Bio-control of Chocolate spot disease of Faba bean using potential rhizobacterial strains under field conditions in Northwestern Ethiopia

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Received: 20 May, 2023; Accepted: 15 June, 2023; Published online: 16 June, 2023

Abstract

Faba bean (Vicia faba L.) is an important legume that is frequently produced in the highlands of Ethiopia. However, the production of faba bean in this country is limited by the chocolate spot disease caused by Botrytis fabae. The purpose of this study was to evaluate the efficacy of Chryseobacterium strain GY04; Chryseobacterium proteolyticum GY05, and Pseudomonas chlororaphis GY07 on chocolate spot disease management; faba bean growth promotion, and improvement of the yield-related parameters under field conditions. All the three rhizobacteria significantly reduced the incidence and severity of chocolate spot disease in both tested varieties (i.e., Local and Dosha), compared with the control. In both faba bean varieties, the highest disease incidence reduction (%) was observed on the plot treated by P. chlororaphis GY07; recording 73.07 % and 75.86 % in the Local and Dosha varieties, respectively. In terms of disease severity, inoculation of P. chlororaphis GY07 in the Local and Dosha varieties resulted in an 84.3 % and 81.5 % reduction in the disease severity, respectively. All the three tested rhizobacterial strains caused growth parameters enhancement with different potentials. In both faba bean varieties, the highest growth promotion was observed on the plot treated with P. chlororaphis GY07, followed by Chryseobacterium strain GY04, and Chryseobacterium proteolyticum GY05. All of the rhizobacteria had a significant enhancement potential on the number of pods per plant and grains weight, compared to the uninoculated treatment. The results of this study give a clue that these rhizobacterial strains may be used as biocontrol agents for chocolate spot diseases, and as bio-inoculants for enhancement of faba bean production.

Keywords: Biological control, Chocolate spot, Faba bean, Incidence, Severity, Rhizobacteria
1. Introduction

Faba bean is an important legume frequently produced in the highlands of Ethiopia (You et al., 2021; Dobocha and Bekele, 2022). It is known of having several advantages, including nutritional and economic potentials, and is ecologically considered as a rotational crop (Gebru and Mesganaw, 2021). However, in Ethiopia; its production is threatened by the biotic and abiotic stresses. Faba bean is affected by over 100 microbial pathogens (Karkanis et al., 2018); where in Ethiopia, more than 26 biotic diseases hinder its production. One of the most economically important diseases of faba beans in Ethiopia is chocolate spot disease, which is incited by Botrytis fabae Sard (Muruts and Alemayehu, 2021; You et al., 2021). This fungal pathogen causes noticeable crop losses ranging from minor to total failure (Torres et al., 2004). In Ethiopia, the chocolate spot disease causes yield losses of up to 67.5 % in the susceptible faba bean cultivars (Samuel et al., 2010).

Various management strategies have been developed to reduce faba bean yield losses caused by the chocolate spot around the world. These include the use of chemical fungicides; tolerant varieties, and the implementation of specific cultural practices. In Ethiopia, the use of fungicides; particularly mancozeb, is common for the management of this disease (Samuel et al., 2008). However, due to the high cost of this fungicide and its negative effects on plant growth and the environment, it is no longer widely used (Walia et al., 2014; Muruts and Alemayehu, 2021). Another control option for management of the chocolate spot disease is the use of resistant varieties (Abera and Semagn, 2022). However; according to EIAR. (2018), this option has not also been further realized in Ethiopia, because almost the entire faba bean varieties used in this country is not fully resistant to the chocolate spot pathogen. Because of these problems, it is critical to find an alternative solution that is also environmentally safe.

Recently, the use of biocontrol agents as an alternative disease management option has gained popularity, in order to manage the plant disease in a sustainable manner (Khunnamwong et al., 2020). The use of beneficial rhizobacteria as biocontrol agents is a good option for managing the sustainability of the phytopathogens (Jiao et al., 2021). In this regard, different bacterial species have revealed antagonistic potential against Botrytis infection in faba beans (El-Banoby et al., 2013; Mbazia et al., 2016; El-Shatoury et al., 2020). Few studies have been conducted in Ethiopia on the bio-control of chocolate spot disease of faba beans using the rhizobacteria (Fekadu and Tesfaye, 2015; Zewdineh et al., 2022; Adal et al., 2022). All of these studies have focused on the well-studied rhizobacteria; mainly P. florescence and Bacillus spp.

