Influences of Biological Control on Damping Off Diseases of Faba beans as well as Physico-Chemical and Technological Properties

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Received: 20 Oct. 2020 / Accepted 05 Dec. 2020 / Publication date: 10 Dec. 2020

ABSTRACT

Faba bean seeds are the most important legumes in terms of consumption, for its positive nutritional properties and the most important diseases of Faba bean seeds are damping off. The field experiments was carried out during the 2017-2018 seasons to study the effect of the biocontrol agents as well as Rizolex T on the incidence of Faba beans damping off disease. The growth parameters, yield and its quality parameters of Faba bean plants were also, studied. The cultures of Paenibacillus polymyxa, Pseudomonas fluorescens and Bacillus megaterium bacteria and the Trichoderma viride, Trichoderma harzianum fungi were used. The results exhibited that, the highest percentage of pre-emergence damping off reduction, the maximum plant height and the maximum seeds weight per plant were recorded with Rizolex T and P. polymyxa followed by B. megaterium and T. harzianum treatments. Significantly increases in the percentage of survived plants compared with the control were found in all treatments. Number of branches, and number of pods per plants were the highest in the Rizolex T treatment. Quality parameters of the fabea bean crops resulted, such as protein, protein digestibility and minerals (Fe and Zn), as well as, total flavonoid and antioxidant activity evaluations were significantly increased in P. polymyxa, B. megaterium followed by Rizolex T compared with control samples. Technological evaluation of density, ratio between cotyledons and seed coats, as well as, water absorption, hydration coefficients and swelling coefficients after soaking and cooking were studied and showed high levels in Faba beans treated with P. polymyxa, B. megaterium and Rizolex T. Also, high level of cookability (stewing) and sensory characteristics were detected in Faba beans treated with P. polymyxa, B. megaterium and Rizolex T compared with other treatments and control.

Keywords: Faba beans, Damping off, Biocontrol agents, Rizolex T, Hydration coefficients, Swelling coefficients

Introduction

Legumes have beneficial health implications related to their nutritional properties. They are an important source of macronutrients, containing almost twice the amount of proteins compared to cereal grains. Faba beans (Vicia faba L.) is one of the important winter legume crop grown in northern eastern of Africa i.e. Egypt, Sudan and Ethiopia (Multari et al., 2015). It has been used as a meat extender or as a substitute due to its high protein contents, it is also, comparatively high in lysine, which is an essential amino acid for human (Robinson et al., 2019).

Faba bean seeds are an excellent nitrogen fixer which makes it suitable for food and feed production. The quality and yield of Faba bean seeds are affected by soil type, climatic conditions and agronomic factors. The key biotic factors responsible for yield loss in Faba bean seeds are fungal diseases (Barlög et al., 2018). Crop losses due to diseases can reach 15% or even 80% in species of the Fabaceae family (Horoszkiewicz-Junka et al., 2013).

Faba bean seeds damping off caused by Rhizoctonia solani Kühn and Fusarium solani Mart are considered the most destructive diseases. However, roots rot diseases caused by Rhizoctonia solani Kühn is a major cause of yield instability and losses for Faba beans production in most areas of the world. Plant pathologists make great efforts to reduce the use of synthetic fungicides and to improve the use of alternative management strategies to control soil-borne pathogens. Acquired resistance that increases plant resistance to subsequent pathogen attack, by using biotic (microorganisms) seemed, to
be one of alternatives to substitute for decreasing the use of fungicides in plant disease control (Walters et al., 2013).

Recently, biological control has intensively been studied by many researchers throughout the world. Bio-inoculants containing living cells of microorganisms could be an effective alternative for chemicals in food production (O’Callaghan, 2016).

*Paenibacillus polymyxa* and *Pseudomonas fluorescens* are known for their ability to produce antimicrobial metabolites and hydrolytic enzymes, such as β-1, 3-glucanase, chitinase, and protease, which are considered key enzymes in control of fungal plant diseases. So, application of *Paenibacillus polymyxa* in seeds can be used to manage pre- and post-emergence damping off in plants. However, *Bacillus megaterium* is a potential bacterial biocontrol agent against *Rhizoctonia solani* (Zhang et al., 2016).

The species of the genus Trichoderma, which contain a group of plant growth-promoting fungi, can colonize the intercellular parts of plant roots and stimulate systemic resistance in all parts of the plant, the actions of these fungi suppress some plant diseases by direct mycoparasitism or antibiosis, as well as indirect induced resistance (Sharma and Sain, 2004).

Faba bean seeds are a potential source of dietary fiber and it has a high content of essential mineral elements. Malnutrition mineral affects millions of people all over the world. Faba bean seeds have higher concentrations of minerals (e.g., Fe, Zn, K and Mg) than some other cereal grains (White and Broadley, 2009).

Phenolics compounds are plant secondary metabolites and have important roles in pigment biosynthesis. They also, provide a scaffolding support and a structural integrity to plants (Bhattacharya et al., 2010). Plants need these compounds for pigmentation, growth, reproduction, resistance to pathogens, repel or kill many microorganisms, defaces or even subvert them to their own advantage (Boudet, 2007).

Phenolic compounds as well as flavonoids are well-known as antioxidant activities agents related with their ability to scavenge free radicals and break radical chain reactions. Likewise, they have several important bioactive agents that have been attractive because of their benefits for the human health (Tungmunnithum et al., 2018).

The antioxidant properties of Faba bean extracts are related to total phenolics and flavonoids contents. Differences in total phenolics and flavonoids were observed for different genotypes collected during three different growing stages. Higher contents of total phenolics and flavonoids were found during vegetative and reproductive stages, which also showed the highest antioxidant activity (Boukhanouf et al., 2016).

