Egypt. J. Bot. **Vol. 61**, No. 1, pp. 241-253 (2021)

Egyptian Journal of Botany
http://ejbo.journals.ekb.eg/

Biological Control of Root Rot Disease and Growth Promotion in Fenugreek (*Trigonella foenum-graecum* L.) Mediated by Microbial Antagonism

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PATHOGENIC fungi are among the most serious biotic agents that attack plants and cause considerable losses in crop yield. Therefore, a sincere effort is being made to provide safe and inexpensive ecofriendly innovative approaches for managing plant diseases and reducing the fungicide pressure in our agro-ecosystem. In this study, *Bradyrhizobium japonicum* Asw1 was successfully used for suppressing the root rot disease of fenugreek (*Trigonella foenum-graecum* L.) caused by *Fusarium solani*. Strain Asw1 exhibited significant antifungal activity revealing remarkable antagonistic effect against the pathogen. The results showed that *T. foenum-graecum* was susceptible to fungal infection which severely affected the growth and metabolite content of the plant. Treatment with strain Asw1 resulted in a sharp reduction of disease incidence from 80 to 13%. Growth attributes, *e.g.*, plant height (cm), number of leaves per plant, fresh and dry weight (g) of treated plants were significantly enhanced. The contents of chlorophylls, proteins, carbohydrates, flavonoids, phenolics and tannins were significantly ($P< 0.05$) reduced in the infected plants. In the treated plants, protein and carbohydrate contents were not enhanced compared to control. However, the content of total flavonoids was significantly accumulated in the treated plants which might trigger their resistance against fungal infection.

Keywords: Antagonism, Biological control, *Bradyrhizobium japonicum,* Fenugreek, *Fusarium solani*, Root rot.

Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual plant that belongs to family Fabaceae. It is worldwidely distributed neighboring the eastern shores of the Mediterranean, and it is broadly spread in India, Egypt and Morocco as a cultivated crop (Snehlata & Payal, 2012). It is a valuable plant in the human life and has been usually used as food and medicine (Syeda et al., 2008). The leaves are eaten as a vegetable, and the seeds are used as a raw material in pharmaceutical industry and traditional medicine for treatment of kidney and digestive problems, wounds, abscesses and arthritis as well as in cooking as a spice. Fenugreek is a source of minerals, protein, vitamin A and C (Bose et al., 2004; Snehlata & Payal, 2012).

In agriculture, the greatest annual serious losses are caused by the fungal plant diseases (Agrios, 1988). *Cercospora* leaf spot, powdery mildew, *Fusarium* wilt and root rot are among the most serious agronomic fungal diseases infecting fenugreek which cause significant economic losses in crop yield (Acharya et al., 2010). Root rot disease of fenugreek caused by *Fusarium solani* which affects seed germination, plant development and crop production was previously reported in Egypt (Ali et al., 2018). The typical symptoms of *F. solani* root rot are yellowing and defoliation of the leaves, wilting and stunting of the plant, browning of the roots and decaying of the cortical tissues (Yadav et al., 2019).

The known recommended fungicides are costly and rarely available in the market. Consequently,

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Received 28/8/ 2020; Accepted 2/11/ 2020
DOI: 10.21608/ejbo.2020.40834.1546
Edited by: Prof. Dr.: Khaled M. Ghanem, Faculty of Science, Alexandria University, Alexandira, Egypt.

the hazardous chemical approaches for controlling plant diseases must be regulated and the search for alternative approaches for the management using economic and eco-friendly biological control by antagonistic microorganisms is being an urgent need (Kumawat, 2014). Biological control by introducing non-pathogenic antagonistic species is healthy and environmentally safe (Schouten et al., 2004).

The active microorganisms, effectively used as biocontrol agents, antagonize the plant pathogens through many mechanisms of action as antibiosis, hyper-parasitism, competition or metabolite production (Heydari & Pessarakli, 2010). Several bacterial species were successfully used for controlling plant fungal diseases such as *Bacillus subtilis, Actinoplanes* spp.*, Burkholderia cepacia* A3R*, Comamonas acidovorans* HF42*, Enterobacter* sp. BF14*, Paenibacillus* sp.*, Rhizobium etli* and *Pseudomonas* spp. (Whipps, 2001; Heydari & Pessarakli, 2010). Therefore, the present study aimed to evaluate the antagonistic efficacy of *B. japonicum* Asw1 against *F. solani* which causes root rot in fenugreek plant in order to manage and reduce the disease incidence.

