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# Effect of host plant resistance and rhizobial inoculants on chocolate spot (*Botrytis fabae*) severity and yield of faba bean (*Vicia faba* L.) in South region, Ethiopia

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**ABSTRACT**

Chocolate spot (*Botrytis fabae*) is one of the major diseases menacing faba bean (*Vicia faba* L.) production and restraining its productivity in Ethiopia. Using newly released varieties that are high yielding and tolerant to biotic stresses can increase faba bean productivity. However, the level of protection provided by the varieties has not been satisfactory. *Rhizobium* spp. might be used to control pathogens of legume crops when used as seed dressing. The objective of this study is to evaluate the synergistic effect of host plant resistance and use of bio-inoculants on faba bean yield and severity of the disease. The field experiments consisted of 12 treatments laid out in a randomized complete block design with three replications during the 2019/2020 crop season. The chocolate spot severity was scored (with a 1-9 scale) at the flowering and final pod filling stage when the disease attained maximum and then grain yield was recorded. Combined application of rhizobia strains reduced disease severity and increased grain yield and hundred-grain weight in all varieties compared to untreated. The lowest disease severity (30.3%) was observed with the application of a combination of rhizobia on the Gebelocho variety at Bulle. The highest yield of 3296 kg/ha was harvested from Gebelcho at Bulle with the application of a combination of rhizobia strains. The result indicated the possible use of rhizobial strains integrating with host plant resistance as an alternative means of management but further study is needed to verify actual use in agricultural production.

**Keywords:** *Botrytis fabae*, released varieties, response, *Vicia faba*.

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## INTRODUCTION

Ethiopia is the largest producer of Faba bean in the world next to China. In spite of its huge importance, the productivity of Faba bean remains far below the crop's potential. Faba bean is highly produced in the highland area of Gedeo zone, South region. The lack of improved varieties and hence low yield of the local cultivars is a major problem of faba bean production in the Gedeo zone (SARI, 2017). Fungal disease chocolate spot severely constrains faba bean production in South Nations Nationalities People region. Using newly released varieties that are high yielding and tolerant to biotic and abiotic stresses can increase faba bean productivity. However, the level of protection provided by the varieties has not been satisfactory (Teklay et al., 2013). The use of fungicides against the disease has been shown to induce negative effects on the environment and results in the appearance of fungicide-resistant pathogen biotypes. It is also unaffordable for farmers in countries like Ethiopia (Butt et al., 2001). Thus it is important to look for ecofriendly management strategies for the disease. Many soil microorganisms possess multiple beneficial traits such as nutrient mobilization, production of plant growth-promoting substances and biocontrol ability (Muleta et al., 2007). Rhizobium spp. can be used to control pathogens of legume crops when used as seed dressing (Baraka et al., 2009). *R. leguminosarum*, and *B. japonicum* are also reported to significantly inhibit the growth of fungi infecting faba beans such as *M. phaseolina*, *R. solani* and *Fusarium* sp. (Esteshamul-

Haque and Ghaffar, 1993). Combined inoculation of Rhizobium sp. and Trichoderma spp. were shown to increase growth, nutrient uptake and yield of chickpea under field conditions (Rudresh et al., 2005). Rhizobial isolates exhibited inhibition of radial growth of *F. solani* under *in vitro* conditions (Tamiru and Muleta, 2018). Therefore, the integration of varieties and beneficial microbes is an important option to increase the productivity of faba beans. This study aims to evaluate the synergistic effect of host plant resistance and use of bio-inoculants on faba bean yield and severity of the disease.

## MATERIALS AND METHODS

### Description of the study area

The study was conducted in Bulle and Gedeb Woredas of the Southern Nations Nationalities People Region, Ethiopia during the 2019/2020 main crop season. Gedeb Woreda is located in the Gedeo zone of the South Nations Nationalities People Region, 160 km from the capital of SNNPR – Hawassa and 74 kilometers from Dilla which is the administrative seat of the Gedeo zone. The area of the Woreda is estimated to be 30,909 hectares. The altitude of Gedeb Woreda ranges from 1950 m.a.s.l up to 2650 m.a.s.l, the annual rainfall ranges from 1290 to 1800 mm and the temperature ranges from 16 to 21°C. The land-use system of the Woreda is mostly a mixed farming system. Most of the land was cultivated by annual crops of 12,756 hectares, perennial crops of 16,372 hectares and pasture land coverage are 244 hectares (GWBA, 2007). Bulle Woreda is located in the Southern part of Hawassa, 117 kilometers (km) from the region's capital in 6°.07'-6°.37' North and 38°.27'-38°.77' East. The Woreda has a total area of 27,300 hectares, with its altitude ranging between 2,001 and 3,000 meters above sea level (m.a.s.l). The mean annual rainfall of the Woreda is 1401 to 1800 mm, with a mean average temperature ranging between 12.6 and 20°C (SARI, 2017).