As a result, additional rhizobacteria must be considered in order to develop effective bio-control agents against B. fabae. Several previous studies have shown that P. chlororaphis has strong antagonistic and bio-control activity against several phytopathogenic fungi infecting different host plants (Arjona-López et al., 2019; Tienda et al., 2020; Xu et al., 2021; Liu et al., 2022). Many studies have demonstrated that Chryseobacterium spp. are effective plant growth-promoting (PGP) rhizobacteria, which suppress a wide range of plant pathogens (Young et al., 2005; Solano et al., 2008; Alsultan et al., 2019). However, the effects of Chryseobacterium spp. and P. chlororaphis on chocolate spot disease management; enhancement of faba bean growth, and improvement of the yield-related parameters, have not been fully evaluated yet under field conditions. Therefore, this study was conducted to evaluate the efficacy of using Chryseobacterium spp. and P. chlororaphis on chocolate spot disease management; enhancing faba bean growth, and improvement of the yield-related parameters under field conditions.
2. Materials and Methods

2.1. Description of the study site

This study was conducted at the University of Gondar Horticulture department field site around Shinta River, Ethiopia, during the 2022 main cropping season. The research site is geographically located at 12°35’N and 37°26’E and has an altitude of 2115 m.a.s.l. The used soil of the study area was a clay soil type. The field was naturally infected with B. fabae; the causal agent of faba bean’s chocolate spot (Ademe et al., 2018), and had no rhizobacterial inoculation history. Gondar has a temperate highland tropical climate with dry winter’s climate. The district’s yearly temperature is 22.73ºC (72.91ºF) and it is 0.5% higher than Ethiopia’s averages. Gondar typically receives about 104.26 millimeters (4.1 inches) of precipitation and has 161.74 rainy days (44.31% of the time) annually (https://tcktcktck.org/2023).

2.2. Sources of the rhizobacteria and faba bean varieties

The rhizobacterial strains used in this study were obtained from the culture collection center of the Biology Department at the University of Gondar. The rhizobacterial strains used in this study, included Chryseobacterium strain GY04 (OQ248108.1); Chryseobacterium proteolyticum GY05 (OQ248109.1), and P. chlororaphis GY07 (OQ248111.1). These strains were tested in vitro for their different plant growth promoting attributes, including phosphate solubilization (Pikovskaya, 1948); ammonia production (Cappuccino and Sherman, 1992), IAA production (Brick et al., 2004), and hydrolytic enzymes production, such as protease (Smibert and Krieg, 1994); amylase (Cappuccino and Sherman, 1992), lipase (Ilesanmi et al., 2020), and pectinase (Raju and Divakar, 2013). The used faba beans varieties, include a Dosha variety that was selected for its moderate resistance to chocolate spot disease; and obtained from the local farmers in Gondar Zuria district.

2.3. Seed surface sterilization, inoculants preparation and seeds coating

Faba bean seeds were surface sterilized with 70% alcohol for 3 min.; followed by 1% sodium hypochlorite (NaOCl) for 5 min., and rinsed 5 times with sterile dist. water (Bello et al., 2018). The surface sterilized seeds were dipped in to 4% of carboxymethyl cellulose (CMC) solution as a sticker for 15 min. at the rate of 3 ml/100 seeds.

A single colony of each rhizobacterial strain was taken from 48 h old culture and inoculated individually in 100 ml flasks; each containing 40 ml nutrient broth (NB). The flasks were incubated at 30°C for 2 d on a rotary shaker at 150 rpm. The late log-phase cells were harvested by centrifugation (10,000 g) at 4°C for 10 min., and then washed twice with sterile NaCl solution (0.85%). The bacterial growth was examined by measuring the absorbance of each culture sample spectrophotometrically at 550 nm. The cell densities were related to the viable cell numbers; measured as colony forming unites per ml (cfu/ml) using the standard plate counts, and the number of all rhizobacterial cells was adjusted to (10^8 cfu/ml) (Elbadry et al., 2006). To enhance the survival and establishment of the inoculated strains in the rhizosphere; a charcoal carrier formulation was used. About 150 g of sterile charcoal (Hi Media) was mixed individually with 150 ml of each bacterial suspension (Trivedi et al., 2005). The surface-sterilized and CMC treated faba bean seeds were mixed with the prepared charcoal carrier formulation. However, the control treatment included the surface-sterilized and CMC treated seeds, which were mixed with 150 g of sterile charcoal and 150 ml of NB.