Materials and Methods

Isolation and identification of causal pathogen

Infected fenugreek plants were collected in sterile plastic bags from the greenhouse at the Research Unit for Study Plants of Arid Lands, Aswan University (39.59°N 32.82°E). Instantly, roots were carefully surface sterilized with 70% ethanol, washed three times with sterile distilled water and cut into pieces. Four pieces were placed on potato dextrose agar (PDA) plates. Then, plates were incubated at 28±2ºC for 7 days. A single colony was isolated, purified and coded as Fen 2020. The morphological and microscopical characteristics of the isolates were determined according to the keys described by Barnett & Hunter (1972) and Moubasher (1993).

Molecular characterization was performed in the molecular Biology Research Unit, Assiut University, Egypt using internal transcribed spacer (ITS) sequence. The total gDNA was extracted using Patho-gene-spin DNA/RNA extraction kit (Intron Biotechnology Company, Korea). DNA samples were sent to SolGent Company, Daejeon, South Korea for polymerase chain reaction (PCR) and ITS sequencing. ITS was amplified using ITS1-F and ITS4-R primers: (ITS1-F: 5′CCGTAGGTGAACCTGCGG-3′ and ITS4-R: 5′-TCCTCCGCTTATTGATATGC-3′). PCR was carried out using Gene Amp® PCR system 9700 (Applied Biosystem, Foster City, CA, USA). PCR products were reconfirmed using 1% agarose gel electrophoreses with a nucleotide marker of 100bp (White et al., 1990). Finch TV version 1.4.0 (www.geospiza.com) was used for sequence assembly. The obtained ITS sequence was blasted with sequences deposited in NCBI GenBank database (https://www.ncbi.nlm.nih.gov/). The evolutionary tree of isolate Fen 2020 and the most closely related fungi derived from NCBI GenBank based on ITS sequence was constructed by Neighbor-joining method with 1000 bootstrap replication using MEGA X 10.1.7 software.

Bacterial strain and in vitro antagonistic test

The plant growth-promoting rhizobacterium (PGPB), *B. japonicum* Asw1 (MN079045), was previously isolated from *Vigna unguiculata* (L.) nodules (Hagaggi & Radwan, 2020). The effect of *B. japonicum* Asw1 against the growth of *F. solani* Fen 2020 was estimated using the dual culture method (Gupta et al., 2006). Seven-day-old fungal PDA disc (5mm in diameter) was inoculated in one side on pre-solidified PDA medium and the opposite side was inoculated with a loopful of 24hrs. old bacterial culture (10^8 CFU/mL) at approximately 2 cm away. The test fungus placed at the center of PDA plates was used as control. Plates were incubated at 28±2ºC for 7 days. Experiment was performed in triplicate and repeated twice. The inhibition percentage over control of fungal growth was calculated using the following formula:

$$\text{Inhibition (\%)} = (C-T)/C \times 100$$

where, C is the growth of the tested pathogen in control (mm), and T is the growth of the tested pathogen against the antagonist (mm).

Antifungal activity and Analysis of secondary metabolites produced by strain Asw1

B. japonicum Asw1 was grown for 72hrs. in yeast extract mannitol broth at 28±2°C under 150rpm. Culture was centrifuged and the supernatant was extracted with equal volume of ethyl acetate. Then, the extract was subjected to various quantitative analyses including total phenolics, total flavonoids, total saponin and total tannins according to the methods described

by Singleton et al. (1999), Zhishen et al. (1999), Ebrahimzadeh (1998) and Julkunen-Tiitto (1985).

The antifungal effect of strain Asw1 against the pathogenic fungus Fen 2020 was evaluated by the poisoned food method (Balouiri et al., 2016). The prepared ethyl acetate extract of strain Asw1 was added at a concentration of 1mg/mL to PDA medium as mentioned above. Mycelial disc (5mm in diameter) was placed in the center of Petri plates. Plates were incubated at 28±2°C for 7 days. The diameters of fungal growth were measured for both the control and treated plates, and the percentage of inhibition was calculated (Singh & Tripathi, 1999).

Bio-priming of seeds with pathogenic and antagonistic inoculum

Fenugreek seeds were surface sterilized by immersion in 2% sodium hypochlorite (3 min) followed by 70% ethanol (2min), and then rinsed five times with sterile distilled water. Seeds were divided into three groups: (1) Seeds soaked in pathogen suspension (1×10^7 spores mL^{-1}) (infected), (2) Seeds soaked in (1:1 v:v) *B. japonicum* suspension (1×10^7 CFU mL^{-1}) plus pathogen suspension (1×10^7 spores mL^{-1}) (treated), (3) Seeds soaked in sterile distilled water (control). All seeds were kept at 25±2°C in a rotary shaker (50rpm) for 3hrs. to facilitate the penetration of the fungus and bacterium inside the seeds. These seeds were then used for subsequent experiments.