### Faba bean varieties

The testing crops were Faba bean varieties, which are already adapted and still perform best in the study area (Table 1). Seeds of Gebelcho and Tumusa varieties that had been released for production until 2010 (EARO, 2010) were obtained from Holleta Agricultural Research Centers, Holleta city, Ethiopia. A 'local cultivar (Bulga 70)' commonly grown by farmers in the respective study areas is included as a check.

**Table 1.** Agronomic characteristics of faba bean varieties used in the study.

| Variety | Pedigree name | Production domain (m.a.s.l) | Maturity days | Yield (qt/ha) | Year of release |
|---|---|---|---|---|---|
| Gebelcho | Tesfa x ILB4726 | 1800-3000 | 103-167 | 20-30 | 2006 |
| Tumusa | EH9965-3 | 2050-2800 | 121-176 | 20-38 | 2010 |

### Treatments

The experiment was done by using two selected rhizobial strains based on their performance as recommended by Menagesha biotech industry, Addis Ababa city, Ethiopia. Carrier peat-based strains of faba bean EAL 110 and EAL 301 were obtained from the Laboratory of Menagesha biotech industry, Addis Ababa city, Ethiopia. Before the experimentation, the viability of inoculates is tested to know whether they are viable or not. Thus, they found that their colony number was above $10^8$ per one carrier (125 g) and was taken as they are viable.

### Experimental design

First, the faba bean seeds were immersed in lukewarm water (36°C) to create an anchoring environment for the strain. Then under the umbrella shade, the bacterial strains opened and mixed with the faba bean seeds in a mixing container. Finally, seeds were allowed to air dry for 15 minutes and planted immediately after air drying. Two rhizobial strains and their combination combined with three faba bean varieties in factorial randomized complete block design with three replications and hence there were 12 treatments in each replication (Table 2). The plot size was 4-m length by 2-m width and spacing between plots and blocks was 0.8 and 1 m, respectively. The spacing between plants and rows was also 0.1 and 0.4 m, respectively (EARO, 2010). The recommended agronomic practices like weeding are uniformly employed in each plot as required in the study period. Faba bean plants in the central two rows were used for disease, yield and yield-related data.

### Data collection procedures

#### *Disease data*

Chocolate spot severity was recorded on each of 15 sample plants from the middle two rows per plot, using a

**Table 2.** Treatments used in the study.

| No. | Treatments | No | Treatments |
|---|---|---|---|
| 1 | Strain 1 (EAL 110) + Gebelcho variety | 7 | Combination (EAL 110+301)+ Tumusa |
| 2 | Strain 2 (EAL 301)+ Gebelcho Variety | 8 | Tumusa without rhizobial strain |
| 3 | Combination (EAL 110+301)+ Gebelcho | 9 | Strain 1 (EAL 110)+ local (Bulga 70) |
| 4 | Gebelcho without rhizobial strain | 10 | Strain 2 (EAL 301) + local(Bulga 70) |
| 5 | Strain 1(EAL 110)+ Tumusa Variety | 11 | Combination (EAL 110+301)+ local |
| 6 | Strain 2 (EAL 301) + Tumusa variety | 12 | Local (Bulga70) without rhizobial strain |

1 to 9 scale; where 1 = no lesions or covering up to 1 % of leaf surface; 3 = lesions covering 1 – 2 % of leaf surface; 5 = lesions common (3 – 5 mm in diameter), covering 2 – 5 % of leaf surface; 7 = lesions that cover 5 – 10 % of leaf surface; 9 = extensive lesions, covering more than 10 % of the leaf surface (ICARDA, 1986). Severity was recorded at the final pod filling stage when the disease attained maximum (Villegas-Fernandez et al., 2012).

