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ORIGINAL PAPER

# Assessment of Bion, Two Commercial Bio-products and Other Organic Amendments for Management of Potato Brown-Rot under Field Conditions

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## ABSTRACT

The efficacy of using Bion, two commercial bio-products and organic amendments on reduction of potato brown rot and assessment of the susceptibility of eight potato cultivars to the disease were evaluated. Field experiments during 2019/20 and 2020/21 growing seasons at Giza governorate, Egypt revealed that all the tested treatments effectively reduced the bacterial wilt under the natural infection. The combination treatments of Bion + Bio-ARC + Bio-Zeid and Bio-ARC + old Balady manure + chicken manure were the highly efficient ones in lowering the infection, being 2.3% wilt, 1.9% infected tubers and 2.0% wilt, 1.4% infected tubers, respectively. Additionally, Mondial potato cultivar was the least susceptible one associated with high rhizospheric microflora content. Using the combination of the resistance inducer Bion (BTH) with two commercial bio-products (Bio-ARC and Bio-Zeid) and two organic manures (Balady and chicken) are promising alternative methods for reducing potato bacterial wilt. Besides, there were remarkable variations in the values of free, conjugated and total phenols of the tested 8 potato cultivars, where Mondial cultivar recorded the highest values, Spunta the lowest values and the other cultivars recorded intermediate values.


**Keywords:** Potato, *Ralstonia solanacearum*, Cultivars, Bion, Bio-products, Organic manure, Phenols.

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## INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important Solanaceous crops in Egypt for local consumption and exportation. It is liable to infection by many Actinomycetes, bacterial and fungal diseases in addition to physiological disorders and nematodes (Mikhail, 1974 and Gudmestad *et al.*, 2007). However, brown-rot caused by *Ralstonia solanacearum* is considered the most constrain soil-borne pathogen to potato (Charkowski *et al.*, 2020). Recently, *R. solanacearum* has been ranked as the second most important bacterial plant pathogen across all crops (Mansfield *et al.*, 2012). The bacterium is an aerobic non-spore forming, Gram-negative and motile with polar flagella (Yabuuchi *et al.*, 1995). It is subdivided into 5 races on the basis of host range and 5 biovars on the basis of biochemical properties (Hayward, 1991).

The dominant race in Egypt and Europe is race 3 biovar 2, which was recently classified on the basis of genetic sequencing as Phylotype II, sequevar 1 (Fegan and Prior, 2005). The bacterium has a broad-spectrum host range throughout the world, including over 450 host species representing 54 plant families (Wicker *et al.*, 2007). Therefore, it becomes an economically significant pathogen and obstacle to the production of Solanaceous plants in tropical, subtropical and temperate regions. The European and Mediterranean Plant Protection Organization (EPPO) has listed *R. solanacearum* as an $A_2$ quarantine pest (Lee *et al.*, 2012). This bacterium attacks plants through the roots and colonizes xylem vessels (García *et al.*, 2019).

Plants have evolved two defense layers, constitutive and inducible, in response to pathogen infection (Jones and Dangl, 2006). In addition, some plants have developed induced resistance (IR) to pathogens after induction by external factors which can be biological, chemical or physical factors. Since discovery the chemical resistance inducer, Bion (benzothiadiazole; BTH), using Bion has become an effective alternative strategy for controlling several plant pathogens (Aslam *et al.*, 2019).

The biological induction factors include fungi, bacteria and their metabolites (Mauch-Mani *et al.*, 2017). Plant rhizosphere contains large bacterial populations capable of exerting beneficial effects on the plant growth. The employed bio-agents have been several mechanisms for disease management such as antagonism and competition in addition to induce plant disease resistance. Potato production in Egypt is annually increased year after year. Therefore, adequate management is the important approach in sustainable agricultural development, which has a role in reducing the crop losses, increasing productivity and minimizing environmental contamination and health hazards (Nwilene *et al.*, 2008).