Faba bean seeds can be fresh consumed (green stage) or after cooking. Their polyphenol profiles are affected by processing such as soaking, sprouting, freezing, boiling, pressure cooking and steaming. Soaking, boiling and autoclaving caused losses in phenolic compounds and antioxidant activities due to leaching of these compounds into the soaking and cooking medium. Boiling was shown to be a better method than autoclaving in keeping phenolic compounds, and it is suggested that home-cooked Faba beans may contain higher levels of phenolic content than industrially processed Faba beans (Siah et al., 2014). Even sprouting, a process involving germination and drying of legume seeds was found to decrease total polyphenol content and was associated with improvements in the nutritive value in sprouted seeds. Due to the aforementioned inhibitory, effect of tannins and other anti-nutritional factors on mineral bioavailability, all the treatments (soaking, germination and boiling) significantly improved *in vitro* availability of minerals and protein digestibility (Luo and Xie, 2014).

The aim of this study was to investigate the effect biotic treatments for controlling damping off diseases and estimate the quality of the Faba bean seeds and its technological characteristics.

**Materials and Methods**

1. **Materials**

Faba bean seeds (*Vicia faba* L.), cultivar Misr 1 were obtained from the Legumes Res. Dept., Field Crops Res. Inst., ARC, Giza, Egypt. Apparently healthy uniformity seeds of Faba beans were surface disinfected by immersing in a sodium hypochlorite solution (1%) for 2 min, and several times washed with sterilized water, then left to dry on screen cloth with paper towels to absorb the excess water at the room temperature for approximately two hour.
2. Biotic inducers

The cultures of the \textit{Paenibacillus polymyxa}, \textit{Pseudomonas fluorescens}, previously isolated by the authors (Shehata et al., 2006), and \textit{Bacillus megaterium} bacteria were activated on fresh slants. After 24 hr bacterial isolates were transferred to many erlenmeyer flasks (250 ml) with 50 ml of a nutrient yeast dextrose broth (NYDB) medium for \textit{P. polymyxa} and \textit{B. megaterium} and 50 ml of a King’s medium broth (KMB) for \textit{P. fluorescens}. The flasks were placed on a rotary shaker to grow at 120 rpm for 66 hr at 24°C±1°C, after growth of bacterial isolates, the liquid cultures media were then centrifuged under cooling (4°C) at 10000 rpm for 10 min. Then, the disinfected Faba bean seeds were soaked in a supernatant for 20 minutes, then spread on screen cloth. The coated seeds were air-dried for 15 hr until planting time.

\textit{Trichoderma harzianum} and \textit{Trichoderma viride} fungi were collected by Plant Pathol. Dept., Fac. of Agric., Ain Shams Univ. and identification according to Rifai (1969). Formulation was prepared by growing the fungus in glass bottles (500 ml) containing 100 gram sterilized sorghum grains medium according to Rini and Sulochana (2007). Air dried fine grinded sorghum grains, which contained 3.9x10^9 (CFU) of \textit{T. harzianum} and \textit{T. viride}, was used to coat the disinfected Faba bean seeds moistened with 1% methyl cellulose in sterile distilled water as a sticker, then the coated seeds were air-dried for 15 hr until planting time (Tewari and Bhanu, 2004).

3. Fungicide treatment:

Seed dressing was carried out to the disinfected Faba bean seeds by applying the Rizolex T 50% WP [(Tolclofos-methyl-thiram), Sumitomo Chemical Company Ltd.] at the recommended dose (3 g/kg) to the 1% methyl cellulose (as a sticker), moistened seeds were packed in polyethylene bags and shaking well, to ensure even distribution of the fungicide, according to the recommendations of the Egyptian Ministry of Agriculture and Land Reclamation.

4. Root-nodule bacteria treatment

Formulation of \textit{Rhizobium leguminosarum} biovar \textit{Viciae} (Faba beans), was obtained from Biofertilizers Production Unit, Soils Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt and it was used to inoculate field soil. \textit{Rhizobium} formulation was mix with approximately 50 kg of moistened fine sandy soil and added to field soil during planting at a rate of 800 g \textit{Rhizobium} formulation / Feddan, according to the recommendations of the Egyptian Ministry of Agriculture and Land Reclamation.

5. Disease assessment

The disease incidence (DI) % was determined by recording pre-emergence damping off, post-emergence damping off and the percentage of survived plants at 15, 30 and 45 days after planting, respectively according to the following formulas (Atwa, 2016):

\begin{align*}
\text{Pre-emergence} \% &= \frac{\text{Total No. of un-germinated seeds}}{\text{Total No. of planted seeds}} \times 100 \\
\text{Post-emergence} \% &= \frac{\text{Total No. of rotted seedlings}}{\text{Total No. of planted seeds}} \times 100 \\
\text{Survived seedlings} \% &= \frac{\text{Total No. of survived seedlings}}{\text{Total No. of planted seeds}} \times 100
\end{align*}

Reduction or increasing % over the infected control was also calculated according to the following formula:

\begin{align*}
\text{Reduction or Increasing} \% &= \frac{\text{DI of Control} - \text{DI of treatment}}{\text{DI of Control}} \times 100
\end{align*}

6. Field experiments

The field experiments was carried out during the two winter growing seasons 2017- 2018 and 2018-2019 at Giza Agricultural Research Station, Giza Governorate, Egypt, in field known to have a root
rot history, in order to investigate the effect biotic treatment for controlling damping off. In the control treatment, the disinfected Faba beans were soaked in distilled water. The field trial (28 plots) was designed in a complete randomized block (four replicates). Each plot (10.50 m²) consisted of five rows; each row was 3.5 m length and 0.6 m width. All treatments were sown in hills 20 cm apart on both sides of the row ridge with one seed/hill. Calcium super-phosphate (15 % P₂O₅) at 150 kg/Feddan was added on rows during the soil preparation. Potassium sulphate (48% K₂SO₄) at 50 Kg/Feddan was applied as soil application at the first irrigation after planting. Ammonium sulphate (20.5% N) at a rate of 75-100 kg/Feddan was added at the planting time as a starter dose of nitrogen. All recommended agricultural practices were monitored according to the recommendations of the Egyptian Ministry of Agriculture and Land Reclamation. The treatments were as follows: (1) T. viride, (2) T. harzianum, (3) P. flourescence, (4) B. megaterium, (5) P. polymyxa, (6) Rizolex T and Water (control). The disease incidence (DI) % was determined as mentioned before. Random samples of twenty Faba bean plants were collected (from the inner rows) at the harvest stage from each plot. Plant growth parameters of plant height (cm), number of pods per plant, number of branches, seed weight per plant and weight of one hundred seeds were recorded as well as seed yield Ton/Feddan were calculated.

