Novel Research in Microbiology Journal (2023), 7(3): 1982-1994
Research Article

(Print) (ISSN 2537-0286)
(Online) (ISSN 2537-0294)
https://nrmj.journals.ekb.eg/
DOI: 10.21608/nrmj.2023.303798

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

Gebeyehu Y. Mengstie[1,2*]; Zewdu T. Awlachew[2]; Atsede M. Degefa[2]

[1]Department of Biology, College of Natural and Computational Sciences, P.O. Box 90, Debark University, Debark, Ethiopia; [2]Department of Biology, College of Natural and Computational Sciences, P.O. Box 196, University of Gondar, Gondar, Ethiopia

[*]Corresponding author E-mail: gebeyehuyibeltie@gmail.com

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 un-inoculated 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. Martials 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 (EIAR. 2018), and obtained from the Gondar Agricultural Research Center. The second faba been seeds belonged to Local variety, which was selected for its susceptibility 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^{o}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 spectrophtometrically 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 m^2) 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 none 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 (10^8 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):

Disease incidence % = Number of infected leaves/ total number of tested leaves × 100

Reduction % (Efficacy) of disease incidence = infection % in control - infection% in treatment / infection% in control × 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.

Disease severity % = n×v/9 × N × 100,

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

Reduction % (Efficacy %) of disease severity = C-T /C × 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):

Increase % = T-C/ T × 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).

Table1: Rhizobacterial species with in vitro multiple plant growth promoting traits

Bacterial strain	Growth promoting traits			Production of hydrolytic enzymes			
	IAA Production	NH_3 Production	P Solubilization	Amylase	Lipase	Protease	Pectinase
Chryseobacterium strain GY04	+++	++	+++	++	++	+++	++
Chryseobacterium proteolyticum GY05	+++	++	++	++	+++	+++	++
P. chlororaphis GY07	+++	+++	++	-	+++	++	-

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

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

Treatments	Faba bean (variety Local)				Faba bean (variety Dosha)			
	% DI	BCE %	% DS	BCE %	% DI	BCE %	% DS	BCE %
Control	78	-	66.08	-	58	-	38	-
Chryseobacterium strain GY04	28^{b}	64.1^{b}	11.91	81.97	18b	68.96^{b}	9.3	75.52
Chryseobacterium proteolyticum GY05	34^{c}	56.41^{c}	20.84	68.46	24c	58.62^{c}	14.2	62.63
P. chlororaphis GY07	21^{a}	73.07^{a}	10.37	84.30	14a	75.86^{a}	7.2	81.05

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

Treatment	Local variety			Dosha variety		
	Plant height (cm)	number of branch	Stem girth (mm)	Plant height (cm)	number of branch	Stem girth (mm)
Control	91.60±0.6	1.33±0.33	1.4±0.04	100±1.15	2.33±0.33	1.53±0.08
Chryseobacterium strain GY04	$98.4±1.44^{b}$	$3.33±0.33^{b}$	$2.00±0.05^{b}$	$107.06±0.52^{b}$	$6.33±0.88^{b}$	$2.19±0.06^{b}$
Chryseobacterium proteolyticum GY05	$93.93±0.78^{bc}$	$1.66±0.33^{ns}$	$1.60±0.05^{c}$	$105±2.08^{b}$	$3.67±0.33^{c}$	$1.71±00^{c}$
P. chlororaphis GY07	$106.73±1.79^{a}$	$7.00±0.57^{a}$	2.97±00a	$115±1.73^{a}$	$8.33±0.57^{a}$	$2.99±00^{a}$
LSD at 0.05	0.06	0.72	0.31	0.05	0.5	0.28