Integration of different management practices such as using disease-free tubers, resistant cultivars, sowing less susceptible crop varieties, rotating with non-host crops and amending soil with bioagents have been employed to manage bacterial wilt and brown-rot of potato (Saddler, 2005). The use of low susceptible cultivars to the infection by soil-borne plant pathogens as well as the biological control have been extensively reviewed by several authors (Youssef, 2007). Application of organic amendments is an effective measure for controlling many soil-borne diseases (Abd El-Ghafar *et al.*, 2004; Hagag *et al.*, 2015 and Messiha, and Elhalag, 2019)

Bacterial wilt in tomato was suppressed using the poultry and farmyard manure and higher microbial activity was likely responsible (Islam and Toyota, 2004).

The present study was carried out to evaluate the susceptibility of 8 commercial potato cultivars to the infection by *R. solanacearum* and demonstrated the correlation between cultivars susceptibility and the total count of rhizospheric microorganisms. The efficacy of combination of the chemical resistance inducer Bion (BTH) and the commercial bio-product Bio-ARC with Balady and chicken manure on the natural infection of potato brown-rot incidence and severity was also estimated. The relation between free, conjugated and total phenols with the susceptibility of eight potato cultivars to the infection of *R. solanacearum* was studied.

## MATERIALS AND METHODS

**Susceptibility of eight potato cultivars to the natural infection of potato bacterial wilt and the total count of rhizospheric microorganisms at Giza governorate, Egypt:**

Three potato fields, frequently cultivated with potato showing noticeable symptoms of brown rot disease caused by *R. solanacearum* were chosen to assess the susceptibility of 8 cultivars *i.e.*, Agria, Diamant, Draga, Hermes, Lady Rosetta, Mondial, Nicola and Spunta at Giza governorate, Egypt during March of 2019. For estimating the percentages of the infected tubers, 5 samples (each of 25 tubers) were carefully examined to determine the percentages of the infected tubers depending on the outer symptoms during May 2019 (Garica *et al.*, 2019). The severity of the disease was assessed 80 days after planting (Kemp and Sequeira, 1983).

Samples representing the rhizospheric soil of each cultivar were collected according to Barillot *et al.* (2013) during April 2019 to isolate the associated microorganisms. Serial dilution plate technique (Johnson *et al.,* 1959) was used to isolate the native microorganisms, *i.e.*, Actinomycetes on arginine-glycerol-salt medium (AGS), bacteria on nutrient agar medium and fungi on soil extract medium (Oedjijono and Dragar,1993).

**Effect of Bion, Bio-ARC and Bio-Zeid on the natural infection of potato by bacterial wilt and the tubers yield:**

The effect of dipping potato tubers in the chemical resistance inducer Bion (BTH) then dressing them with 2 commercial bio-products, Bio-ARC (*Bacillus megaterium* $25x10^6$ cfu/ g), Bio-Zeid (*Trichoderma album* $10x10^6$ cfu/ g) each alone or in combination, on the natural infection of bacterial wilt and the produced tubers yield was assessed. The field was prepared for planting potato and divided into plots, each plot of 42 $m^2$. Imported potato tubers (Spunta cultivar) were dipped in 50 mM of Bion (benzothiadiazole; BTH) for 2 hours then left for semi-air dry and dressed with any of Bio-ARC and Bio-Zeid, each alone or in different combinations at the rate of 10 g/ kg tubers, before planting. Also, potato tubers were dressed with the bactericide Starner 20% (Oxolínico 20%) at the rate of 10 g/ kg tubers. The adherent material Sedo-film (100 mL/l water) was sprayed on the tubers in order to adhere the commercial bio-products on the treated tubers. Untreated tubers were used as control treatment. The tubers were planted during 20 and 24 January of 2019 and 2020, respectively. Four plots were used for each treatment. All agricultural practices were carried out according to the recommendation of the Egyptian Min. of Agric. and Land Rec., Egypt.