7. Technological characteristics: (on 2018-2019 seasons)
7.1. Preparation of seeds for soaking and cooking
Faba bean seeds were manually cleaned from broken or damaged seeds, stones, dust and other foreign materials. The cleaned Faba bean seeds were soaked for 12 hr at the room temperature (30±2ºC) in tap water. A ratio of 1: 4 (w/v) seeds to water was used according to Avila et al., (2015), water absorption was determined after soaking.

Cooking: seeds were cooked in oven for 12 hr at 100ºC. After cooking the following measurements were determined in cooked seeds according to the method of Fahmy et al. (1996).

Water absorption of soaking and cooking Faba bean seeds were calculated by Fahmy et al. (1996) as follows:

\[
\text{Water absorption (\%) of soaking} = \frac{\text{Seeds weight after soaking} - \text{Seeds weight before soaking}}{\text{Seeds weight before soaking}} \times 100
\]

\[
\text{Water absorption (\%) of cooking} = \frac{\text{Seeds weight after cooking} - \text{Seeds weight before cooking}}{\text{Seeds weight before cooking}} \times 100
\]

7.2. Hydration coefficient of Faba bean seeds
Hydration coefficient of Faba bean seeds soaking was calculated according to El-Refai et al., (1988).

\[
\text{Hydration coefficient (\%) of soaking} = \frac{\text{Seeds weight after soaking}}{\text{Seeds weight before soaking}} \times 100
\]

\[
\text{Hydration coefficient (\%) of cooking} = \frac{\text{Seeds weight after cooking}}{\text{Seeds weight before cooking}} \times 100
\]

7.3. Swelling coefficient of Faba bean seeds: Swelling coefficient of soaking and cooking Faba bean seeds calculated according to El-Refai et al., (1988).

\[
\text{Swelling coefficient (\%) of soaking Faba beans} = \frac{\text{Seeds volume after soaking}}{\text{Seeds volume before soaking}} \times 100
\]

\[
\text{Swelling coefficient (\%) of cooking Faba beans} = \frac{\text{Seeds volume after cooking}}{\text{Seeds volume of before cooking}} \times 100
\]

7.4. Water soluble solids
After cooking, the solution containing soluble material was poured into a porcelain pot placed in an oven at 60ºC until all the water was evaporated (Fahmy et al., 1996). The pot was weighed and water soluble solids calculated as follows:
Water soluble solids (%) = \frac{\text{Weight of pot after drying} - \text{Weight of empty pot}}{\text{Initial weight of seeds}} \times 100

7.5. Stewing % (cook ability)

The ability of seeds to be cooked was measured by means of using the normal press of fingers and comparing between the cooked seeds for their hardness (Ismail et al., 2007). Stewing percentage is calculated as follows:

\text{Stewing} (%) = \frac{\text{Initial number of seeds} - \text{non stewing seeds number}}{\text{Initial number of seeds}} \times 100

8. Physical characteristics

8.1. Ratio of cotyledons to seed coat

The seeds were emergent in water for 12hr, after that, weight the cotyledons and the seed coat of soaked Faba beans and calculate the ratio of cotyledons to seed coat (Osman et al., 2010).

8.2. Determination of color

The color of Faba bean samples were measured using a hand-held Tristimulus Reflectance Colorimeter, Minolta chromameter (CR-400 model, Konica Minolta, Japan) using different color parameters \(L\) (lightness with \(L = 100\) for lightness, and \(L = 0\) for darkness), \(a\) [(chromaticity on green (−) to red (+)), \(b\) [(chromaticity on a blue (−) to yellow (+)]. Reported values are the means of triplicate determinations.

9. Chemical analysis:

9.1. Chemical composition

The contents of moisture, protein, crude fiber, ash and fat of Faba bean seeds \((n = 3)\) were determined following the official methods of analysis (AOAC, 2012). \textit{In vitro} protein digestibility was determined according to Akeson and Stahmann (1964). Total carbohydrates were calculated by difference \([100-(\text{Protein} + \text{Fat} + \text{Ash} + \text{Crude fiber})]\). Minerals content in Faba bean seeds were determined according to AOAC (2012) by using Agilent Technologies, Microwave Plasma Atomic Emission Spectroscopy (MP-AES 4210).

9.2. Determination of total phenols content (TP)

Total phenols were estimated by the Folin-Ciocalteu method reported by Singleton and Rossi (1965).

9.3. Determination of total flavonoids content (TF)

The amount of total flavonoids in the extracts was measured spectrophotometrically following the method reported by Zhishen et al. (1999).

9.4. DPPH radical scavenging activity

The antioxidant activity of Faba bean samples was determined based on the radical scavenging ability in reacting with a stable DPPH free radical solution according to Brand-Williams et al. (1995).

9.5. Determination of tannins content

Tannin content was estimated using the vanillin HCl method (Price et al., 1978).

10. Sensory evaluation of cooked Faba beans

Organoleptic characteristics (color, texture, taste, odor and overall quality) of Faba beans were evaluated by 10 panelists from Food Technology Research Institute, Agricultural Research Center, Giza, Egypt. A numerical hedonic scale, which ranged from 1 to 10 (1 is very bad and 10 for excellent), was used for sensory evaluation (Larmond, 1977).