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

Treatment	Local variety				Dosha variety			
	Pod no. per plant	Increase pod no. per plant over the control	Seed per pod	Hundred grain weight (g)	Pod no. per plant	Increase pod no per plant over the control	Seed per pod	Hundred grain weight (g)
Control	15.66±0.88		3.00±00	69.99±0.40	18.00±0.57		3.33±0.33	73.16±0.31
Chryseobacterium strain GY04	25.00±1.52^{b}	37.36%	3.66±0.33^{b}	78.44±0.12^{b}	26.66±1.85^{b}	32.48%	3.66±0.33	85.19±1.74^{b}
Chryseobacterium proteolyticum GY05	20±0.57c	21.7%	3.66±0.33^{b}	72.67±0.42^{c}	23.00±0.57^{c}	21.73%	3.66±0.33	81.05±0.26^{c}
P. chlororaphis GY07	30.66±0.33^{a}	50%	4.00±00^{a}	82.83±0.19^{a}	33.00±0.57^{a}	45.45%	4.00±00	92.75±0.28^{a}
LSD at 0.05	0.26		0.12	0.06	0.23		0.13	0.09

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*.,

2011). 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 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 varities; 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. desertion 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.

5. References

Abera, M. and Semagn, M. (2022). 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. American Journal of Plant Sciences. 13: 588-599. https://doi.org/10.4236/ajps.2022.135039

Adal, M.; Wubie, G. and Adem, K. (2022). Isolation and Characterization of Rhizobacteria with Biocontrol activities against Faba bean (*Vicia faba L*.) Chocolate spot disease causing *Botrytis fabae*. Abyssinia Journal of Science and Technology. 7(1): 1-10. https://doi.org/10.20372/ajst.2022.7.1.429.

Ademe, A.; Ebabuye, Y.; Gelaye, M.; Gezachew, S. and Telahun, G. (2018). Survey of faba bean (*Vicia faba* L.) diseases in major faba bean growing districts of North Gondar. African Journal of Plant Science. 12(2): 32-36. https://doi.org/10.5897/AJPS2016.1615

Alsultan, W.; Vadamalai, G.; Khairulmazmi, A.; Saud, H.M.; Al-Sadi, A.M.; Rashed, O. et al. (2019). Isolation, identification and characterization of endophytic bacteria antagonistic to *Phytophthora palmivora* causing black pod of cocoa in Malaysia. European Journal of Plant Pathology .155(4): 1077 1091. https://doi.org/10.1007/s10658-019-01834-8.

Antoun, H. and Prévost, D. (2005). Ecology of plant growth promoting rhizobacteria. In: Siddiqui, Z.A. (Editor). PGPR: Biocontrol and Biofertilization. Dordrecht: Springer. pp. 1-38.

Arjona-López, J.M.; Tienda, S.; Arjona-Girona, I.; Cazorla, F.M. and López-Herrera, C. J. (2019). Combination of low concentrations of fluazinam and antagonistic rhizobacteria to control avocado white root rot. Biological Control. 136:103996. https://doi.org/10.1016/j.biocontrol.2019.05.015

Arrebola, E.; Tienda, S.; Vida, C.; de Vicente, A. and Cazorla, F.M. (2019). Fitness features involved

in the biocontrol interaction of *Pseudomonas chlororaphis* with host plants: The case study of PcPCL1606. Frontiers in Microbiology.10: 439018. https://doi.org/10.3389/fmicb.2019.00719.

Bello, O.A.; Esan, E.B. and Obembe, O.O. (2018). Establishing surface sterilization protocol for nodal culture of *Solanecio biafrae*. IOP Conference Series: Earth and Environmental Science. 210: 012007.

https://doi.org/10.1088/1755-1315/210/1/012007

Bertani, I.; Zampieri, E.; Bez, C.; Volante, A.; Venturi, V. and Monaco, S. (2021). Isolation and Characterization of *Pseudomonas chlororaphis* Strain ST9; Rhizomicrobiota and in Planta Studies. Plants. 10(7): 1466. https://doi.org/10.3390/plants10071466

Brick, J.M.; Bostock, R.M. and Silverstone, S.E. (2004). Rapid *in situ* assay for indole acetic acid production by bacteria immobilized on the nitrocellulose membrane. Applied and Environmental Microbiology.57 (2): 535-538. https://doi.org/10.1128/aem.57.2.535-538.1991

Cappuccino, J.C., and Sherman, N. (1992). Microbiology: "A Laboratory Manual". Benjamin/ Cummings, New York, NY, USA.