**Efficacy of the combination of Bion and the commercial bio-product Bio-ARC with old Balady and chicken manure on the natural infection of potato by bacterial wilt:**

The efficacy of the combination of the commercial bio-product Bio-ARC with old Balady and chicken manure, each alone or in combination, on the natural infection of potato by bacterial wilt and the produced tubers yield, was evaluated. Experimental blocks design was completely randomized, the field was prepared for planting potato and divided into plots, each plot of 42 $m^2$. The imported potato tubers (Spunta cultivar) were sprayed with the adherent material Sedo-film (100 mL/l water) in order to adhere the commercial bio-product Bio-ARC on the treated tubers. Old Balady and chicken manures were added to the furrow of the prepared land for planting potato at the rate of $15m^3$/ feddan then the tubers were put on the spreading manure (at 25-30 cm distance) then covered with the soil and the rows were prepared. Untreated tubers were planted in soil free from old Balady and chicken manure as control treatment. Four plots were used for each treatment. All agricultural practices were carried out according to the recommendation of the Egyptian Min. of Agric. and Land Rec. At the end of the experiment of each season the tuber yield was harvested and assessed for each plot and the average was calculated and recorded.

**Disease assessment:**

Disease severity (DS) was determined 80 days after planting periodically according to the key proposed by Kemp and Sequeira (1983) describing the wilt symptoms in the plant based on the visual observation using the scale (0.0-5.0), where: 0.0 = No symptoms, 1= up to 25 % wilt, 2 = more than 25-50 % wilt, 3 = more than 50-75 % wilt, 4 = more than 75-100% wilt and 5 = Dead plants. The percentage of disease severity (DS) was calculated by the following formula:

$$\%DS = \frac{\sum R.T}{5 \times N} \times 100$$

**Where:**

**T** = Total number of plants with each category.
**R** = Disease rating scale (0.0, 1, 2, 3, 4 and 5).
**N** = Total number of tested plants.

Also, 25 tubers were randomly collected from each plot and examined for infection with *R. solanacearum* depending on the tuber symptoms (Garica *et al.,* 2019).

**Assessment of phenolic compounds in the eight potato cultivars:**

The Folin–Ciocalteu assay was employed to determine the total phenolic content in the tubers of each potato cultivar extract. Determination of total phenols was carried out as described by Simons and Ross (1971). Concentrate hydrochloric acid (0.25 mL) was added to 0.2 mL of the sample extract in test tube and mixed. The mixture was then boiled for about 10 min. After cooling, 1mL Folin reagent and 5 mL sodium carbonate solution (20%) were added and diluted to 10 mL using distilled water. After 30 min the density of the developed blue color was determined at 520 nm using chatichole as standard. Determination of free phenols was carried out using the same described method with some exception, since 1mL Folin reagent and 3mL sodium carbonate solution (20%) were added to 0.2 mL of the sample extract of each cultivar, diluted with distilled water up to 10 mL. After 30 min, the density of the developed blue color was determined at the same wavelength.

**Statistical analysis:**

Analysis of variance (ANOVA) was carried out according to Snedecor and Cochran (1989). ANOVA was carried out using compatible computer basic language. LSD test was used to compare treatments means at 5% level of significance.

## RESULTS

**Susceptibility of eight potato cultivars to the natural infection of potato by bacterial wilt at Giza governorate:**

Presented data in Table (1) reveal that all the tested cultivars were liable to the infection by *R. solanacearum* showing typical symptoms of potato bacterial wilt. In addition, the tested cultivars were significantly varied in their susceptibility to the disease. Concerning, Spunta was the highest susceptible cultivar; being 12.4% wilt and 14.0% infected tubers, on the average, followed by Agria, being 10.8 and 6.2%, on the average, then Diamant, being 9.6 and 5.0 % on the average, respectively. Meanwhile, Mondial was the lowest susceptible cultivar, being 7.2 and 4.0%, respectively, followed by Lady Rosetta, being, 7.8 and 4.2% on the average, respectively. The other 3 cultivars (Draga, Hermes and Nicola) were of intermediate values of infection.