11. Statistical analysis

Randomized blocks design (RBD) were conducted in field experiment. The obtained data were subjected to computer statistical software (ASSI STAT) originated by Silva and Azevedo (2009).
for quality and technology traits, the collected date were statistically analyzed for mean values and standard deviation and are reported using Costat statistical software according to Steel and Torrie (1980). The obtained data were subjected to one-way analysis of variance (ANOVA) at (P ≤ 0.05) followed by Duncan’s new multiple range tests to assess differences between samples mean.

Results and Discussion

1. Field experiments
1.1. Effect of some biocontrol agents on the incidence of Faba beans damping off disease

In these experiments, the effect of treatments by different biocontrol and the chemical fungicide (Rizolex T) agents on damping off incidence and survived plants of Faba beans under field conditions at Giza, Agricultural Research (during 2017/2018 and 2018/2019 seasons) were studied. Results in Table (1) exhibited that all the tested treatments significantly decreased the percentages of pre and post-emergence damping off compared with untreated control sample. The highest percentage of pre-emergence damping off reduction over the control was obtained from treatments with Rizolex T and *P. polymyxa*. Also, results showed that all the tested treatments significantly increased the percentage of survived plants compared with the control.

He et al., (2007) found that, *P. polymyxa* is known for its ability to produce antimicrobial compounds. More than that, *P. polymyxa* strains are capable of producing several hydrolytic enzymes, including β1,3-glucanases and chitinases which are considered key enzymes in control of fungal plant diseases (Jung et al., 2003 and Raza et al., 2009).

Table 1: Effect of some chemical and biocontrol agents as seed treatments on the percentage of damping off disease of Faba bean plants grown under field conditions at Giza Agricultural Research during winter growing 2017-2018 and 2018-2019 seasons

| Treatments       | Pre-emergence (%) | Post-emergence (%) | Survived plants (%) | Increasing (%) |
|------------------|-------------------|--------------------|---------------------|---------------|
|                  | Incidence | Reduction | Incidence | Reduction |               |                  |
| *T. viride*      | 12.5 b      | 55.4      | 2.5 b   | 43.2      | 85.0 ab     | 25.7             |
| *T. harzianum*   | 12.1 b      | 56.8      | 2.7 b   | 36.4      | 85.2 ab     | 26.0             |
| *P. flourescence*| 11.3 b      | 59.4      | 2.6 b   | 41.0      | 86.1 ab     | 27.4             |
| *B. megaterium*  | 10.3 b      | 63.2      | 2.3 b   | 47.7      | 87.4 a      | 29.3             |
| *P. polymyxa*    | 9.7 b       | 65.4      | 2.0 b   | 54.5      | 88.3 a      | 31.0             |
| Rizolex T        | 6.5 c       | 76.8      | 1.6 b   | 63.6      | 91.8 a      | 35.8             |
| Control          | 28.0 a      | 0.0       | 4.4 a   | 0.0       | 67.6 b      | 0.0              |

Table 1: Effect of some chemical and biocontrol agents as seed treatments on the percentage of damping off disease of Faba bean plants grown under field conditions at Giza Agricultural Research during winter growing 2017-2018 and 2018-2019 seasons

Values are the average of 3 experiments. Mean values (within the same column) followed by different superscripts are significantly different at the 5% level.

1.2. Effect of some inducers on growth parameters and yield of Faba bean plants

Under the tested field conditions, bioagents treatments significantly improved growth and yield parameters compared to the untreated control treatment as presented in Table (2). The plant height was affected by seed treatments with different biocontrol agents, all treatments at two growing seasons significantly increased the plant height compared with untreated control. The maximum plant height was recorded with Rizolex T and *P. polymyxa* treatments followed by *B. megaterium* and *T. harzianum* treatments and it differed from rest of all treatments.

Number of branches per plant was affected by seed treatments with some different biocontrol agents. All the biocontrol agents at Giza Agricultural Research at two seasons significantly showed an
increase in number of branches as compared with untreated control. Highest significant increase in the 
number of branches per plant was recorded with Rizolex T treatment.

All treatments significantly increased number of pods per plant as compared with untreated control. The maximum number of pods per plant was recorded with Rizolex T treatments which significantly differed from other treatments, also increased seed weight per plant in two seasons as compared with untreated control. At 2017-2018 growing seasons, the maximum seed weight per plant was recorded with Rizolex T followed by P. polymyxa, B. megaterium and T. harzianum. Meantime, there were no significant differences among the treatments at 2018-2019 growing seasons.

Table 2: Effect of some chemical and biotic inducers as seed treatments on some growth parameters of Faba bean plants grown under field conditions at Giza Agricultural Research during winter growing 2017-2018 and 2018-2019 seasons

| Treatments       | 2017-2018 | 2018-2019 |
|------------------|-----------|-----------|
|                  | Plant height (cm) | Number of branches/pod plant | Number of pods/plant | Seeds weight/Plant (g) | 100 seeds weight (g) | Seeds yield (ton/fed) |
| T. viride        | 105.8 b   | 2.9 b     | 16.8 b   | 31.5 c   | 73.4 b   | 1.250 c    |
| T. harzianum     | 106.8 b   | 3.0 b     | 17.2 b   | 32.0 b   | 73.6 b   | 1.270 c    |
| P. fluorescens   | 100.1 c   | 2.8 b     | 16.4 c   | 30.9 c   | 71.8 b   | 1.260 c    |
| B. megaterium    | 108.5 b   | 3.2 b     | 16.8 bc  | 32.4 b   | 73.6 b   | 1.298 c    |
| P. polymyxa      | 111.3 b   | 3.3 b     | 17.8 b   | 35.3 b   | 77.7 a   | 1.390 b    |
| Rizolex T        | 119.0 a   | 3.9 a     | 18.7 a   | 40.8 a   | 79.6 a   | 1.540 a    |
| Control          | 90.0 d    | 2.4 c     | 13.1 d   | 27.1 d   | 69.8 c   | 0.981 d    |

All treatments significantly increased the weight of one hundred seeds in two seasons as compared with untreated control. The seed treatments with Rizolex T and P. polymyxa significantly increased the weight of one hundred seed and it differed from the rest of all treatments at 2017-2018 growing seasons. Meantime, there were no significant differences among the treatments with at 2018-2019 growing seasons.