Evaluation of seed germination, seedling emergence and seedling vigor

To evaluate the effect of pathogen and antagonistic bacterial treatment on seed germination, infected, treated and control seeds were plated at equal distances on moistened filter paper placed in glass Petri plates. Germination percentage was calculated using the following formula:

Germination (%)= (No. of germinated seeds)/ (Total no. of seeds)×100

After seedling establishment, the seedling emergence and seedling vigor index were assessed according to Abdul-Baki & Anderson (1973).

In vivo pot experiment

The antagonistic effect of *B. japonicum* Asw1 against *F. solani* isolate Fen 2020 was studied using pot culture following the method described by Tamiru & Muleta (2018). Plastic pots were surface sterilized with 5% sodium hypochlorite and washed many times with sterile distilled water. Pots were packed with a mixture of pre-sterilized clay and sand (2:1, w/w). Surface sterilized healthy seeds of fenugreek were sown (10 seeds/pot) and kept at 28±2°C for one week until the development of seedlings. Treatments were carried out as follows: control [uninoculated]; infected [inoculated with 20mL of pathogen (1×10^7 spores mL^{-1})] and treated [inoculated with a combination of 20mL of the pathogen (1×10^7 spores mL^{-1}) plus 20mL of the antagonist (1×10^8 CFU mL^{-1})]. Inoculation was applied to each seedling near the root at soil depth about 3cm. Then, the pots were maintained under normal climatic conditions in a randomized complete block design. The experiment was repeated three times during 2019-2020.

Up to 30 days after inoculation, the percent of disease incidence was evaluated according to Singh & Rao (2015) as follows:

Disease incidence (%)= (No.of diseased plants in the treatment)/ (Total no of plants in the treatment) × 100

Growth attributes and metabolite analysis

Growth attributes, i.e., plant height (cm), number of leaves per plant, fresh and dry weight (g) of fenugreek plants were recorded. Then, the contents of chlorophylls, proteins, carbohydrates and some secondary metabolites of the seedlings were evaluated.

Chlorophyll content

Fresh leaves (0.1g) of control, infected and treated seedlings were homogenized with 80% acetone at the end of the experiment. Then, the homogenates were filtered and the contents of chlorophyll a (Chl. *a*) and chlorophyll b (Chl. *b*) were measured in the filtrates according to Mackinney (1941).

The content of total carbohydrates

The content of carbohydrates in the seedlings was spectrophotometrically measured using anthrone reagent according to Morris (1948). Glucose was used as a standard and the content of carbohydrates was measured as mg/ g dry weight.

Protein content

The content of total proteins of the dried seedlings was evaluated using Folin-Ciocalteau reagent according to Lowry et al. (1951). Egg albumin was prepared in a diluted series and used as a standard to illustrate the calibration curve. The content of protein was expressed as mg/g dry weight.

The content of total phenolics, total flavonoids and tannins

Five mL of methanol (80%) were added to 100mg of dried seedlings and vortexed for 1min. Then, the mixtures were kept in water bath (60ºC) for 1hr., centrifuged at 800rpm for 10min and the supernatants were used for determination of phenolics, flavonoids and total tannins as previously stated.

Statistical analysis

The obtained data were analyzed using one-way analysis of variance (ANOVA; from Minitab version 18.1). A P value less than 0.05 ($P< 0.05$) was statistically significant. Principal component analysis (PCA) was run (Minitab version 18.1) to discriminate the different treatments concerning their content of metabolites. Data were collected from three biological replicates (n= 3).

Results

Identification and evolutionary relationships of the causal pathogen

Only one fungal species coded as Fen 2020 was detected and isolated from the rotten roots of infected fenugreek plants collected from the greenhouse. The isolate was morphologically and microscopically characterized. It produced white cottony mycelia. Morphological characteristics of conidia of 7-day-old culture grown on PDA medium at 28±2°C were examined under microscope. The macroconidia were slightly curved, hyaline and broad having 1 to 3 septa. The microconidia were the most abundant, oval, hyaline and smooth (Fig. 1). The Fen 2020 isolate was molecularly identified using ITS region genomic sequence (632bp). Blast analysis with NCBI Genbank database revealed that the highest similarity of Fen 2020 sequence (100% sequence identity, E value 0.0) was with *F. solani* CBS 140079 (NR163531). Using MEGA X 10.1.7 software, the evolutionary relationships of isolate Fen 2020 and the closest fungi from NCBI Genbank was illustrated by neighbor-joining phylogram (Fig. 2).