In general, the total counts of the rhizospheric microflora *i.e.,* Actinomycetes, bacteria and fungi were corresponded to somewhat, with susceptibility of the tested cultivars to the infection, where the highly susceptible cultivars were of low counts of the rhizospheric microflora compared with high counts in case of the less susceptible ones. In this regard, the recorded counts of the rhizospheric microflora of Spunta cultivar were $0.15 \times 10^3$, $1.62 \times 10^3$ and $1.20 \times 10^6$ for Actinomycetes, bacteria and fungi, respectively. Meanwhile, in case of the less susceptible cultivar, Mondial, higher figures were counted, being $0.45 \times 10^3$, $4.32 \times 10^6$ and $3.22 \times 10^3$, respectively. Intermediate counts were recorded for the other cultivars.

**Table (1): Susceptibility of the eight potato cultivars to the natural infection of potato by bacterial wilt and the total counts of rhizospheric microorganisms.**

| Cultivars | % Wilted plants[1] | % Infected tubers[2] | Total count of the rhizospheric | | |
|---|---|---|---|---|---|
| | | | Actinomyctes[3] | Bacteria[4] | Fungi[5] |
| Agria | 10.8 | 6.2 | 0.23 | 3.41 | 2.23 |
| Diamant | 9.6 | 5.0 | 0.26 | 3.63 | 2.34 |
| Draga | 8.8 | 4.6 | 0.27 | 3.68 | 2.64 |
| Hermes | 8.2 | 4.6 | 0.32 | 3.83 | 2.71 |
| Lady Rosetta | 7.8 | 4.2 | 0.40 | 4.15 | 3.14 |
| Mondial | 7.2 | 4.0 | 0.45 | 4.32 | 3.22 |
| Nicola | 9.4 | 4.8 | 0.30 | 3.87 | 2.80 |
| Spunta | 12.4 | 14.0 | 0.15 | 1.62 | 1.20 |
| LSD at 0.05 | 2.1 | 1.4 | n.s. | 0.70 | 0.42 |

1: Assessed during March, 2: Assessed during May, 3 and 5: Both Actinomycetes and fungi were counted at$10^{-3}$ and 4: the bacteria were counted at$10^{-6}$.

**Effect of Bion, Bio-ARC and Bio-Zeid on the natural infection of potato by bacterial wilt**

The effect of dipping potato tubers in the chemical resistance inducer Bion (BTH) then dressing them with commercial bio-products was tested under the natural infection of bacterial wilt disease. Table (2) indicates that treated potato tubers with BTH then dressing them with commercial bio-products, each alone or in combination, resulted in a significant reduction to the natural infection by bacterial wilt than the control treatment. In addition, using each of the tested treatments *i.e.*, BTH, Bio-ARC and Bio-Zeid was of low efficiency in lowering the infection, being 5.1, 7.3 and 6.4% wilt, on the average and 5.6, 6.1 and 6.2% infected tubers, on the average when compared with using different combinations of the treatments. In addition, the treatment with the bactericide Starner recoded 1.3 and 0.9% on the average, respectively. Control treatment recorded 13.0 and 15.2%, on the average, respectively. No significant difference was recorded due to the effect of the growing season on the wilted plants and the infected tubers.

**Table (2): Effect of Bion, Bio-ARC and Bio-Zeid on the natural infection of potato by bacterial wilt.**

| Treatments | % Wilted plants | | | % Infected tubers | | |
|---|---|---|---|---|---|---|
| | 2019/20 | 2020/21 | Mean | 2019/20 | 2020/21 | Mean |
| Bion (BTH) | 5. 2 | 5.0 | 5.1 | 5. 4 | 5.8 | 5.6 |
| Bio-ARC(BA) | 7.2 | 7.4 | 7.3 | 6.0 | 6.2 | 6.1 |
| Bio-Zeid (BZ) | 6.8 | 6.0 | 6.4 | 6.4 | 6.0 | 6.2 |
| BTH+BA | 4.4 | 4.2 | 4.3 | 4.6 | 4.4 | 4.5 |
| BTH+BZ | 4.6 | 4.4 | 4.5 | 4.6 | 4.4 | 4.5 |
| BA+BZ | 5.4 | 5.6 | 5.5 | 5.0 | 5.2 | 5.1 |
| BTH+BA+BZ | 2.2 | 2.4 | 2.3 | 1.8 | 2.0 | 1.9 |
| Starner | 1.4 | 1.4 | 1.3 | 0.8 | 1.0 | 0.9 |
| Control | 12.8 | 13.2 | 13.0 | 15.4 | 15.0 | 15.2 |
| Mean | 5.6 | 5.5 | - | 5.6 | 5.6 | - |
| LSD at 0.05 | | | | | | |
| Treatments (T) | | 1.3 | | | 1.5 | |
| Season (S) | | n.s. | | | n.s. | |
| T × S | | 2.1 | | | 2.2 | |