2.4. Experimental design and procedure

The field experiment included the three potential rhizobacterial strains and four treatments were established for the experiment; namely, T1:
Chryseobacterium strain GY04, T2: *Chryseobacterium proteolyticum* GY05, T3: *P. chlororaphis* GY07), and T0: Control treatment (non-bacterized seeds). The experiment was laid out in a randomized complete block design (RCBD) with three replicates for each treatment.

The land that is naturally infected with B. botrytis was prepared by oxen plowing, and the seedbeds were leveled and compacted. A plot size of 2 m × 2.5 m (5 m²) with 40 cm row spacing, 10 cm spacing between plants, and a total of 24 plots were used. The spacing between plots and blocks was 0.5 m. The bacterized and non-bacterized seeds were sown in the prepared seed beds. Fifteen days after seedling emergence, a second bacterial inoculation was performed; in which 5 ml of each bacterial inoculum (10⁸ cfu/ml) was added individually per plot, while 5 ml of sterilized NB was added to the control plot. Weeding practices were made manually by hand. The experiment was performed during the main faba bean production season (June-October) under rain feed conditions without any inorganic fertilization.

2.5. Disease assessment and data collection

At the end of August (i.e., fully flowering stage); disease assessments were carried out in accordance with El-Kholy, (2014). In detail, a total of 500 leaves from each plot were collected randomly, and the number of infected leaves was determined to calculate disease incidence (% of infected leaves) and reduction in chocolate spot incidence (% of efficacy), by using the following formula of El-Shennawy, (2011):

\[
\text{Disease incidence} \% = \frac{\text{Number of infected leaves}}{\text{Total number of tested leaves}} \times 100
\]

\[
\text{Reduction (Efficacy)} \% \text{ of disease incidence} = \frac{\text{infection} \% \text{ in control} - \text{infection} \% \text{ in treatment}}{\text{infection} \% \text{ in control}} \times 100
\]

The severity of the disease was determined using ICARDA. (1986). The disease severity was rated on plants from each plot using a 0-9 scale; where 0, 1, 2, 3, 4, 5, 6, 7 and 8, represent no visible leaf infection (0), or disease covering less than 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 % or 80 % of the foliar tissue, respectively. While 9 represents disease covering more than 90 % of the foliar tissue.

\[
\text{Disease severity} \% = \frac{n \times v}{9 \times N \times 100},
\]

Where: \( n \) = number of plants in each grade, \( v \) = numerical grade (disease grade), \( N \) = total number of plants, and 9 = maximum disease grade

\[
\text{Reduction} \% \text{ (Efficacy)} \% \text{ of disease severity} = \frac{C - T}{C} \times 100.
\]

Where: \( C \) = Disease severity (%) in the control, and \( T \) = Disease severity % in the treatment

Approximately 150 d after sowing, the plants were harvested by hand and allowed to dry for 5 d under natural conditions before the following parameters were evaluated: Plant height (cm), (10 plants per plot); Number of pods per plant, (10 plants per plot); Weight of 100 grains (g) in each plot; Stem girth (mm), (10 plants per plot); and Number of seed per pods, (10 plants per plot).

Also, the % of increase in all parameters was calculated using the following formula of El-Kholy, (2014):

\[
\text{Increase} \% = \frac{T - C}{T} \times 100,
\]

Where; \( T \) = the value of the parameter in the treatment and \( C \) = the value of the parameter in the control

2.6. Statistical analysis

All collected data were analyzed statistically by analysis of variance test (ANOVA), and all numeric differences were considered significantly different at the probability level of \( p \leq 0.05 \).

3. Results

3.1. Plant growth promoting properties of the rhizobacterial strains

The results presented in Table (1) revealed that all the three tested rhizobacterial strains were positive for
(IAA) production; NH₃ Production, P solubilization, lipase and protease production; with different potentials. The multiple plant growth promoting traits of the tested strains are summarized in Table (1).