The severity grades were converted into percentage disease severity index (PDSI) for analysis:

$$Disease\ severity\ index(DSI) = \frac{\sum(score\ x\ number\ of\ plants\ with\ this\ score)}{Total\ number\ of\ plants\ x\ greater\ score}$$

Percentage of disease reduction (DR%) was calculated by Edginton et al. (1971):

$$DR\ (\%) = \frac{Dc - Dt}{DC}\ X\ 100$$

Dc is the disease on the control plants that are treated with only pathogen and
Dt is the disease treated with antagonist and pathogen

### *Grain yield*

Grain yield of each plot was also determined after threshing. Finally, yield per plot was converted to grain yield per hectare basis. The grain yield was adjusted to 10% moisture content.

### Data analysis

The data were subjected to analysis of variance (ANOVA) using the general linear modeling (GLM) procedure of SAS-9.2 software (SAS, 2008). LSD was performed at a 5% level of significance to denote a significant difference between the treatments.

## RESULTS AND DISCUSSION

### Chocolate spot severity

The results showed that there was a significant ($p < 0.05$) interaction effect among the types of varieties used and the application of the bio-inoculant strains in the reduction of disease severity (Table 3). A combination of rhizobia strains reduced disease severity in all varieties compared to untreated. At Gedeb, the lowest severity (48.9%) was recorded with the treatment of a combination of rhizobial strains on the Gebelcho variety. At Bulle, the lowest (30.3%) disease severity was recorded with the treatment of a combination of rhizobia on the Gebelcho variety. Rhizobia have been reported as the best control of root infecting fungi on leguminous plants (Ehteshamul-Haque and Ghaffar, 1993). The combined application of plant growth-promoting rhizobacteria has significantly lowered the Fusarium wilt disease of *Capsicum annum* L. caused by *F. solani* compared to individual isolates (Sundaramoorthy et al., 2012). Advantages of strain mixtures include the broad spectrum of action, enhanced efficacy, reliability and also allowing a combination of various traits without genetic engineering (Janisiewicz, 1996). It seems that the establishment of Rhizobium protected the plant. It might have been due to induced systemic resistance.

### Grain yield

Results reported herein indicate that Rhizobial strains treatments not only suppressed disease severity but also enhanced grain yield of Faba bean plants compared to untreated control. At Bulle, the highest yield of 3296 kg/ha was harvested from Gebelcho with the application of a combination of rhizobia strains (Table 4). This work agrees with the work of Rugheim and Abdelgani (2012) who indicated that co-inoculation of rhizobial strains significantly increased faba bean grain yield. Sameh et al.

**Table 3.** Interaction effect of host plant resistance and bio-inoculants on disease severity of faba bean chocolate spot.

| Varieties | Bio-inoculants | DSI (Disease severity index) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Gedeb | | | | Bulle | | | |
| | | Year-2019 | Year-2020 | Average | DR% | Year-2019 | Year-2020 | Average | DR% |
| Gebelcho | EAL 110 | 51.8 | 52.5 | 52.15[ef] | 15.4 | 31.4 | 42.6 | 37[d] | 4.7 |
| | EAL 301 | 59.2 | 57.2 | 58.2[cd] | 5.5 | 32.6 | 44.8 | 38.7[d] | 0.3 |
| | EAL 110+ EAL 301 | 48.1 | 49.8 | 48.95[f] | 20.6 | 30.3 | 41.3 | 35.8[d] | 7.8 |
| | Untreated | 62.2 | 61.1 | 61.65[b] | 0 | 33.3 | 44.4 | 38.85[d] | 0 |
| Tumusa | EAL 110 | 44.4 | 60.6 | 52.5[ef] | 11.3 | 39.7 | 63.4 | 51.55[a] | 3.9 |
| | EAL 301 | 51.8 | 62.4 | 57.1[ed] | 3.5 | 40.7 | 65.3 | 53[a] | 1.2 |
| | EAL 110+ EAL 301 | 42.4 | 58.6 | 50.5[ef] | 14.6 | 38.2 | 64.1 | 51.15[ba] | 4.6 |
| | Untreated | 54.6 | 63.8 | 59.2[cd] | 0 | 40.7 | 66.6 | 53.65[a] | 0 |
| Bulga 70 | EAL 110 | 59.2 | 61.4 | 60.3[cb] | 14.7 | 35.5 | 50.5 | 43[bc] | 4.1 |
| | EAL 301 | 59.2 | 62.8 | 61[b] | 13.7 | 37 | 52.4 | 44.7[bc] | 0.3 |
| | EAL 110+ EAL 301 | 55.5 | 59.6 | 57.55[ed] | 18.6 | 35 | 49 | 42[dc] | 6.3 |
| | Untreated | 77.7 | 63.8 | 70.75[a] | 0 | 37 | 52.7 | 44.85[bc] | 0 |
| Average | | 55.5 | 59.5 | 57.5 | 9.8 | 35.9 | 53.1 | 44.5 | 2.8 |
| CV (%) | | | | 5.01 | | | | 11.82 | |
| LSD (0.05) | | | | 4.93 | | | | 8.97 | |