The two seasons showed nearly similar seeds yield results which indicated that all treatments significantly increased the seed yield as compared with untreated control sample. The maximum seed yield was recorded from Rizolex T treatment which significantly followed by P. polymyxa and differed from the rest of all treatments.

From the result, treatment with P. polymyxa, had a significant effect on damping off reduction and significantly enhanced the vegetative and seed growth parameters of Faba bean plants at field conditions compared with untreated control. Previous reports have shown that P. polymyxa controls many soil in the greenhouse and in the field (Raza et al., 2015). P. polymyxa and P. fluorescens can be produce a wide variety of antimicrobial metabolites such as the antibiotic 2,4-diacyl phloroglucinol and pyrrolnitrin, hydrolytic enzymes from bacteria such as chitinase, β-1,3-glucanase, and protease (Zhang et al., 2016).

The Trichoderma treatment had, also, a significant effect on damping off reduction and significantly enhanced the vegetative and seed growth parameters of Faba bean plants under field conditions compared with untreated control, identified a potential Trichoderma isolate (T. viride T14) that could stimulate the growth of cucumber and bottle gourd plants and which acted as an antagonist against Sclerotium rolfsii and Rhizoctonia solani. Many T. harzianum isolates were previously found to control the development of Rhizoctonia solani of many crops under greenhouse and field conditions.
(Basak and Basak, 2011). *T. harzianum* was demonstrated to be very efficient producer of a wide range of extracellular enzymes and some of these were implicated in the biological control of plant diseases. Meanwhile, *T. harzianum* is well known producers of antibiotic (produced by some but not all strains) that are toxic for phytopathogenic fungi (Vinale et al., 2009). It could be concluded that using biocontrol agent is a promising methods for controlling damping off and root rot diseases. Soil inoculation with phosphorein, known as phosphate dissolving bacteria, has been reported to improve soil fertility and plant productivity. (Mohammed 2004).

2. **Chemical composition of Faba bean seeds**

The chemical composition of Faba bean seeds (2018-2019 seasons) treated with biocontrol agents *T. viride*, *T. harzianum*, *P. fluorescens*, *B. megaterium*, *P. polymyxa* and the fungicide Rizolex T are presented in Table 3. Moisture content showed significant differences among the treatments, moisture content was recorded slightly high amounts in Rizolex T and *B. megaterium* treatments (7.94 and 8.06 %, respectively), while the lowest value of such content reached to 7.62% in control seeds under the same conditions. Same results agreed with Hendawey and Younes (2013).

| Sample          | Moisture (%) | Fat   | Ash (%) | Crude fiber (%) | Protein (%) | TC (%) | IVPD (%) |
|-----------------|--------------|-------|---------|-----------------|-------------|--------|---------|
| *T. viride*     | 7.70±0.02    | 2.13±0.01 | 3.34±0.04 | 7.09±0.01 | 29.47±0.01 | 57.97±0.05 | 52.23±0.07 |
| *T. harzianum*  | 7.77±0.02    | 2.18±0.01 | 3.35±0.02 | 7.09±0.02 | 29.50±0.01 | 57.88±0.06 | 60.54±0.06 |
| *P. fluorescens*| 7.78±0.01    | 2.03±0.13 | 3.34±0.03 | 6.40±0.02 | 30.21±0.01 | 58.02±0.08 | 64.30±0.03 |
| *B. megaterium* | 8.06±0.04    | 1.86±0.05 | 3.35±0.01 | 6.65±0.04 | 30.51±0.04 | 57.53±0.06 | 65.57±0.06 |
| *P. polymyxa*   | 7.80±0.05    | 1.85±0.03 | 3.38±0.03 | 6.74±0.00 | 31.46±0.02 | 56.57±0.08 | 65.67±0.05 |
| Rizolex T       | 7.94±0.06    | 1.91±0.13 | 3.45±0.03 | 6.96±0.03 | 30.35±0.02 | 57.33±0.07 | 64.84±0.16 |
| Control         | 7.62±0.03    | 1.83±0.04 | 3.31±0.01 | 6.77±0.04 | 28.56±0.02 | 59.53±0.12 | 53.70±0.01 |

TC: Total carbohydrates

Values are the average of 3 experiments ± SD. Mean values followed (within the same column) by different superscripts are significantly different at the 5% level.

The range in fiber content extended from 6.40 to 7.09% for seeds treated with *P. fluorescens* and *T. harzianum* or *T. viride*. On the other hand, treated seeds with *T. harzianum* and *T. viride* recorded the highest value of fat content, compared with the other treated seeds. The results showed that ash content ranged from 3.31 to 3.45% for seeds under study. These results agreed with Perez-Maldonado et al. (1999). Total carbohydrates of Faba bean seeds ranged from 56.57 to 59.53%. There are significant differences in protein content among the total samples, wherein protein values ranged from 28.56 to 31.46% where the highest value was in *P. polymyxa* treatment seeds (31.46%) followed by seeds treated with *B. megaterium* (30.51%) and Rizolex T fungicide (30.35%), while the lowest amount was (28.56%) in control seeds. Protein content of Faba bean seeds ranged from 20.35 to 23.02%, with an average of 21.73% (Yahia et al., 2013). This may be due to the biocontrol agents effect on formation of amino acids, which led to increase protein content (Agamy et al., 2012).

Biocontrol agent bacteria improved the *In vitro* protein digestibility (IVPD) compared with control seeds (Table 3). Non-significant differences were found between *P. polymyxa* and *B. megaterium* where recorded the highest values (65.67 and 65.57 %, respectively) followed by the fungicide Rizolex T (64.84%) and *P. fluorescens* (64.30%), then the lowest value for *T. viride* (52.23%). The IVPD in Faba beans affected by many factors such as genotype and tannin contents (Osman et al. (2010).