Results in Table (3) show that the reduction in the infection by *R. solanacearum* due to the aforementioned treatments was reflected on increasing the produced tubers yield, where the highest yield was obtained by the combination treatment (BTH+BA+BZ), being 143.8 kg/ plot ($42m^2$), on the average, compared with the other treatments. However, the treatment with bactericide Starner resulted in producing the superior yield, being 148.8 kg/ plot ($42m^2$), on the average, while the control treatment produced poor yield, being 101.3 kg/ plot ($42m^2$), on the average. No significant difference was found due to the effect of the growing season on the weight of tubers yield.

**Table (3): Effect of Bion, Bio-ARC and Bio-Zeid on the average weight of tubers yield under the natural infection of potato by bacterial wilt.**

| Treatments | Average weight of tubers yield (kg)/ plot ($42m^2$) during | | |
|---|---|---|---|
| | 2019/20 | 2020/21 | Mean |
| Bion (BTH) | 126. 0 | 127.2 | 126.6 |
| Bio-ARC (BA) | 125.4 | 124.6 | 125.0 |
| Bio-Zeid (BZ) | 125.5 | 125.3 | 125.4 |
| BTH + BA | 140.4 | 140.5 | 140.5 |
| BTH + BZ | 140.4 | 140.8 | 140.6 |
| BA + BZ | 128.4 | 129.6 | 129.0 |
| BTH + BA + BZ | 144.0 | 143.6 | 143.8 |
| Starner | 148.1 | 149.5 | 148.8 |
| Control | 100.8 | 101.7 | 101.3 |
| Mean | 131.0 | 131.4 | - |
| LSD at 0.05 for: | | | |
| Treatments (T) | | 3.9 | |
| Season (S) | | n.s. | |
| T × S | | 5.2 | |

**Efficacy of the combination of the commercial bio-product Bio-ARC with old Balady and chicken manure on the natural infection of potato by bacterial wilt**

Results in Table (4) show that the combination between the commercial bio-product Bio-ARC with old Balady and chicken manure, each alone or in combination, resulted in significant reduction in the natural infection of potato bacterial wilt compared with the control treatment. In this regard, when the commercial bio-product Bio-ARC, old Balady and chicken manure were used each alone, low efficiency in lowering the infection was recorded, being 7.3, 9.4 and 5.3 % wilt, on the average and 6.1 and 6.2 and 5.2 % infected tubers, on the average, respectively, compared with using the combination between two of them .This reduction was the highest in case of the combination among the bio-agent, old Balady manure and chicken manure, being 2.0% wilt and 1.4% infected tubers, on the average. No significant effect of the growing season on the wilted plants and the infected tubers was found.

**Table (4): Efficacy of the combination of the Bio-ARC, old Balady and chicken manure on the natural infection of potato by bacterial wilt.**

| Treatments | % Wilted plants | | | % Infected tubers | | |
|---|---|---|---|---|---|---|
| | 2019/20 | 2020/21 | Mean | 2019/20 | 2020/21 | Mean |
| Bio-ARC (BA) | 7.2 | 7.4 | 7.3 | 6.0 | 6.2 | 6.1 |
| Old Balady manure (OBM) | 9.6 | 9.2 | 9.4 | 6.4 | 6.0 | 6.2 |
| Chicken manure (CM) | 5.4 | 5.2 | 5.3 | 5.4 | 5.0 | 5.2 |
| BA + OBM | 5.4 | 5.6 | 5.5 | 5.6 | 5.2 | 5.3 |
| OBA + CM | 5.0 | 5.2 | 5.1 | 5.0 | 5.2 | 5.1 |
| BM + CM | 6.4 | 6.6 | 6.5 | 5.8 | 5.4 | 5.6 |
| BA + OBM + CM | 2.0 | 2.0 | 2.0 | 1.4 | 1.4 | 1.4 |
| Control* | 12.8 | 13.2 | 12.0 | 15.4 | 15.0 | 15.2 |
| Mean | 6.7 | 6.8 | - | 6.4 | 6.2 | - |
| LSD at 0.05 for: | | | | | | |
| Treatments (T) | | 1.4 | | | 1.3 | |
| Season(S) | | n.s. | | | n.s. | |
| T × S | | 2.0 | | | 2.3 | |