Dimkpa, C.O.; Zeng, J.; McLean, J.E.; Britt, D.W.; Zhan, J. and Anderson, A.J. (2012). Production of indole-3-acetic acid *via* the indole-3-acetamide pathway in the plant-beneficial bacterium *Pseudomonas chlororaphis* O6 is inhibited by ZnO nanoparticles but enhanced by CuO nanoparticles. Applied and Environmental Microbiology.78 (5): 1404-1410. https://doi.org/10.1128/AEM.07424-11

Dobocha, D. and Bekele, D. (2022). Faba Bean Agronomic and Crop Physiology Research in Ethiopia. Legumes Research. 1: 289. https://doi.org/10.5772/intechopen.101542

EIAR. (2018). Faba bean Production Guideline Using Rhizobial Bio-fertilizer Technology. Addis Ababa, Ethiopia. 1-37.

El-Banoby, F.; Abd-Alla, M.A.; Tolba, I.H.; Morsy, A.A.; El-Gamal, N.G. and Khalil. M. (2013). Biological control of chocolate spot disease of faba bean using some bioagents under field conditions. The Journal of Applied Sciences Research. 9*:* 4021-4029.

Elbadry, M.; Taha, R.M.; Eldougdoug, K.A. and Gamal-Eldin, H. (2006). Induction of systemic resistance in faba bean (*Vicia faba* L.) to *bean yellow mosaic potyvirus* (BYMV) *via* seed bacterization with plant growth promoting rhizobacteria. Journal of Plant Diseases and Protection. 113: 247-251. https://doi.org/10.1007/BF03356189

El-Kholy, R.M.A. (2014). Chemical and biological control of chocolate spot disease in faba bean under field conditions. Middle East Journal of Agriculture Research. 3(2): 368-377.

El-Shatoury, S.A.; Ameen, F.; Moussa, H.; Wahid, O.A.; Dewedar, A. and AlNadhari, S. (2020). Biocontrol of chocolate spot disease (*Botrytis cinerea*) in faba bean using endophytic actinomycetes Streptomyces: A field study to compare application techniques. PeerJ Microbiology. 8: e8582. https://doi.org/10.7717/peerj.8582

El-shennawy, R. (2011). Biological control of root-rot and wilt disease of faba bean using some bioagents. Journal of Plant Protection and Pathology. 2(2): 195-202. https://doi.org/10.21608/jppp.2011.84676

Fekadu, A. and Tesfaye, A. (2015). *Pseudomonas fluorescens* isolates used as a plant growth promoter of faba bean (*Vicia faba*) *in vitro* as well as *in vivo* study in Ethiopia. American Journal of Life Sciences. 3(2): 100-108. https://doi.org/10.11648/j.ajls.20150302.17

Gebru, A. and Mesganaw, M. (2021). Improving faba bean production and productivity through the integrated management of *Orobanche Crenatae* at

Kutaber, Amhara Region, Ethiopia. International Journal of Agricultural Science and Food Technology. 7(1): 114-117. https://doi.org/10.17352/2455-815x.000097

Goswami, D.; Thakker, J.N. and Dhandhukia, P.C.A (2016). Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. Cogent Food & Agriculture. 2: 1127500. https://doi.org/10.1080/23311932.2015.1127500

ICARDA. (1986). Screening techniques for disease resistance in faba bean. International Center for Agricultural Research in the Dry Areas, Aleppo. Syria. pp: 59.

Ilesanmi, O.I.; Adekunle, A.E.; Omolaiye, J.A.; Olorode, E.M. and Ogunkanmi, A.L. (2020). Isolation, optimization and molecular characterization of lipase producing bacteria from contaminated soil. Scientific African. 8: e00279. https://doi.org/10.1016/j.sciaf.2020.e00279

Jiao, X.; Takishita, Y.; Zhou, G. and Smith, D.L. (2021). Plant Associated Rhizobacteria for Biocontrol and Plant Growth Enhancement. Frontiers in Plant Science. 12: 634796. https://doi.org/10.3389/fpls.2021.634796.