Means with the same letter are not significantly different at p = 0.05 according to LSD significant difference. DR%: Disease reduction % = (Untreated-treated)/Untreated*100.

**Table 4.** Interaction effect of host plant resistance and bio-inoculant on yield of faba bean.

| Varieties | Bio-inoculants | Yield (kg/ha) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Gedeb | | | Bulle | | |
| | | Year-2019 | Year-2020 | Average | Year-2019 | Year-2020 | Average |
| Gebelcho | EAL 110 | 3268 | 3214 | 3241[a] | 3154 | 3119 | 3136.5[a] |
| | EAL 301 | 3121 | 3067 | 3094[b] | 3012 | 2977 | 2994.5[b] |
| | EAL 110+ EAL 301 | 3296 | 3242 | 3269[a] | 3184 | 3149 | 3166.5[a] |
| | Untreated | 2901 | 2847 | 2874[d] | 2887 | 2852 | 2869.5[c] |
| Tumusa | EAL 110 | 2802 | 2748 | 2775[e] | 2730 | 2695 | 2712.5[d] |
| | EAL 301 | 2743 | 2689 | 2716[e] | 2632 | 2597 | 2614.5[e] |
| | EAL 110+ EAL 301 | 3011 | 2957 | 2984[c] | 2994 | 2959 | 2976.5[b] |
| | Untreated | 2661 | 2607 | 2634[f] | 2556 | 2521 | 2538.5[f] |
| Bulga 70 | EAL 110 | 2602 | 2548 | 2575[g] | 2512 | 2477 | 2494.5[g] |
| | EAL 301 | 2573 | 2519 | 2546[g] | 2492 | 2457 | 2474.5[g] |
| | EAL 110+ EAL 301 | 2724 | 2670 | 2697[f] | 2705 | 2670 | 2687.5[e] |
| | Untreated | 2571 | 2517 | 2544[g] | 2402 | 2367 | 2384.5[h] |
| Average | | 2856.1 | 2802.1 | 2829.1 | 2771.7 | 2736.6 | 2754.2 |
| CV (%) | | | | 1.14 | | | 1.21 |
| LSD (0.05) | | | | 54.48 | | | 56.44 |

Means with the same letter are not significantly different at p = 0.05 according to LSD significant difference. DR%: Disease reduction % = (Untreated-treated)/Untreated*100.

(2017) reported that the application of effective strains increases the grain yield of faba bean up to 44 to 47%. Unlike the above-mentioned findings, Zerihun and Abera (2014) reported that rhizobium inoculation didn't increase faba bean grain yield over the control. The increments in grain yield due to the inoculation of rhizobium strains indicated that the nitrogen of the soil is a limiting factor. Thus, the grain yield could be strongly improved by means of inoculation or fertilization.

## CONCLUSION

The result of the study demonstrated that rhizobial strains were found to reduce chocolate spot disease severity, and enhanced faba bean growth relative to the control. This finding indicated that rhizobial biological control has considerable promise in the suppression of *B. fabae*. A combination of the strains applied was found to be the most efficient in reducing disease severity compared to other rhizobial treatments. The result indicated the possible use of rhizobial strains integrating with host plant resistance as an alternative means of management but further study is needed to verify actual use in agricultural production.

## Competing interests

The authors declare no competing interests.

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