In vitro antagonistic test

Strain Asw1 significantly inhibited the mycelial growth of *F. solani*. The inhibition was 75% compared with control (Fig. 3).

Antifungal activity and secondary metabolites of strain Asw1

The antifungal activity of strain Asw1 against Fen 2020 isolate was estimated. Results showed that the incorporation of Asw1 ethyl acetate into the PDA medium resulted in significant inhibition of fungal growth (90%) compared with the control (Fig. 4). The secondary metabolites produced by strain Asw1 were quantified. The ethyl acetate extract of strain Asw1 contained high amount of tannins (137.95±15.35mg/g extract) and saponins (114.54±3.16mg/g extract) (Table 1).

Fig. 1. Isolate Fen 2020 on PDA medium and under microscope

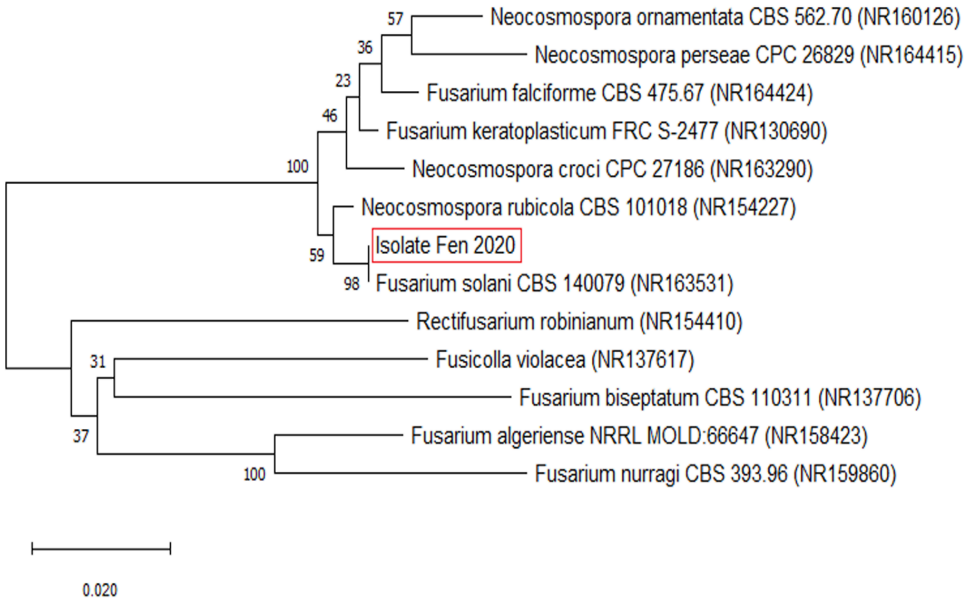

Fig. 2. The neighbor-joining phylogram with 1000 bootstrap replication revealing the evolutionary relationships of Fen 2020 isolate and closely related fungi based on ITS sequences from NCBI Genbank