Kang, B.R.; Yang, K.Y.; Cho, B.H.; Han, T.H.; Kim, I.S.; Lee, M.C. et al. (2006). Production of indole-3-acetic acid in the plant-beneficial strain *Pseudomonas chlororaphis* O6 is negatively regulated by the global sensor kinase *GacS*. Current Microbiology. 52: 473-476. https://doi.org/10.1007/s00284-005-0427-x

Karkanis, A.; Ntatsi, G.; Lepse, L.; Fernández, J.A.; Vågen, I.M.; Rewald, B. et al. (2018). Faba Bean Cultivation - Revealing Novel Managing Practices for More Sustainable and Competitive European Cropping Systems. Frontiers in Plant Science. 9: 1115. https://doi.org/10.3389/fpls.2018.01115

Khunnamwong, P.; Lertwattanasakul, N.; Jindamorakot, S.; Suwannarach, N.; Matsui, K. and Limtong, S. (2020). Evaluation of antagonistic activity and mechanisms of endophytic yeasts against pathogenic fungi causing economic crop diseases. Folia Microbiologica. 65(3): 573-590. https://doi.org/10.1007/s12223-019-00764-6

Lahlali, R.; Ezrari, S.; Radouane, N.; Kenfaoui, J.; Esmaeel, Q.; El Hamss, H. et al. (2022). Biological Control of Plant Pathogens: A Global Perspective. Microorganisms. 10(3): 596. https://doi.org/10.3390/microorganisms10030596.

Liu, F.; Yang, S.; Xu, F.; Zhang, Z.; Lu, Y.; Zhang, J. et al. (2022). Characteristics of biological control and mechanisms of *Pseudomonas chlororaphis* zm-1 against peanut stem rot. BMC Microbiology. 22: 9. https://doi.org/10.1186/s12866-021-02420-x

Mbazia, A.; Omri Ben Youssef, N. and Kharrat, M. (2016) .Tunisian Isolates of *Trichoderma* spp. and *Bacillus subtilis* Can Control *Botrytis fabae* on Faba Bean. Biocontrol Science and Technology. 26 (7): 915-927. https://doi.org/10.1080/09583157.2016.1168775.

Muruts, L.W. and Alemayehu, C. (2021). Management of faba bean chocolate spot (*Botrytis fabae*) through varieties and fungicide application frequencies in Southern Tigray, Ethiopia. Archives of Phytopathology and Plant Protection. 54(19-20): 2233-2246. https://doi.org/10.1080/03235408.2021.1925516

Nadeem, S.M.; Zahir, Z.A.; Naveed, M. and Arshad, M. (2007). Preliminary investigation on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC deaminase activity. Canadian Journal of Microbiology. 53(10): 1141-1149. https://doi.org/10.1139/W07-081

Pikovskaya, R.E. (1948). Mobilization of phosphorus in soil in concentration with vital activity of some microbial species. Microbiology. 17: 362-337.

Raju, E.V.N. and Divakar, G. (2013). Production of pectinase by using *Bacillus circulans* isolated from dump yards of vegetable wastes. International Journal of Pharmaceutical Sciences and Research. 4(7): 2615-2622. https://doi.org/10.13040/IJPSR.0975-8232

Rana, A.; Saharan, B.; Joshi, M.; Prasanna, R. and Kumar, K. (2011). Identification of multi-trait PGPR isolates and evaluating their potential as inoculants for wheat. Annals of Microbiology. 61: 893-900. https://doi.org/10.1007/s13213-011-0211-z

Samuel, S.; Chemeda, F.; Sakhuja, P. K. and Seid, A. (2010). Yield loss of faba bean (*Vicia faba*) due to chocolate spot (*Botrytis fabae*) in sole and mixed cropping systems in Ethiopia. Archives of Phytopathology And Plant Protection. 43(12): 1144-1159. https://doi.org/10.1080/03235400802343791

Samuel, S.; Ahmed, S.; Fininsa, C.; Abang, M.M. and Sakhuja, P.K. (2008). Survey of chocolate spot (*Botrytis fabae*) disease of faba bean (*Vicia faba* L.) and assessment of factors influencing disease epidemics in northern Ethiopia. Crop Protection. 27(11), 1457-1463. https://doi.org/10.1016/j.cropro.2008.07.011

Smibert, R.M. and Krieg, N.R. (1994). Phenotypic characterization. In: Gerhardt, P.; Murray, R.G.E.; Wood, W.A. and Krieg, N.R. (Editors). Methods for General and Molecular Bacteriology. Washington DC: American Society of Microbiology. pp. 607-654.

