Days after Sowing, LSD (0.05) = Least significant Difference at $P \leq 0.05$; M = *Mancozeb*; CV = Coefficient of Variations; WP = Wettable powder.

Fungicide rate also significantly different from each other for disease incidence, the mean disease incidence was at initial 45.6% (62 DAS) and final 97.8% (112 DAS) on unsprayed plots and 33% at initial (62 DAS) and 90% at final (112 DAS) on 3.5 kg/ha (**Table 3**). This result was agreed with [3] who showed that Mancozeb at the rate of 2.5 kg/ha significantly reduce the incidence of chocolate spot disease.

2) Disease severity

Based on the interaction effects of varieties and fungicide rates, maximum disease severity was recorded from the unsprayed varieties local (60%), and (40% and 23%) from *Gora* and *Walki* respectively and the least disease severity was recorded from varieties treated with 3.5 kg/ha of *Mancozeb* 80% WP fungicide rate *i.e. Walki* (12.7%), and (20.8% and 18.1%) on local and *Gora* respectively at the final dates of disease assessment (112 DAS) (**Table 4**). This finding disagreed with the research done by [16] who considered that *Mancozeb* fungicide at a rate of 2 kg/ha integrated with faba bean varieties is enough to reduce severity of chocolate spot disease. [3] also reported that *Mancozeb* fungicide at a rate of 2.5 kg/ha integrated with faba bean varieties can reduce severity of chocolate spot disease.

The maximum amount of disease severity was recorded from unsprayed local variety (60%) compared with other treatments and the least from variety *Walki* treated with 2.5 kg/ha and 3.5 kg/ha of *Mancozeb* 80% WP fungicide rate (13%

Table 4. Interaction effect of faba bean varieties and fungicide rates on severity of chocolate spot disease under field condition at Tach Gayint District during 2020/2021.

Variety	Fungicide rate	62DAS	72DAS	82DAS	92DAS	102DAS	112DAS
Local	Unsprayed	12.6^{a}	22.8^{a}	33.7^{a}	47.1^{a}	53^{a}	60^{a}
	1.5 kg/ha M 80% WP	11.5^{b}	17.2^{b}	21^{b}	27.2^{b}	31.^{c}	36.6^{b}
	2.5 kg/haM 80% WP	10^{c}	12^{d}	15.3^{c}	17.6^{c}	21.4^{ed}	24.2^{d}
	3.5 kg/haM 80% WP	8.9^{d}	9.6^{e}	12.3^{d}	14.3^{de}	17.6^{gf}	20.8^{ed}
Walki	Unsprayed	3.5^{ih}	7.8^{f}	11.1^{ed}	16.5^{dc}	20.1^{ef}	23^{d}
	1.5 kg/haM 80% WP	3.1^{i}	5^{h}	8.3^{hgf}	10.7^{fg}	14.1^{ih}	15.8^{gf}
	2.5 kg/haM 80% WP	2.9^{i}	3.3^{i}	7.1^{hg}	10^{fg}	12.1^{ih}	13^{g}
	3.5 kg/haM 80% WP	2.7^{i}	3.8^{i}	6^{h}	8.4^{g}	11.2^{i}	12.7^{g}
Gora	Unsprayed	6.9^{e}	15.6^{c}	21.5^{b}	28^{b}	35^{b}	40.2^{b}
	1.5 kg/haM 80% WP	4.8^{gf}	8^{f}	12.6^{d}	18.2^{c}	23.9^{d}	29.1^{c}
	2.5 kg/haM 80% WP	5.6^{f}	7.4^{f}	10.6^{edf}	15.7^{dc}	19.1^{ef}	22.4^{d}
	3.5 kg/haM 80% WP	4.43^{gh}	6.5^{g}	9.2^{egf}	11.4^{fe}	15.5^{gh}	18.1^{ef}
	LSD (0.05)	1.11	0.8522	2.3384	2.9552	3.48	3.6
	CV (%)	10.2	5.07	9.8	9.3	9.0	8.07

Means within the same column followed by the same letter(s) are not significantly different; DAS = Days after Sowing, LSD (0.05) = Least significant Difference at $P \leq 0.05$; M = *Mancozeb*; CV = Coefficient of Variations; WP = Wettable powder.

and 12.7%) respectively at the final date of disease assessment (112DAS) (Table 4). This finding was similar with [29] who reported that highest mean disease severity (47%) was recorded from unsprayed plot compared with highly protected plots with mean value (2%). Similarly, [30] showed that, applications of fungicide integrated with resistance varieties of faba bean increases the potential of reducing the severity of chocolate spot diseases than susceptible varieties.

3) Area under disease progress curve (AUDPC)

The analysis of variance showed that there was a significant difference ($P \leq 0.05$) among main effect of varieties, fungicide rates and the interaction effects of varieties and fungicide rates for AUDPC (Table 5).

Based on the interaction effect of varieties and fungicide rate the local variety of faba bean sprayed with 1.5 kg/ha, 2.5 kg/ha and 3.5 kg/ha of *Mancozeb* 80%

Table 5. Interaction effect of faba bean varieties and fungicide rates on area under disease progress curve and apparent infection rate of chocolate spot disease under field condition at Tach Gayint District during 2020/2021.