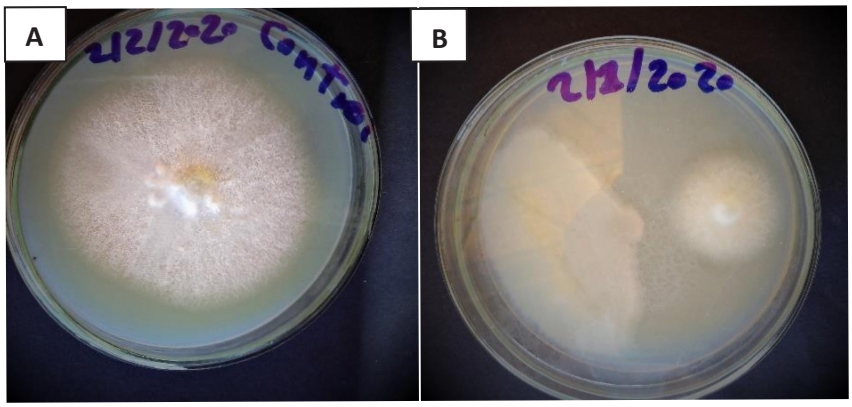

Fig. 3. Antagonistic activity of strain Asw1 against *Fusarium solani*

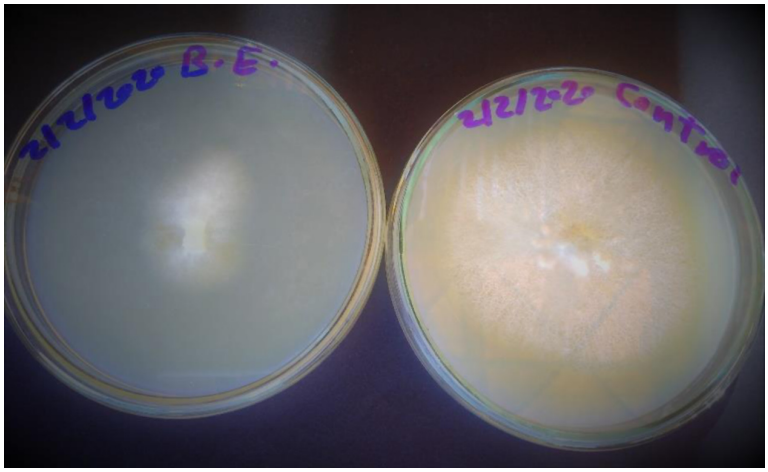

Fig. 4. Antifungal activity of ethy acetate extract of strain Asw1

TABLE 1. Secondary metabolites of strain Asw1

Metabolites	Concentration (mg/g extract)
Total phenolics	16.34±4.37
Total flavonoids	85.46±7.12
Total saponins	114.54±3.16
Total tannins	137.95±15.35

Evaluation of seed germination, seedling emergence and seedling vigor

Seed treatment with strain Asw1 significantly (P< 0.05) enhanced early emergence (3 days after sowing) compared with the infected and control seeds (Table 2). The highest percentage of germination (88%), and seedling vigor index (11880) were recorded for the treated seeds. It was observed that the inoculation of seeds with the pathogenic fungus isolate Fen 2020 attributed to significant suppression of seed germination, seedling emergence and seedling vigor (Table 2).

In vivo pot experiment

Plants treated with strain Asw1 offered significant disease suppression after 30 days of sowing where the disease incidence was reduced from 80 to 13 %, and the disease protection percentage was significantly (P< 0.05) raised (Fig. 5 a &b).

Growth attributes and metabolite analysis

Compared with the control group, the treatment of plants with strain Asw1 inoculant significantly enhanced the heights of the plants, increased the number of leaves for each individual plant and improved the fresh and dry weight of plants (Table 3). In contrast, the infection with *F. solani* caused considerable reduction of all studied growth attributes (Table 3).

TABLE 2. Seed germination, seedling emergence and seedling vigor index

	Germination (%)	Seedling emergence	Root length (mm)	Shoot length (mm)	Seedling vigor index
Control	76±0.7^{a}	4 days	17±0.5^{b}	80±2.5^{b}	7372^{c}
Infected	32±1.4^{c}	6 days	10±1.7^{c}	50±1.1^{c}	1920^{b}
Treated	88±1.3^{b}	3 days	30±1.5^{a}	105±2.5^{a}	11880^{a}

- Each value represents mean ± SD (n= 3).
- Different letters indicate significant difference at P<0.05.