Solano, R.B.; Barriuso Maicas, J.; Pereyra de la Iglesia, M.T.; Domenech, J. and Gutiérrez Mañero, F.J. (2008). Systemic disease protection elicited by plant growth promoting rhizobacteria strains: relationship between metabolic responses, systemic disease protection, and biotic elicitors. Phytopathology. 98(4): 451-457. https://doi.org/10.1094/PHYTO-98-4-0451

Tienda, S.; Vida, C.; Lagendijk, E.; de Weert, S.; Linares, I.; Guirado, E. et al. (2020). Soil Application of a Formulated Biocontrol *Rhizobacterium*, *Pseudomonas chlororaphis* PCL1606, Induces Soil Suppressiveness by Impacting Specific Microbial Communities. Frontiers in Microbiology. 11: 1874. https://doi.org/10.3389/fmicb.2020.01874.

Torres, S.A.M.; Roman, B.; Avila, C.M.; Satovic, Z.; Rubials, D.; Sillero, J.C. et al. (2004). Faba bean breeding for resistance against biotic stresses: Towards application of marker Technology. Euphytica. 147: 67-80. http://dx.doi.org/10.1007/s10681-006-4057-6

Trivedi, P.; Pandey, A. and Palni, S. (2005). Carrier-based preparations of plant growth-promoting bacterial inoculants suitable for use in cooler regions. World Journal of Microbiology and Biotechnology. 21: 941-945. https://doi.org/10.1007/s11274-004-6820-y

Walia, A.; Mehta, P.; Guleria, S.; Chauhan, A. and Shirkot, C.K. (2014). Impact of Fungicide Mancozeb at Different Application Rates on Soil Microbial Populations, Soil Biological Processes, and Enzyme Activities in Soil. The Scientific World Journal. 2014: 9. https://doi.org/10.1155/2014/702909

Xu, W.; Xu, L.; Deng, X.; Goodwin, P.H.; Xia, M.; Zhang, J. et al. (2021). Biological Control of Take-All and Growth Promotion in Wheat by *Pseudomonas chlororaphis* YB-10. Pathogens. 10(7): 903. https://doi.org/10.3390/pathogens10070903

You, M.P.; Eshete, B.B.; Kemal, S.A.; van Leur, J. and Barbetti, M.J. (2021). *Physoderma*, not *Olpidium*, is the true cause of faba bean gall disease of *Vicia faba* in Ethiopia. Plant Pathology. 70(5): 1180-1194. https://doi.org/10.1111/ppa.13359

Young, C.C.; Kämpfer, P.; Shen, F.T.; Lai, W.A. and Arun, A.B. (2005) .*Chryseobacterium formosense* sp. nov., isolated from the rhizosphere of *Lactuca sativa* L. (garden lettuce). International Journal of Systematic and Evolutionary Microbiology. 55(1): 423-426. https://doi.org/10.1099/ijs.0.63331-0

Youseif, S.H. (2018). Genetic diversity of plant growth promoting rhizobacteria and their effects on the growth of maize plants under greenhouse conditions. Annals of Agricultural Sciences. 63(1): 25-35. https://doi.org/10.1016/j.aoas.2018.04.002

Zewdineh, F.; Tesfaye, A. and Fassil, A. (2022) . The synergistic effects of *Trichoderma harzianum* AAUT14 and *Bacillus subtilis* AAUB95 on faba bean (*Vicia faba* L.) growth performance and control of chocolate spot compared to chemical fungicides under greenhouse conditions. Archives of Phytopathology and Plant Protection. 55(2): 129-142. https://doi.org/10.1080/03235408.2021.2000179