Varieties	Fungicide rate	AUDPC (%-day)	Apparent infection rate (unit/day)
Local	Unsprayed	1927.5[a]	0.028318
	1.5 kg M 80% WP	1205[b]	0.014985
	2.5 kg M 80% WP	835.3[c]	0.012150
	3.5 kg M 80% WP	686.4[d]	0.011784
Walki	Unsprayed	686.87[d]	0.016301
	1.5 kg/haM 80% WP	475.7[fe]	0.013034
	2.5 kg/ha M 80% WP	403.8[fg]	0.009879
	3.5 kg/ha M 80% WP	371.8[g]	0.008941
Gora	Unsprayed	1238.6[b]	0.020897
	1.5 kg/ha M 80% WP	795.6[c]	0.018299
	2.5 kg/ha M 80% WP	668[d]	0.013828
	3.5 kg/ha M 80% WP	539[e]	0.012122
	LSD (0.05)	82	0.0031
	CV (%)	5.9	12.2

Means within the same column followed by the same letter(s) are not significantly different; AUDPC = Area under disease progress curve; LSD (0.05) = Least significant Difference at $P \leq 0.05$; M = *Mancozeb*; CV = Coefficient of Variations; WP = Wettable powder.

WP fungicide rate showed that mean AUDPC value of 1205, 835.3 and 686 (% day) respectively, but the unsprayed local variety showed mean AUDPC value of 1927.5% day. The improved varieties *Walki* and *Gora* showed that the mean AUDPC value of (475.7, 403.8 and 371.8) %-day and (795.6, 668 and 539) % day sprayed with 1.5 kg/ha, 2.5 kg/ha and 3.5 kg/ha of fungicide rate respectively. However, the unsprayed varieties *Walki* and *Gora* showed mean AUDPC value of 686.7 and 1238.6 (% day) respectively (**Table 5**). Highest mean AUDPC value was observed from local unsprayed 1927.5% day and lowest AUDPC from *Walki* sprayed with 3.5 kg/ha of *Mancozeb* 80% WP fungicide rate 371.8% day (**Table 5**). This result was agreed with [31] who reported that lowest AUDPC recorded from *Walki* variety treated with *Mancozeb* fungicide. Similarly, [32] showed that area under disease progress curve exhibited significant difference ($P \leq 0.05$) on the interaction effect of varieties and fungicide sprayed treatments. [33] indicated that AUDPC is used to make comparison between treatments and [34] also showed that comparisons of disease progress curves and AUDPC between treatments are the most commonly used tools for evaluating practical disease management strategies.

4) Disease progress rate

Both logistic $\ln[(y/1-y)]$, [25] and Gompertz, $-\ln[-\ln(y)]$ models were tested

to choose the best fitted one in describing the rate of the disease development. The goodness of fit of models was tested based on the magnitude of the coefficient of determination (R^2). Then based on their coefficient of determination values (R^2), Gompertz model was better than the logistic model for the chocolate spot disease and was used to determine the disease progress rate parameters in the study. This showed 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 [23].

Significant ($P < 0.05$) differences were observed on disease progress rate among varieties, fungicide rates by their main effects and their interaction.

Local variety sprayed with 1.5 kg/ha, 2.5 kg/ha and 3.5 kg/ha gave 0.01498467, 0.009879 and 0.008941 units/day compared with its respective unsprayed local variety (0.028318 units-day) and variety *Walki* sprayed with 1.5 kg/ha, 2.5 kg/ha and 3.5 kg/ha gave 0.013034, 0.01215033 and 0.01178367 units/day compared with its respective unsprayed (0.01630133) units/day. The disease progress rate of variety *Gora* sprayed with 1.5 kg/ha, 2.5 kg/ha and 3.5 kg/ha also gave 0.018299, 0.013828 and 0.01212167 units/day compared with unsprayed *Gora* variety which had 0.020897 units/day (**Table 5**). In this study, all varieties sprayed with 3.5 kg/ha of *Mancozeb* fungicide rate gave maximum apparent infection rate than their respective other combinations (unsprayed, 1.5 kg/ha, and 2.5 kg/ha). These results indicated that the disease has progressed at faster rate on the unsprayed plot than plots which were sprayed with *Mancozeb* fungicide. This result is in agreement with [35] who showed that plots sprayed with *Mancozeb* retarded the apparent infection rate than unsprayed plots. Similarly, [16] reported that varieties sprayed with *Mancozeb* 80% WP were effective to reduce the apparent infection rate of chocolate spot disease than unsprayed varieties. Maximum apparent infection rate was recorded from Local unsprayed (0.028318 units/day) and the minimum was from *Walki* sprayed with 3.5 kg/ha (0.008941 units/day). This finding becomes in agreement with [31] who showed that integrated use of resistance varieties with fungicide effectively reduced apparent infection rate of chocolate spot disease.

4. Conclusions and Recommendations

Use of host plant resistance and application of *Mancozeb* fungicide considerably reduced the severity of the disease. Variety *Walki* retarded the effect of chocolate spot disease compared with *Gora* and Local varieties. High disease epidemics (AUDPC values of 1927.5%, 1238.6% and 686.87% unit/day) occurred on unsprayed plots of the varieties; Local, *Gora* and *Walki*, respectively. The present study has determined that an application of Mancozeb fungicide at a rate of 2.5 kg/ha for variety *Walki* and at a rate of 3.5 kg/ha for Local and *Gora* varieties were more feasible for the management of chocolate spot and increases the grain yields markedly.

Based on the results obtained, variety *Walki* was best in performance by re-

tarding the effect of chocolate spot disease and it should be addressed to farmers. Application of *Mancozeb* 80% WP at 2.5 and 3.5 kg/ha of on different faba bean varieties was effective to minimize the yield loss of faba bean and could be recommended to manage chocolate spot of faba bean. However, the experiment should be repeated across different environments over years in order to give the right recommendations and its rate may need to be well refined.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this manuscript.

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