Inoculation of Faba bean seeds with bacteria (Biocontrol agent) led to increase the availability of various nutrients that positively reflected in growth, yield and its quality (Abo El-Soud et al., 2003).

2.1. **Minerals content**

The minerals content of Faba bean seeds treated with biocontrol agents and fungicide Rizolex T are shown in Table (4). Data showed significant differences in minerals content among treatments. The
calcium and magnesium values seemed to be similar and recorded the highest values in fungicide Rizolex T and P. polymyxa treated seeds. The lowest value were detected in T. viride seeds (982.5 ppm) for calcium and (1062.0 ppm) for magnesium, while control seeds had 1042.5 and 1379.00 ppm for calcium and magnesium, respectively.

Potassium content was abundant mineral in Faba bean seeds, with a range of 11021.0 to 13571.5 ppm where, the highest value was in case of Rizolex T treated seeds (13571.5 ppm) followed by P. polymyxa, B. megaterium and P. fluorescens treated seeds (13560.0, 13481.0 and 13313.0 ppm, respectively). Iron content ranged from 28.55 to 43.76 ppm, the highest values were found in P. polymyxa and the lowest value for T. viride treated seeds.

**Table 4:** Effect of some chemical and biocontrol agents as seed treatments on minerals (ppm) quantified (as dry weight basis) of Faba bean seeds

| Sample       | Ca ( ppm )       | Mg (ppm)      | K (ppm)      | Fe (ppm)      | Zn (ppm)      |
|--------------|------------------|---------------|--------------|---------------|---------------|
| T. viride    | 982.5±2.12       | 1062.0±4.24   | 11021.0±1.41 | 28.55±0.35    | 22.42±0.11    |
| T. harzianum | 1006.5±3.54      | 1186.5±4.95   | 11458.5±4.95 | 32.65±0.21    | 24.10±0.14    |
| P. fluorescens| 1019.0±1.41     | 1354.75±3.18  | 13313.0±2.83 | 34.10±0.01    | 27.81±0.28    |
| B. megaterium| 1027.5±2.12     | 1407.5±6.36   | 13481.0±2.83 | 40.15±0.21    | 28.90±0.14    |
| P. polymyxa  | 1074.0±1.41      | 1406.75±1.10  | 13560.0±0.00 | 43.76±0.30    | 31.53±0.39    |
| Rizolex T    | 1084.2±0.50      | 1427.5±3.54   | 13571.5±2.12 | 36.55±0.35    | 27.20±0.28    |
| Control      | 1042.5±2.95      | 1379.00±1.41  | 11843.5±4.95 | 34.54±0.35    | 26.31±0.27    |

Values are the average of 3 experiments ± SD. Mean values (within the same column) followed by different superscripts are significantly different at the 5% level.

Biocontrol agents bacteria treated seeds had the highest zinc level followed by fungicide Rizolex T treated seeds where the highest value for P. polymyxa (31.53 ppm) and the lowest value for T. viride treated seeds (22.42 ppm).

Khazaei and Vandenbergh (2020) found that Faba bean seeds had generous amounts of K (11315 ppm), P (5118 ppm), S (1903 ppm), Mg (1334 ppm), and Ca (971 ppm). The Fe and Zn concentrations were 51 and 42 ppm, respectively. Ray et al. (2014) found that, the low- tannin white-flowered Faba beans were rich in Ca, Mg, Fe, and Zn, which are minerals often lacking in the human diet globally. The bio-fertilizers (Bacillus megaterium) have a positive integrative roles in growing of plants by increasing available nitrogen, phosphorus, potassium and calcium (Mahdi et al., 2019).

### 2.2. Phytochemicals

Phytochemicals, especially phenolics are known to be major bioactive compounds for health benefits. Plant extracts containing different classes of polyphenols are very attractive in the food industry (Pasricha et al., 2014).

Data presented in Table (5) show the effect of some biocontrol agents as well as the fungicide (Rizolex T) on total phenolics compounds content, total flavonoids content, tannins content and antioxidant activity. Significant differences were observed among all treatments. Fungicide Rizolex T and biocontrol agent P. polymyxa treated seeds recorded high total phenols content [132.83 and 132.28 mg gallic acid equivalent (GAE)/100g respectively]. The lowest value was found in Faba bean seeds treated with T. viride (125.79 mg GAE/100g). Journi et al. (2015) found that, the value of total phenols content in the methanol extract of Faba beans were (0.991 mg GAE/g). The total phenolic contents are near those mentioned by Baginsky et al. (2013) in Faba bean values ranging from 0.81 mg GAE/g to 1.33 mg GAE/g.

Total flavonoids content showed significant differences among all treatments of Faba beans. Paenibacillus polymyxa treated seeds recorded the highest value [53.00 mg Quercetin equivalent (QE)/100g], followed B. megaterium treated seeds 42.22 mg QE/100g. The results of P. fluorescens treated seeds were close to those of the fungicide Rizolex T treated seeds, recording 38.21and 38.55 mg QE/100g, respectively. The least effect was in T. viride, where treated seeds recorded 33.50 mg QE/100g. Journi et al. (2015) found that the amount of flavonoids content was registered in Faba beans methanolic extract (0.128 mg QE/g).
Table 5: Effect of some chemical and biocontrol agents as seed treatments on phytochemical analysis of Faba bean seeds.

| Treatments       | T. phenols (mg GAE/100g) | T. flavonoids (mg QE/100g) | Tannins (mg CE/100g) | AOA % |
|------------------|---------------------------|-----------------------------|----------------------|-------|
| T. viride        | 125.79±0.08               | 33.56±0.14                  | 45.02±0.01           | 46.82±0.01 |
| T. harzianum     | 128.15±0.18               | 35.07±0.05                  | 43.70±0.01           | 48.91±0.00 |
| P. fluorescens   | 129.65±0.10               | 38.21±0.01                  | 35.42±0.06           | 51.81±0.00 |
| B. megaterium    | 130.15±0.10               | 42.22±0.02                  | 32.24±0.04           | 53.65±0.04 |
| P. polymyxa      | 132.28±0.08               | 53.00±0.15                  | 32.34±0.10           | 55.12±0.03 |
| Rizolex T        | 132.83±0.03               | 38.55±0.21                  | 39.43±0.02           | 52.22±0.04 |
| Control          | 126.18±0.06               | 33.46±0.03                  | 40.39±0.04           | 47.82±0.01 |

Values are the average of 3 experiments ± SD. Mean values (within the same column) followed by different superscripts are significantly different at the 5% level. GAE: gallic acid equivalent; QE: Quercetin equivalent; CE: catechin equivalent.