*Fertilized with the recommended doses of the mineral fertilizers.

Results in Table (5) demonstrate that the aforementioned treatments significantly increased the produced tubers yield of potato plants grown under the natural infection of bacterial wilt compared with the control. Moreover, using each of Bio-ARC, old Baldy and chicken manure alone was of low efficiency in increasing the produced yield compared with using their combination. In this regard, the highest yield was obtained with the combination among the three items, being 162.6 kg/ plot (42m$^2$), on the average, compared with the other treatments. Control treatment produced 101.3 kg/ plot (42m$^2$), only.

**Assessment of phenolic compounds in tubers of the eight potato cultivars**

Presented data in Table (6) reveal that there were noticeable variations in the values of free, conjugated and total phenols of the tested eight cultivars. In this respect, Mondial recorded the highest values, being 0.29, 0.40 and 0.69 mg/g fresh weight of the tubers, followed by Nicola, being 0.28, 0.38 and 0.66 mg/g fresh weight of the tubers, on the average, respectively. Meanwhile, Spunta gave the lowest values, being 0.20, 0.26 and 0.46, followed by Agria, being 0.23, 0.32 and 0.45 mg/g fresh weight of the tubers, on the average, respectively. The other cultivars recorded intermediate values. Moreover, the estimated values of phenolic compounds demonstrated that there was a correlation between the potato roots content of free, conjugated and total phenols with the plant resistance, where the lowest values were recorded in the highly susceptible cultivar (Spunta) than the less susceptible one (Mondial).

**Table 5: Efficacy of Bio-ARC, old Balady and chicken manure on the average weight of tubers yields under the natural infection of potato by bacterial wilt.**

| Treatments | Average weight of tubers yield (kg)/ plot (42m$^2$) during | | |
|---|---|---|---|
| | 2019/20 | 2020/21 | Mean |
| Bio-ARC (BA) * | 125.4 | 124.6 | 125.0 |
| Old Baldy manure (OBM) | 138.5 | 139.3 | 138.9 |
| Chicken manure (CM) | 140.4 | 141.7 | 141.1 |
| BA + OBM | 149.3 | 150.4 | 149.9 |
| BA + CM | 151.4 | 152.1 | 151.8 |
| BM + CM | 152.0 | 153.0 | 152.5 |
| BA + OBM + CM | 162.1 | 163.0 | 162.6 |
| Control* | 100.8 | 101.7 | 101.3 |
| Mean | 140.0 | 140.6 | - |
| LSD at 0.05 for | | | |
| Treatments (T) | | 4.4 | |
| Season (S) | | n.s. | |
| T × S | | 5.7. | |

* Fertilized with the recommended doses of the mineral fertilizers.

**Table (6): Values of free, conjugated, and total phenolic contents in tubers of the eight potato cultivars.**

| Cultivars | Phenolic compounds (mg/g tuber) | | |
|---|---|---|---|
| | Free | Conjugated | Total |
| Agria | 0.23 | 0.32 | 0.45 |
| Diamant | 0.25 | 0.33 | 0.58 |
| Draga | 0.25 | 0.35 | 0.60 |
| Hermes | 0.27 | 0.35 | 0.62 |
| Lady Rosetta | 0.27 | 0.37 | 0.62 |
| Mondial | 0.29 | 0.40 | 0.69 |
| Nicola | 0.28 | 0.38 | 0.66 |
| Spunta | 0.20 | 0.26 | 0.46 |
| L.S.D. at 5% | n.s.* | 0.07 | 0.11 |

* n.s.=Not significant.