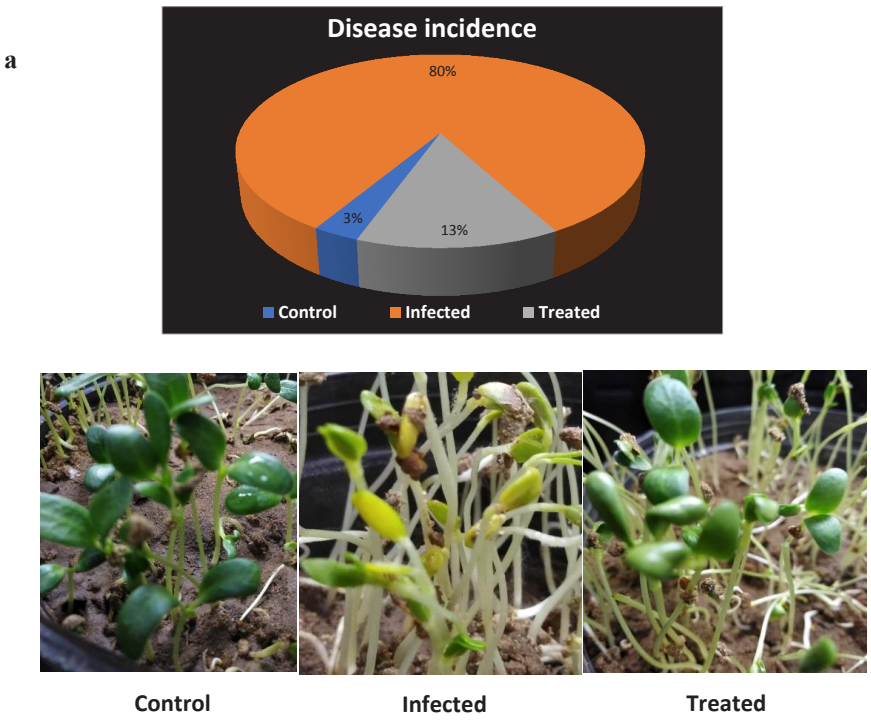

Fig. 5 a &b. Disease incidence in both infected and treated plants vis control

TABLE 3.Growth attributes of fenugreek plants

	Plant height (cm)	No. of leaves / plant	Fresh weight (g)	Dry weight (g)
Control	70±1[b]	45±0.5[b]	10±1.1[b]	6±0.7[b]
Infected	40±1.7[c]	21±1.1[c]	6±0.5[c]	4.3±1.1[c]
Treated	74±2[a]	51±3[a]	13.4±1.2[a]	8.1±0.4[a]

- Each value represents mean ± SD (n= 3).
- Different letters indicate significant difference at P<0.05.

The contents of both Chl a and Chlb were significantly (P< 0.05) reduced in the infected seedlings, while, they were enhanced in the treated seedlings (Table 4). The protein content of the infected fenugreek seedlings was significantly (P< 0.05) reduced compared to control (Table 4). However, treatment by *B. japonicum* significantly increased protein content (P< 0.05) in the treated plants compared to the infected plants (Table 4).

A significant decrease in the carbohydrate level was recorded in the infected seedlings and inoculation with *B. japonicum* did not increase carbohydrate content (Table 4).

In the present study, the flavonoids in the treated seedlings were significantly increased by 76% more than the control seedlings (Table 4). A severe significant reduction (by about 86.7%) in the content of total phenolics was measured in the infected fenugreek plants compared to control plants (Table 4). Also, the content of tannins was significantly decreased in fenugreek plants due to *Fusarium* infection.

PCA indicated the multivariate analysis of the different metabolites in control, treated and infected plants (Fig. 6). The infected plants were deviated as their contents of metabolites were adversely affected as described above. Control plants were characterized by high contents of total carbohydrates, phenolics and tannins (upper-most right side of the plot). On the other hand, the treated plants acquired high accumulation of flavonoids (down-most right side of the plot) which might be the response for fungal resistance in the plant.

Discussion

A variety of soil borne fungi are recognized as the most aggressive pathogens causing critically dangerous plant diseases in agricultural systems resulting in yield reduction and economic losses (Gal-Hemed et al., 2011; Passera et al., 2017).

Since ancient times in Egypt, fenugreek has been used as a nutritive and medicinal herb (Al-Asadi, 2014). Among the most important diseases attacking fenugreek is the root rot caused by *F. solani* which in turn affects seed germination, seedling development and crop yield (Ali et al., 2018). In agriculture, the use of inexpensive ecofriendly biocontrol agents for the management of plant diseases caused by soil borne pathogens instead of hazardous chemicals is of a great interest (Benítez et al., 2004; Jegathambigai et al., 2009; Ali et al., 2018). In this research, the efficacy of using *B. japonicum* strain Asw1 as growth-promoting rhizobacterium for controlling the root rot disease of fenugreek caused by the soil borne *F. solani* was studied.

TABLE 4. Metabolite analysis of fenugreek plants

	Chlorophyll (mg/g FW)		Primary metabolite (mg/g DW)		Secondary metabolite (mg/g extract)		
	Chl. *a*	Chl. *b*	Total protein	Total carbo-hydrates	Total flavonoids	Total phenolics	Total tannins
Control	0.85±0.000[a]	0.47±0.005[a]	100.83±0.59[a]	121.48±0.00[a]	67.17±5.59[b]	25.53±6.83[a]	161.75±18.00[a]
Infected	0.55±0.003[b]	0.22±0.027[b]	27.05±4.48[c]	37.12±3.51[b]	40.85±3.4[c]	3.39±0.91[b]	59.14±6.58[c]
Treated	0.92±0.001[a]	0.44±0.000[a]	85.01±1.176[b]	43.49±0.28[b]	118.27±9.85[a]	14.12±3.78[ab]	98.86±11.00[b]

- Each value represents mean ± SD (n= 3).
- Different letters indicate significant difference at P<0.05.