### Table 1: Rhizobacterial species with in vitro multiple plant growth promoting traits

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Growth promoting traits</th>
<th>Production of hydrolytic enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IAA Production</td>
<td>NH₃ Production</td>
</tr>
<tr>
<td>Chryseobacterium strain GY04</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Chryseobacterium proteolyticum GY05</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>P. chlororaphis GY07</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Where; - = not detected; + = low production; ++ = moderate production; +++ = high production

3.2. Chocolate spot disease assessment

Data presented in Table (2) clearly demonstrate that all the tested rhizobacterial strains significantly reduced the DI and DS of chocolate spot disease in both varieties, compared with the control. The *P. chlororaphis* GY07 recorded the highest reduction of the chocolate spot DI in both the Local and the Dosha varieties by 21% and 14%; respectively, compared with the control, which expressed 78 % in Local and 58 % in Dosha varieties. Regarding to the DS, the highest reduction result was obtained on inoculation of the Local and Dosha varieties of faba bean with *P. chlororaphis* GY07, recording 84.3 % and 81.05, respectively. The *Chryseobacterium* strain GY04 showed moderate reduction of DI and DS of chocolate spot disease in both varieties. The treatment that resulted in the lowest reduction of chocolate spot DI and DS in both varieties was displayed in the faba bean seedlings inoculated with the *Chryseobacterium proteolyticum* GY05, compared to the other rhizobacterial treatments.

3.3. Effects of the rhizobacterial strains on the growth parameters of faba bean

The three rhizobacterial strains were tested for their effects on the faba bean plant growth parameters, including plant height; number of branches, and stem girth. Results shown in Table (3) indicate that all the tested strains caused significant growth parameters enhancement with different potentials (*p* <0.05). *P. chlororaphis* GY07 displayed the highest values of all growth parameters in both Local and Dosha varieties; recording plant height (106.73, 115 cm); number of branches (7, 8.33), and stem girth (2.97, 2.99 mm); respectively. Meanwhile, *Chryseobacterium proteolyticum* GY05 displayed the lowest growth parameters enhancement.
enhancements of plant height (93.93, 105 cm); number of branches (1.66, 3.77), and stem girth (1.60, 1.71 mm), in the Local and Dosha varieties, respectively. The third rhizobacterial *Chryseobacterium* strain GY04 recorded moderate values in all the tested parameters in both faba bean varieties; recording plant height of 98.4, 107.06 cm, number of branches of 3.33, 6.33, and stem girth of 2.00, 2.19 mm, in the Local and Dosha varieties, respectively.

Table 2: The effect of rhizobacterial strains on incidence and severity of faba bean chocolate spot disease incited by *B. fabae* under field conditions

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Faba bean (variety Local)</th>
<th>Faba bean (variety Dosha)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% DI BCE %</td>
<td>% DS BCE %</td>
</tr>
<tr>
<td>Control</td>
<td>78 -</td>
<td>66.08 -</td>
</tr>
<tr>
<td><em>Chryseobacterium</em> strain GY04</td>
<td>28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Chryseobacterium proteolyticum</em> GY05</td>
<td>34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>56.41&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. chlororaphis</em> GY07</td>
<td>21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Where; DI: disease incidence; DS: disease severity; BCE: biological control efficacy. Means in each column followed by the same superscript letter are not significantly different at *p* < 0.05 according to Fisher’s LSD.

Table 3. Effect of rhizobacterial strains treatments on the growth parameters of faba bean

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Local variety</th>
<th>Dosha variety</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plant height (cm)</td>
<td>number of branch</td>
</tr>
<tr>
<td>Control</td>
<td>91.60±0.6</td>
<td>1.33±0.33</td>
</tr>
<tr>
<td><em>Chryseobacterium</em> strain GY04</td>
<td>98.4±1.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.33±0.33&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Chryseobacterium proteolyticum</em> GY05</td>
<td>93.93±0.78&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.66±0.33&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. chlororaphis</em> GY07</td>
<td>106.73±1.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.00±0.57&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LSD at 0.05</td>
<td>0.06</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Where; Values are mean ± Standard error of three replications. Means in each column followed by the same superscript letter are not significantly different at *p* < 0.05 according to Fisher’s LSD, ns: not significant at *p* <0.05.
3.4. Effect of the rhizobacterial strains on the yield related parameters of faba bean