## DISCUSSION

Potato brown-rot has been recorded in Egypt early in the last Century (Briton-Jones, 1925). *R. solanacearum* race 3 biovar 2 (R3Bv2) is an economically important soil borne plant pathogen which causes great economic loss to the productivity of potato yield and hinders potato exportation to the foreign markets (Farag, 2013). *R. solanacearum* became endemic in some Egyptian soil due to its ability to live as a saprophyte in the soil with long survival ability (Gado, 2013). Recently, there has been an increasing demand for searching of effective alternative methods for controlling this bacterium.

In the present study, all the eight tested cultivars were liable to the infection by *R. solanacearum*, showing typical symptoms of potato bacterial brown-rot disease and were significantly varied in their susceptibility. Referring to, Spunta was the most susceptible one, followed by Agria then Diamant. Meanwhile, Mondial was the lowest susceptible one, followed by Lady Rosetta. The other three cultivars were of intermediate values of infection. These results are in harmony with those reported by Youssef (2011) who noted that the potato plant cultivar "Spunta" was the highly susceptible one and produced severe symptoms on the leaves.

Obtained results revealed that the total count of the rhizospheric microflora was corresponded to somewhat, with susceptibility of the tested cultivars to the infection by the disease, where the highest susceptible cultivars were of low count of the rhizospheric microflora compared with high count in case of the lowest susceptible ones. Results suggest that increasing of rhizospheric microflora with the lowest susceptible cultivars may be due to their root exudates that contain high nutrients content, which in turns play an important role in suppression the disease. The rhizosphere is considered a "hot-spot" for microbial abundance and metabolic activity due to the nutrients released by plant roots (Kuzyakov and Blagodatskaya, 2015). Plant cultivar is a primary factor that determining overall fungal, pathogen and saprotrophic community composition (Loit *et al.*, 2020). Consideration that, root exudates contain released ions *i.e.*, $H^+$, inorganic acids, oxygen and water, but mainly consist of carbon-based compounds. These organic compounds can often be separated into two classes: low-molecular weight compounds, which include amino acids, organic acids, sugars, phenolics and an array of secondary metabolites, and high-molecular weight compounds like mucilage and proteins (Bais *et al.*, 2006)

Recently, numerous researchers have been demonstrated that Plant Growth-Promoting Rhizobacteria are characterized through their ability to induce plant growth in diverse ways including solubilizing nutrients such as iron and phosphorus, changing host physiology, fixing atmospheric nitrogen, along with producing secondary metabolites and phytohormones (Idris *et al.*, 2007) as well as inducing plant resistance to different pathogens (Youssef, 2016).

In this study, field experiments revealed that using Bion (BTH) individually or in combinations with two commercial bio-products *i.e.*, Bio-ARC (*Bacillus megaterium*) and Bio-Zeid (*Trichoderma album)* significantly reduced the natural infection of potato by bacterial wilt. Besides, all the treatments were positively reflected on the tubers yield. The positive influence of BTH and the bio-products in reducing the infection and increasing the tubers yield could be due to the hormone-like activities exhibited by the tested treatments that are involved indirectly in respiration, photosynthesis, protein synthesis, oxidative phosphorylation, anti-oxidant reactions, and different enzyme activities (Zhang *et al.*, 2003). The obtained results are in harmony with those reported by Barilli *et al.*, (2015) who stated that BTH is a systemic acquired resistance elicitor, a chemical analogue of SA, which reduces many bacterial diseases. Bion efficiency has been reported in many crop species for its performance in controlling a large number of pathogens and/or in inducing or priming multiple immune responses (Bektas and Eulgem, 2015).

It is noteworthy to report that *Trichoderma* species are efficient plant growth promoting fungi (PGPF). Brotman *et al.*, (2013) suggested that Trichoderma is similar to1-aminocyclopropane-1-carboxylate (ACC) deaminase producing bacteria that alter the expression of several genes that related to the osmo-protection resulted in improving the growth of plants under adverse conditions, by increasing the ethylene levels as well as promoting the elevated antioxidative capacity. In this concern, Schonfeld *et al.* (2003) reported that there was a decrease in *R. solanacearum* population in soil amended with decomposed organic fertilizer and the decomposed organic manure provides nutrients to the bio-control agents (Liu *et al.*, 2012).