*Trichoderma viride* treatment showed the highest seed tannins content [45.02mg Catachin equivalent (CE)/100g] followed by *T. harzianum* where recorded (43.70mg CE/100g) and the lowest value recorded in *B. megaterium* (32.24mg CE/100g). These results agreement with Osman *et al.* (2010) who found that, tannins content ranged from (0.041 to 0.363%).

Antioxidant activity (AOA) measured the free radical scavenging (%) using the DPPH method. The results ranged from 46.82 to 55.12% (Table 5). The highest value was recorded in *P. polymyxa* bioagent compared with other treatments. Yehia *et al.* (2013) found that the free radical scavenging activity determined by DPPH ranged from 0.374 to 0.578 mM.

3. Technological evaluation

3.1. Physical properties

Five biocontrol agents *i.e.* *T. viride*, *T. harzianum*, *P. fluorescens*, *B. megaterium*, *P. polymyxa* and fungicide Rizolex T with a recommended dose (3g/kg seeds) were tested to study their effect on density, seeds parts (cotyledons/seed coat) and color measurements.

No significant differences were observed among biocontrol agents bacteria treated seeds. Density ranged from 1.10 to 1.27g/ml. Rizolex T treatment had the highest density value (1.27 g/ml) followed by *B.megaterium* and *P. polymyxa* (1.22 g/ml), while *T. viride* had the lowest value (1.10g/ml). Kaur *et al.* (2014) found that the density of the *Vicia faba* seeds was 1.27 g/ml.

The ratio between cotyledons and seeds coat indicates how full the seed is. Some of the biocontrol bacteria recorded the highest value, as well as Rizolex T fungicide, as there were no significant differences between them. The lowest value was for control, *T. harzianum* and *T. viride* (7.02, 6.85 and 6.48, respectively).

Table 6: Effect of some chemical and biocontrol agents as seed treatments on the tested physical properties of Faba bean seeds.

| Treatments       | Density (g/ml) | Cotyledons/seed coat | Color | L      | a    | b        |
|------------------|----------------|----------------------|-------|--------|------|---------|
| T. viride        | 1.10±0.03      | 6.48±0.23            | 59.90±0.88 | 11.26±0.78 | 30.54±0.41 |
| T. harzianum     | 1.18b±0.00     | 6.85±0.05            | 60.01±1.05 | 9.74±0.44 | 30.62±0.36 |
| P. fluorescens   | 1.17b±0.01     | 7.10b±0.01           | 64.75b±0.43 | 12.41b±0.73 | 30.81±0.53 |
| B. megaterium    | 1.22ab±0.01    | 7.16b±0.06           | 65.74ab±0.43 | 9.33b±0.49 | 30.06b±0.74 |
| P. polymyxa      | 1.22ab±0.00    | 7.26b±0.05           | 66.31b±0.71 | 8.62±0.61 | 26.73b±0.40 |
| Rizolex T        | 1.27±0.06      | 7.20ab±0.03          | 65.05ab±0.55 | 8.67±1.10 | 30.46b±0.23 |
| Control          | 1.16b±0.01     | 7.02b±0.01           | 64.70b±0.73 | 10.02b±0.56 | 30.69b±0.24 |

Values are the average of 3 experiments ± SD. Mean values (within the same column) followed by different superscripts are significantly different at the 5% level.

The effect of different biocontrol agents and the fungicide Rizolex T on color measurements of Faba beans noticed that, the highest lightness values were in Faba bean seeds treated with biocontrol *P. polymyxa* and *B. megaterium* followed by the fungicide Rizolex T (66.31, 65.74 and 65.05 respectively).
The lowest values were recorded in Faba bean seeds treated with biocontrol T. viride (59.90). In addition, increase in the redness values (a) and yellowness (b) values showed in Faba bean seeds treated with T. viride biocontrol agent than other treatments and control. Abdel-Aleem et al. (2019) found that, color parameters of Faba bean seeds L, a and b recorded 82.86, 6.27 and 8.07 respectively.

3.2. Water absorption, hydration and swelling coefficients of Faba bean treated seeds.

The results of water absorption of Faba bean seeds after soaking and after cooking are shown in Fig 1. The results showed that, high value of water absorption after soaking recorded for P. polymyxa, B. megaterium and Rizolex T treatments (116.03, 115.98 and 115.21 %), respectively, compared with control (106.93%). The corresponding values after cooking were 159.57, 158.87 and 155.16 %, respectively, compared with control (140.92%). Abdel-Aleem et al. (2019) reported that, water absorption of Faba bean seeds was 144.30% after cooking as compared with 84.35% after soaking.

Fig. 2 presents the hydration coefficient of Faba bean seeds after soaking and after cooking. Biocontrol agents P. polymyxa, B. megaterium treated seeds showed the highest value for soaking (210.54 and 209.67%). Hydration coefficient of Faba bean seeds after cooking takes the same trend of absorbing water compared with control. Where the high value was recorded in P. polymyxa, B. megaterium and Rizolex T (260.84, 258.07 and 249.61% respectively) compared with control (240.94%). Abdel-Aleem et al. (2019) revealed that hydration coefficient increased after cooking and ranged from 220.47 to 257.10%.
Fig. 3: Effect of some chemical and biocontrol agents as seed treatments on swelling coefficients after soaking and cooking of Faba bean seeds.