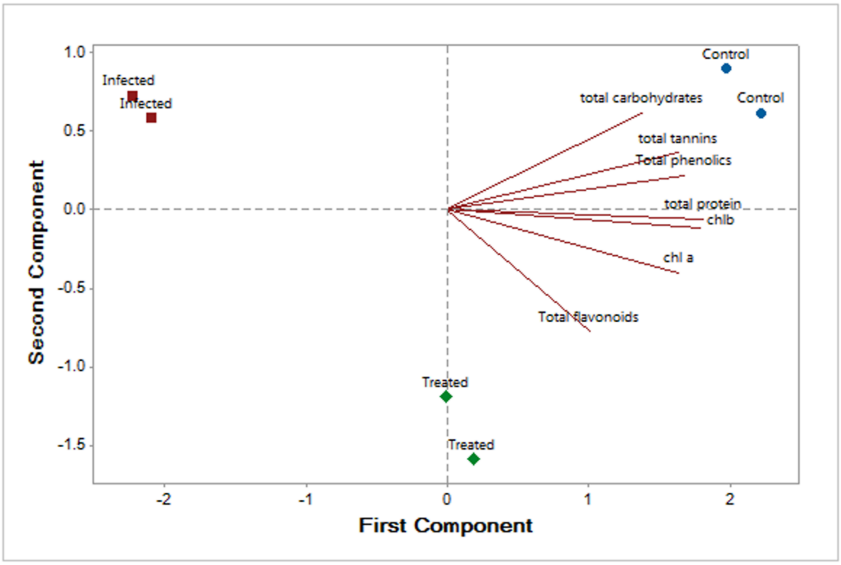

Fig. 6. Biplot of principal component analysis (PCA) showing discrimination of control, infected and treated vis-à-vis their content of metabolites

The replacement of chemical antifungal agents with antagonistic microbes for controlling fungal plant diseases in agriculture has become an important approach in environmental risk management (Zafari et al., 2011). Many investigations reported the antagonistic activities of rhizobacterial species as *Rhizobium trifolii*, *R. leguminosarum*, *R. japonicum* and *B. japonicum* against different plant pathogenic fungi by producing bioactive compounds having antimicrobial activity (Joseph et al., 1983; Rodelas et al., 1998; Nandi et al., 2019).

Numerous microorganisms used in biological control produce bioactive metabolites that can inhibit the growth and activities of different pathogens (Knowles, 1976). *Bradyrhizobium* spp. were recorded by many researches for their potential to produce bioactive and antimicrobial metabolites used against phytopathogens to control and induce plant diseases resistance (Marks et al., 2013; Tjamos et al., 2013; Meena et al., 2018).

Among the phases of crop cycle, seed germination and seedling emergence are the most important (Schut et al., 2014). It was stated that the poor quality of seed germination and seedling emergence have negative effects on plant performance, i.e., reduces plant density and yield, lowers the competition of crops against weeds and increases the development of diseases (Lamichhane et al., 2019). Therefore, the bio-priming of seeds with beneficial microorganisms could be effective for enhancing seed germination and seedling emergence in the early stages (Lamichhane et al., 2019). The effect of seed inoculation with *B. japonicum* on the germination and emergence performance of different crops was previously studied (Kozieł et al., 2013; Masciarelli et al., 2014).

The percentage of disease incidence among plants grown in inoculated soil was 80% compared with control. This agreed with the findings of other researchers who stated that among the fungi causing root rot in the fenugreek, *F. solani* was responsible for the highest incidence of disease (Ehteshamul-Haque et al., 1992; Khokhar et al., 2012; Ali et al., 2018; Ramteke, 2019). In plant disease management, the biological approaches become of an interest. Bio- controlling of plant diseases by rhizobia as an alternative to chemical methods has been reported because they showed satisfactory results in this regard *via* lytic enzymes and antimicrobial secondary metabolite production (Volpiano et al., 2019).

Zafari et al. (2011) reported that *Trichoderma brevicompactum* and *B. japonicum* can be successfully used for disease management caused by *Phytophthora sojae* in soybean. Soybean root rot disease has been limited by a combination of *Bradyrhizobium japonicum* and *Trichoderma harzianumon* (Chakraborty et al., 2003). *Bradyrhizobium* sp. has been used against

Macrophomina phaseolina charcoal rot of peanut (Deshwal et al., 2003).

Pathogenesis is accompanied by many biochemical changes, e.g., cell permeability modification, reduction in chlorophyll levels and nutrient decreases (Warzecha et al., 2015). The enhancement of growth attributes and chlorophyll content was reported in *Citrullus lanatus* treated with *Trichoderma* spp. and *Pseudomonads* spp. and *Lycopersicon esculentum* when treated with *Pseudomonas aeruginosa* and *Burkholderia gladioli* (Alharbi, 2015; Khanna et al., 2019).