Obtained data demonstrated that, the tested mixture (BTH + Bio-ARC + Bio-Zeid) exceeded the effectiveness in suppressing the disease incidence with a pronounced increase in potato tubers yield. These results could be attributed to the synergistic effect of the antagonistic mixed application of the bio-products and inducer resistance BTH resulting into a complex interaction, which reduced the damage caused by the pathogen. Combining antagonists with different mode of action can therefore be effective in disease control (Aguk *et al.*, 2018).

The obtained results, also, showed that using the commercial bio-product Bio-ARC with old Balady and chicken manure, individually or in different combinations, resulted in significant reduction in the natural infection of potato bacterial wilt. The tested mixture of Bio-ARC + old Balady manure + chicken manure was the most effective treatment in reducing the disease incidence with a pronounced increase in the produced tubers yield. These results are in agreement with those reported by Islam and Toyota (2004) who concluded that the influence of organic amendments on soil-borne diseases by increasing the biological buffering capacity of the soil, reducing pathogen numbers during the hydrolysis of organic material, affecting nitrification which influences the form of nitrogen predominating. Also, with those demonstrated by Messiha *et al.,* (2020) who revealed that using composted chicken manure causing a significant increase in soil bacterial and fungal biodiversity with dominance of antagonism resulted in reduction of potato bacterial wilt. The population of *R. solanacearum* was declined at the early stage of composting as a consequence of pH decreasing along with production of organic acids that causing further acidification due to the microbial activity (Yadess *et al.*, 2010). Recheigl (1995) mentioned that organic soil amendment led to physical effects on soil properties including: (a) reducing the bulk density of soil, (b) increasing water holding capacity, (c) increasing water infiltration and drainage in fine-textures and, (d) improving soil aggregation.

There were noticeable variations in the values of free, conjugated and total phenols of the tested 8 potato cultivars. In that matter, Mondial cultivar recorded the highest values. Meanwhile, Spunta cultivar was of the lowest values. The other cultivars recorded intermediate values. In general, there was a correlation between the phenols content and the plant resistance, these results are in accordance with those reported by Field *et al.* (2006) who mentioned that the phenolic compounds are among the most influential and widely distributed secondary products in the plants. Such compounds govern disease resistance in many crop plants. Phenolic compounds can offer an alternative to the chemical control of pathogens on agricultural crops. Also, accumulation of phenolic compounds at the challenge site can reinforce cell wall that is accompanied by localized production of reactive oxygen species driving cell wall cross linking, antimicrobial activity and defense signaling (Dakora and Phillips 1996). Furthermore, the phenolic compounds are necessary for the lignin biosynthesis, which plays an important role in the plant cell walls. The increasing in phenolic compounds content has been found due to an increase in synthesis of phenylalanine ammonia lyase in plants structure (Mittelstrass *et al.*, 2006).

## CONCLUSIONS

Based on the obtained results, it is highly recommended using less susceptible potato cultivar *e.g*., Mondial which is associated with highly microflora content to reduce the natural infection of potato bacterial wilt. Additionally, the present investigation suggests that using the combination of two commercial bio-products with the resistance inducer BTH, also using combination of the commercial bio-product Bio-ARC with Balady and chicken manure are promising strategies for managing the natural infection of potato bacterial wilt. Studies on the efficacy of using these bio-products on inducing resistance of potato plants are needed for further elucidation.

## LIST OF ABBREVIATIONS:

**BTH**: Benzothiadiazole
**BA**: Bio-ARC.
**BZ**: Bio-Zeid.
**OBM**: Old Baldy Manure.
**CM**: Chicken Manure.
**PGPF**: Plant Growth Promoting Fungi.
**PGPR**: Plant Growth-Promoting Rhizobacteria
**SAR**: Systemic Acquired Resistance.
**SA**: Salicylic Acid.

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