As indicated in Table (4), inoculation of faba bean seedlings with all the tested rhizobacterial strains had a statistically significant effect \( (p < 0.05) \) on the number of pods per plant and 100 grain weight, compared with the un-inoculated treatment. In the faba bean Local variety, the highest pod number per plant was obtained on treatment of plot with the \( P. \) chlororaphis GY07 \( (30.66) \); the moderate effect was recorded on the plot treated with \( Chryseobacterium \) strain GY04 \( (25) \), and the minimum recorded plot was that treated by \( Chryseobacterium \) proteolyticum GY05 \( (20) \).

Similar results were obtained on the Dosha variety of faba bean. Regarding the grains weight; for the Local and Dosha varieties, the maximum results scored from the plot treated with \( P. \) chlororaphis GY07 were 82.83 g and 92.75 g, followed by \( Chryseobacterium \) strain GY04; recording 78.44 g and 85.19 g, and finally \( Chryseobacterium \) proteolyticum GY05, which expressed grain weights of 72.67 g and 81.05 g, respectively. Regarding the number of seeds per pod; all the tested rhizobacterial strains inoculated in both faba bean varieties did not show a statistically significant effect \( (p < 0.05) \), compared with the un-inoculated control treatment.

Table 4: Effect of rhizobacterial strains treatments on yield related parameters of faba bean

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Local variety</th>
<th>Dosha variety</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pod no. per plant</td>
<td>Increase pod no. per plant over the control</td>
</tr>
<tr>
<td>Control</td>
<td>15.66±0.88</td>
<td>3.00±00</td>
</tr>
<tr>
<td>( Chryseobacterium ) strain GY04</td>
<td>25.00±1.52( ^a )</td>
<td>37.36%</td>
</tr>
<tr>
<td>( Chryseobacterium ) proteolyticum GY05</td>
<td>20±0.57( ^c )</td>
<td>21.7%</td>
</tr>
<tr>
<td>( P. ) chlororaphis GY07</td>
<td>30.66±0.33( ^d )</td>
<td>50%</td>
</tr>
<tr>
<td>LSD at 0.05</td>
<td>0.26</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Where; Values are mean ± Standard error of the three replications. Means in each column followed by the same superscript letter are not significantly different at \( p<0.05 \) according to Fisher’s LSD.

4. Discussion

Chocolate spot disease caused by \( B. \) fabae is one of the most yield limiting constraints of faba bean in the Northern West of Ethiopia (Samuel et al., 2008; You et al., 2021). The severity and significance of damage caused by this disease has demanded the development of effective strategies for its management. Different management options have been developed worldwide to reduce the yield losses in faba bean crop due to chocolate spot (Samuel et al., 2010). Biological control using antagonistic microorganisms is a feasible alternative option to the use of synthetic chemicals, and is now becoming a critically needed component for disease management (Lahlali et al., 2022). The effectiveness of the plant growth-promoting rhizobacteria (PGPR) is directly related to the presence of multiple plant growth-promoting traits in these microorganisms. The results of this study are in harmony with those of Antoun and Prévost (2005), who reported that a single PGPR may have multiple modes of action; mainly acting as a bio-control agent. A single rhizobacterium may have multiple plant growth promoting traits (PGP) traits (Rana et al.,...
Rhizobacteria with multiple PGP characteristics can benefit the plants in a variety of ways, including improving root functions; suppressing the disease causing agent, and accelerating the plant growth and development.

The PGPR defend their host plants from the phytopathogens via a variety of mechanisms, including antibiosis; competition for nutrients and space, and through the production of lytic enzymes. In this study, all the three rhizobacterial strains produced at least one hydrolytic enzyme. Goswami et al., (2016) reported that the hydrolytic enzymes are involved in lysis of the cell walls of the fungal pathogens by deforming the cell wall components. In the present study, inoculation of both varieties of the faba bean plant with the three rhizobacterial strains reduced the incidence and severity of the chocolate spot disease. Similarly, a previous study conducted by El-Shennawy, (2011) reported that different bacterial biocontrol agents had shown different protection potentials against the root-rot and wilt disease of faba bean. The observed differences in the biocontrol activities may be attributed to the difference in the rhizobacterial strains’ mechanisms.