The participation of protein constituents is known in plant pathogenic interactions as a mechanism of plant disease resistance (Hameed et al., 2017). Increased protein content was previously reported in plants due to their treatment with PGPR (Junqueira et al., 2004; Pandey et al., 2018).

A decreased content of carbohydrates in plant tissues was reported as a probable reason for the limited resistance to certain fungal diseases (Morkunas & Ratajczak, 2014). On the other hand, the carbohydrate level was enhanced in the resistant plants as a potential mechanism for inducing fungal resistance (Mohammadi et al., 2020).

There was no effect on the carbohydrate content of soybean inoculated with *B. japonicum* (Sanz-sáez et al., 2015). In the same context, endophytic bacterial inoculation of sugarcane did not increase carbohydrate content (Marcos et al., 2016). It could be assumed that carbohydrates were consumed for feedback defense mechanism to accumulate secondary metabolites, e.g., flavonoids (Morkunas et al., 2010; Sanz-sáez et al., 2015). The accumulation of flavonoids was stated for defense mechanism against fungal infection due to their antimicrobial properties (Treutter, 2005; Lu et al., 2017). Inoculation of infected plants with PGPR induced antimicrobial defense mechanism (Stefan et al., 2013) and induced accumulation of flavonoids (Jugran et al., 2015).

Phenolics are eminent antimicrobial secondary metabolites acting as precursors to structural polymers or as signal molecules activating plant defense genes (Hameed et al., 2017). Reduction in the phenolics in the plant tissues indicated the susceptibility of the plant to fungal infection (Junqueira et al., 2004; Ewané et al., 2012). Inoculation of plants with PGPB induced accumulation of phenolics (Cappellari et al., 2017).

Tannins are considered as important bioactive secondary metabolites of fenugreek (Rahmani et al., 2018). In previous studies, Fusarium infected plants exhibited higher tannin composition (Omodamiro et al., 2015).

Conclusion

Inoculation of fenugreek with *B. japonicum* improved its growth attributes and resistance against fungal infection. *B. japonicum* could be successfully used for the bio-management of the disease caused by *F. solani* in fenugreek.

Acknowledgement: Gratitude to Dr. Fatma Fakhry, assistant professor of mycology, Aswan University for confirming the identification of the fungal isolate. The authors appreciate the facilities provided in the Botany Department, Faculty of Science, Aswan University for accomplishing this study.

Conflicts of interest. The authors declare that they have no conflict of interest.

Authors' contribution: Both authors conceived, designed research, conducted experiments and wrote the manuscript. NH contributed new analytical tools and AM analyzed data. Both authors read and approved the manuscript.

Ethical approval: Not applicable.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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المكافحة البيولوجية لمرض تعفن الجذور وتعزيز النمو في الحلبة بوساطة التضاد الميكروبي

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تعتبر الفطريات المسببة للأمراض من أخطر العوامل الحيوية التي تهاجم النباتات وتسبب خسائر كبيرة في المحاصيل. لذلك،كان من الضرورى التركيز على تناول هذه الظاهرة بالبحث والدرسة لتوفير أساليب مبتكرة صديقة للبيئة وآمنة وغير مكلفة لمعالجة أمراض النباتات وتقليل ضغط مبيدات الفطريات في نظامنا البيئي الزراعي. تم في هذه الدراسة استخدام *Bradyrhizobium japonicum* Asw1 بنجاح في قمع مرض تعفن جذور الحلبة الناجم عن *Fusarium solani*. أظهرت السلالة Asw1 نشاطًا مضادًا للفطريات وكشفت عن تأثير ملحوظ ضد مسبب المرض. أظهرت النتائج أن الحلبة كانت عرضة للإصابة بالفطر التي أثرت بشدة على النمو والمحتوى الأيضى للنبات. أدت المعالجة بالسلالة Asw1 إلى انخفاض حاد في حدوث المرض من 80 إلى 13 %. كما أدت إلى تحسين صفات النمو مثل طول النبات، وعدد الأوراق لكل نبات، وكذلك الوزن الرطب والجاف للنباتات المُعالجة. انخفض محتوى الكلوروفيل والبروتينات والكربوهيدرات والفلافونويدات والفينولات والتانينات انخفاضا معنويا في النباتات المُصابة. لم يتم تحسين محتوى البروتين والكربوهيدرات في النباتات المُعالجة مقارنة بالمعاملة الحاكمة. ومع ذلك، فقد تراكم محتوى الفلافونويدات الكلي بشكل كبير في النباتات المُعالجة مؤدياً إلى مقاومتها ضد العدوى الفطرية.