ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# Biological Control of Potato Late Blight by Means of Induction Systemic Resistance and Antagonism.

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**ABSTRACT**

Biological control of the fungus-like microorganism *Phytophthora infestans* causing late blight of potato was investigated using four other microorganisms viz., *B. subtilis*, *P. fluorescens*, *T. harzianum* and *T. viride*. Interactions between the microorganisms and *P. infestans* were studied in the laboratory and field (during two successive growing seasons). In the *in vitro* tests, *T. harzianum* and *B. subtilis* antagonists highly restricted the growth of the late blight pathogen by 83.3 and 84.4 %; respectively over the control in agar assays. While *T. viride* and *P. fluorescens* restricted the growth of *P. infestans* by 75.1 to 77.6 %, respectively. In a field experiment, foliar spray of all bioagents suspensions significantly protected potato plants from late blight disease during the two growing seasons. It was observed that, the integration between induction of systemic resistance treatment and antagonists treatment by each of bioagents showed a stronger effect in reducing the severity of late blight. *T. harzianum* and *B. subtilis* was found to be more efficient than *P. fluorescens* and *T. viride*. The highest reduction in late blight severity was obtained with foliar spray of *B. subtilis* suspensions as both induction of systemic resistance treatment and antagonists treatment, which reduced disease severity by 84.6 and 86.1 %, during the two growing seasons, respectively. The above treatment highly reduced the *P. infestans* sporangial / $cm^2$ of potato leaves and increased the tuber yield by 55.0 and 53.6 %, during the two growing seasons, respectively. The above treatment also significantly increased the systemic resistance enzyms viz., chitinase and *β*.1, 3-glucanase activities more than 139.0 and 142%; respectively. It could be suggested from the present study that, potato plants treated with each of *B. subtilis* and *T. harzianum*, applied as integrated management of induction of systemic resistance treatment and as antagonists retarded the severity of late blight, increase the defenced enzymes viz., chitinase and *β*.1, 3-glucanase activities and increased the yield of potato tubers.

**Keywords:** Potato plants, late blight disease, biocontrol agents, inducing systemic resistance under field conditions.

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ISSN: 0975-8585

## INTRODUCTION

Potato (*Solanum tuberosum* L.) is a worldwide cultivated tuber-bearing plant which is the fourth main food crop in the world after rice, maize and wheat, in terms of both area cultivated and total production [1, 2, 3]. In Egypt, potato crop has an important position among all vegetable crops, where about 20% of total area devoted for vegetable production is cultivated with potato. In addition, the total cultivation of potatoes reached 197,250 feddans (one feddan = 1.035 acres) which produce 2,039,350 tons of tubers with an average yield of 10.34 tons/feddan [4]. Late blight disease caused by the fungus-like microorganism, *Phytophthora infestans* (Mont.) de Bary, is a potentially serious fungal disease of potato. Disease management strategies primarily depend on sanitary practices and well-timed fungicide applications [5, 6, 7]. The problem associated with the use of hazardous chemicals for plant disease control has received increasing attention worldwide, because pathogens become resistant to chemical pesticides, environmental pollution and ecological imbalances which may occur [8]. Biological control of late blight is one alternative treatment to chemical control that deserves more research. Plant growth-promoting rhizobacteria (PGPR) such as *Bacillus subtilis* & *Pseudomonas fluorescens*, and the plant-growth promoting fungi (PGPF) such as *Trichoderma harzianum* & *T. viride*, are used in a wide range of crop plants as biocontrol agents for management of different pathogens [9, 10, 11]. Jindal *et al*. [12] found that application of *Trichoderma* spp. spores on potato plants significantly reduced the intensity of late blight.

Arora [13] studied the biological control of late blight of potato by using the antagonist, *Trichoderma*. The antagonist either prevented the germination of sporangia or inhibits the development of late blight. In greenhouse experiments, a prophylactic spray of *T. viride* at concentrations of $10^8$ cfu/mL on two blight susceptible potato cultivars 3 to 4 h before inoculation with the pathogen, restricted development of late blight between 1.5 and 14.0 percent compared to 61.6 and 88.8 percent in control without the antagonists. However, the disease control in the field was less effective compared to the laboratory and greenhouse tests. Zhinong *et al*. [14] found that *Bacillus puimilus* and *Pseudomonas fluorescens* elicited systemic protection against late blight on tomato and reduced disease severity.

Results of studies carried out by Daayf *et al*. [15] showed that bacteria with biocontrol activity against late blight were from the genera *Bacillus, Pseudomonas, Rahnella* and *Serratia*. Ghorbani *et al*. [16] reported that among 223 fungal and bacterial bioagents evaluated against *P. infestans*, 13 antagonists strains showed between 10 to 50 percent reduction in blight development compared to controlplants. Lamsal *et al*. [17] reported that seven bacterial isolate, a majority of them are members of *Bacillus* inhibited *P. infestans* by more than 60% *in vitro*. However, the isolate AB15was the most effective, inhibiting mycelial growth of the pathogen by more than 80% *in vitro* and suppressing disease by 74% compared with control plants under greenhouse conditions. Hossain *et al*. [18] found that biofungicide based *Trichoderma harzianum* treated seed tubers resulted lower late blight incidence and severity followed by the fungicide Bavistin. Yuan-Hang *et al*. [19] found that two *Trichoderma* isolates R-5 and T-15 showed significantly antifungal activities against *P. infestans*. An antagonistic assay showed that the supernatant of these two isolates inhibited mycelium growth and sporangium germination of *P. infestans*. Greenhouse and field experiments indicated R-5 and T-15 reduced the disease incidence by 72.4% and 70.0%; respectively.