Swelling coefficients of Faba bean treated seeds are shown in Fig 3. *P. polymyxa*, *B. megaterium* and Rizolex T treatments seeds had the high values of swelling coefficients after soaking and after cooking compared with other treatments. The hydration and swelling coefficients reflect the capacity to absorb water during soaking process. Both consumers and processors prefer beans that have high hydration and swelling coefficients as these produce greater quantity with better quality (Nasar-Abbasa et al., 2008).

4. Cooking quality

Effect of treated Fabe bean seeds with biocontrol agents (bacteria and fungi) and fungicide Rizolex T on cooking quality of Faba bean treated seeds are shown in Table (7). Regarding to stewing percent, the highest value recognized in biocontrol agents bacteria *P. polymyxa* treatment (100%), no significant differences were obtained among *P. fluorescens*, *B. megaterium*, and fungicide Rizolex T (98.67, 98.33 and 98.21%, respectively). Significant differences were observed among control and other treatments. In general biocontrol agents treatments (bacteria) and fungicide Rizolex T improved stewing character compared with control and *P. polymyxa* treatment was the best treatment.

Total soluble solids (TSS), showed the highest values in *P. fluorescens*, *B. megaterium* and *P. polymyxa* treatments (9.86, 9.78, and 9.77%, respectively) where no significant differences were found among them, followed by Rizolex T and control (9.59 and 9.31%). *T. harzianum* and *T viride* treatments had the lowest TSS values (8.84 and 8.47%, respectively).

Table 7: Effect of some chemical and biocontrol agents as seed treatments on the percentage of cooking quality and total soluble solids of Faba bean seeds

| Treatments | Stewing % | TSS % |
|------------|-----------|-------|
| *T. viride* | 82.32 ± 0.00 | 8.47 ± 0.23 |
| *T. harzianum* | 85.00 ± 0.00 | 8.84 ± 0.07 |
| *P. fluorescens* | 98.67 ± 0.58 | 9.86 ± 0.07 |
| *B. megaterium* | 98.33 ± 1.15 | 9.78 ± 0.04 |
| *P. polymyxa* | 100.00 ± 0.00 | 9.77 ± 0.01 |
| Rizolex T | 98.21 ± 1.15 | 9.59 ± 0.06 |
| Control | 89.33 ± 1.15 | 9.31 ± 0.04 |

Values are the average of 3 experiments ± SD. Mean values (within the same column) followed by different superscripts are significantly different at the 5% level.

5. Sensory characteristics of cooked Faba bean seeds

Sensory evaluation for color, texture, taste, odor and overall acceptability (OAA) of the cooked Faba bean seeds as influenced by biocontrol agent and Rizolix T fungicide were done in order to determine consumer acceptability (Table 8). The results revealed an increase in color value in the
sample treated with *P. polymyxa*, *B. megaterium* and Rizolex T (9.1, 9.00 and 8.65%, respectively), followed by *P. fluorescens* (8.4%), and the lowest value was in *T. viride* treatment (7.3%). No significant differences were observed in odor among all samples. The same trend was observed in taste at all treatments except *T. viride*. Over all acceptability recorded low value in *T. Viride* seeds treatment. It could be seen that Faba beans which treated with biocontrol bacteria and Rizolix T fungicide recorded the highest sensory quality in terms of color, texture, taste, odor and overall acceptability followed by *T. harzianum* and control. On the other hand, Abdel-Aleem et al. (2019) showed that, sensory quality of Faba bean seeds were recorded color (50%), texture (50%), taste (60%), odor (60%) and overall acceptability (50%).

**Table 8:** Sensory characteristics of cooked Faba bean seeds treated with chemical and biocontrol agents

| Treatments  | Color   | Texture | Taste  | Odor    | OAA      |
|-------------|---------|---------|--------|---------|----------|
| *T. viride* | 7.3±0.67| 6.9±0.88| 7.7±0.82| 8.8±0.92| 6.95±0.60|
| *T. harzianum* | 7.9±0.74| 7.8±0.63| 9.1±0.88| 8.9±0.74| 7.7±0.95 |
| *P. fluorescens* | 8.4±0.70| 8.1±0.57| 8.6±1.17| 9.0±0.94| 7.9±0.76 |
| *B. megaterium* | 9.0±0.67| 8.9±0.99| 9.1±0.88| 9.3±0.48| 8.5±0.53 |
| *P. polymyxa* | 9.1±0.52| 9.2±0.50| 8.9±0.74| 9.3±0.67| 8.7±0.48 |
| Rizolex T | 8.6±0.47 | 8.7±0.82| 8.9±0.88| 9.2±0.79| 8.45±0.60 |
| Control | 8.4±0.50 | 7.3±0.67| 9.1±0.99| 9.0±0.82| 7.1±0.74 |

Values are the average of 10 experiments ± SD. Mean values (within the same column) followed by different superscripts are significantly different at the 5% level. OAA: Overall acceptability

**Conclusion**

All the tested biocontrol agents *P. fluorescens*, *B. megaterium* and *P. polymyxa* *T. hazianum* and *T. viride*, as well as, the chemical fungicide namely Rizolex T were used and significantly reduced damping off were detected. High reduction was observed in Rizolix T fungicide *P. polymyxa*, *B. megaterium* and compared with control. They, also, caused the increasing in the yield and 100 seeds weight. Quality parameters, also, were improved by using Rizolix T fungicide, *P. polymyxa* and *B. megaterium*. Protein content, protein digestibility and minerals contents, as well as, total phenols, total flavonoids and antioxidant activity were increased. Technological evaluation of density, ratio between cotyledons and seed coats as well as water absorption, hydration coefficients and swelling coefficients after soaking and cooking showed high values in Faba bean seeds treated with *P. polymyxa*, *B. megaterium* followed by Rizolex T. They, also, improved the cook ability, and increase the sensory evaluation parameters. Therefore, it could be concluded that the inoculation of Faba bean seeds with bacteria increase the availability of nutrients which affect the yield and quality of seeds.

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