In the present study conducted on both faba bean varieties; the highest disease incidence reduction (%) and the highest biocontrol efficacy was observed on the plot treated by P. chlororaphis GY07. Several reports showed that P. chlororaphis has a strong antagonistic and biocontrol activity against several phytopathogenic fungi of different plants (Arjona-López et al., 2019; Tienda et al., 2020; Xu et al., 2021; Liu et al., 2022). P. chlororaphis is an aerobic, Gram-negative bacterium, which is associated with the soil and plant roots, and is characterized by its ability to form biofilm; antifungals production, and/or exoenzyme secretion (Arrebola et al., 2019). The effectiveness of P. chlororaphis GY 07 may be attributed to its single or multiple biocontrol mechanisms. In addition to reducing the incidence and severity of chocolate spot disease of faba bean, P. chlororaphis GY07 enhanced the growth and the yield related parameters significantly (p <0.05), compared with the control. In accordance, Xu et al., (2021) confirmed that inoculation of wheat seedlings with P. chlororaphis significantly promoted their growth. Several studies revealed that P. chlororaphis can produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Nadeem et al., 2007); cause P solubilization (Bertani et al., 2021), and produce indole-3-acetic acid (IAA) (Dimkpa et al., 2012). The production of IAA is important in the microbe-microbe and microbe-plant signaling, and can also results in the promotion of plant growth (Kang et al., 2006). To the best of our knowledge, this is the first report of P. chlororaphis having antagonistic potential against the B. fabae; the causative agent of faba bean chocolate spot disease, in addition to improving the growth and several yield parameters of faba bean. This study provided a clue that P. chlororaphis may be used as a biocontrol agent against the chocolate spot diseases, and as a biofertilizer for enhancing the faba bean production.

Inoculation of faba bean seedlings with the rhizobacterial strains; Chryseobacterium strain GY04 and Chryseobacterium proteolyticum GY05, significantly (p <0.05) reduced the percentage of chocolate spot disease incidence and severity in both faba bean varieties under field conditions. Many studies have demonstrated that Chryseobacterium spp. are potent plant growth-promoting rhizobacteria that suppresses a wide range of plant pathogens (Young et al., 2005; Solano et al., 2008; Alsultan et al., 2019). In addition, inoculation of rhizobacterial strains Chryseobacterium strain GY04 and Chryseobacterium proteolyticum GY05 significantly (p <0.05) enhanced the faba bean growth and yield related parameters in both tested varieties. Youseif, (2018) reported that inoculation Chryseobacterium sp. strain NGB-29 has increased the shoot and root fresh and dry weights of maize plants. To the best of our knowledge, this is the first report of Chryseobacterium strains having antagonistic potential against B. fabae and improving the growth and yield parameter of faba bean. This study provided evidence that Chryseobacterium spp. may be used as biocontrol agents of chocolate spot disease.
disease and as bio-inoculants to promoting the faba bean production.

Conclusion

Faba bean field inoculation with *Chryseobacterium* strain GY04; *Chryseobacterium proteolyticum* GY05, and *P. chlororaphis* GY07 significantly reduced the incidence and severity of chocolate spot disease in both of the Local and Dosha varieties. In addition, these three rhizobacterial strains showed great potential to increase the growth and yield parameters of faba bean varieties. Studying the effects of these potent rhizobacterial strains on other faba bean varieties; different years, and different places is mandatory, in order to use them as potent biocontrol agents of *B. fabae* and as effective biofertilizers of the faba bean crop.

Acknowledgements

This work is a part of PhD. dissertation that was sponsored by the Ministry of Education of Ethiopia. Moreover we would like to acknowledge the Department of Biology, College of Natural Sciences of the University of Gondar, for the kind assistance in providing the laboratory facilities, and all the required consumables and equipment during the whole period of this study. Finally, we would like to acknowledge the Gondar Agricultural Research Centre for providing the faba bean seeds.

Conflict of interest

The authors confirm that there are no known conflicts of interest associated with this publication.

Ethical approval

Non-applicable.

Funding source

This research did not receive any fund from any governmental; commercial, or non-profit funding organizations.

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