Mechanisms of inhibition characterized included those occurring directly, through antibiosis, and (or) indirectly, through the induction of plant defense systems. Induced systemic defense responses in plants have been reported as one of the mechanisms by which these organisms reduce the diseases in plants in conjunction with other mechanisms including direct antagonism, antibiosis and siderophore production [20, 21, 22]. Induction of defense responses by *Bacillus* spp. and *Trichoderma* spp. is largely associated with production of pathogenesis related proteins like *ß*-1,3-glucanase and the defense enzyme phenylalanine ammonia-lyase and oxidative enzymes like peroxidase, polyphenol oxidase and superoxide dismutase [23, 24, 20, 25, 26]. Apart from controlling diseases, these biocontrol organisms also promote plant growth by production of plant growth hormones like IAA and GA3 coupled with increased availability of nutrients [11, 26]. The objectives of the present study were to evaluate the efficiency of some bioagents applied as induction systemic resistance and as antagonism to management late blight disease of potato plants under field conditions.

ISSN: 0975-8585

## MATERIALS AND METHODS

### Fungi and cultural conditions

An extremely aggressive isolate of the fungus-like microrganism, *Phytophthora infestans*, used throughout this study were kindly provided by Plant Pathology Department, National Research Center, Giza, Egypt, and had been isolated from potato plants in previous study [27]. It was grown on V8 juice agar (200 mL V8 juice, 800mL distilled water, 3g $CaCO_3$ and15g agar) medium and incubated in 17±2$^{o}$C for 7days. Actively growing mycelia were then selected from plates, subcultured in freshly prepared agar medium, and incubated at their optimal growth temperature for further use.

### Plant material

Potato tubers (cv. Nicola) obtained from Dept., of Vegetables Crop Research, Agricultural Research Centre, Giza, Egypt, were used in this study.

### Biocontrol agents

The biocontrol agents viz., *Pseudomonas fluorescens*, *Bacillus subtilis*, *Trichoderma harzianum* and *T. viride*, were kindly obtained from Plant Pathology Dept., National Research Centre, Giza, Egypt. Inocula of each *P. fluorescens* and *B. subtilis*, was prepared for all experiments by harvesting cells from nutrient broth cultures grown at 28 ± 1$^{o}$C for 48 h, prior to the date of application, followed by centrifugation at 6000 rpm for 15 min. The inoculum was re-suspended in sterile distilled water and then the concentration was adjusted to $10^8$ CFU/mL [28, 29]. For each of *T. harzianum* and *T.viride* inoculum production, cultures were grown on potato dextrose agar plates incubated at 25 ± 1$^{o}$C for 10 days prior to the date of application. Spores were harvested by flooding the surface of the Petri dish with sterile distilled-deionized water (5 ml) and gently scraping the surface of the media with an L-shaped glass rod to dislodge the spores. The resulting suspension was strained through four layers of cheesecloth to remove mycelial fragments and the concentration then was adjusted to $10^6$ spore /mL using a hemacytometer slide [30]. A few drops of the emulsifier Tween 20 (Sigma Co.) and sticker were added.

### *In vitro* inhibition assay

A dual culture inhibition assay was conducted on V8-PDA (150mL V8 juice, 10g PDA, 3g $CaCO_3$, 10g agar, and 850mL $dH_2O$) in Petri dishes (9-cm diameter). The antagonistic activity of each fungal bioagents viz., *T. harzianum* and *T. viride* against the pathogen *P. infestans* was studied via the dual culture technique using the method described by Amel *et al*. [31]. The method consists of placing an active mycelial disc (5-mm in diameter) of the pathogen, 1cm from the edge of a 9-cm-diameter Petri plate containing freshly prepared PDA medium. Another disc (5-mm in diameter) of the antagonist fungus was deposited in a diametrically opposed position 1cm away from the other set of the plate. For untreated plates, an agar disc (5-mm in diameter) of the pathogen only was placed at 1cm from the edge of a 9-cm-diameter Petri plate containing freshly prepared PDA medium.

The bacterial isolates viz., *P. fluorescens* and *B. subtilis* were also screened for their antagonistic ability against the pathogen *P. infestans in vitro* via the dual culture technique using the method described by Estrella *et al.* [32]. Therefore, each bacterial isolate was cultured (by streaking) at 1cm from the edge of a 9-cm diameter Petri plate containing freshly prepared PDA medium. On the opposed position 1cm away from the other set of the plate a 5-mm plug from the leading edge of a 5-days old culture of *P. infestans*, cultured on PDA medium were inoculated individually. For untreated plates, an agar disc (5-mm in diameter) of the pathogen only was placed at 1cm from the edge of a 9-cm diameter Petri plate containing freshly prepared PDA medium.

Five plates were used as replicates for each treatment as well as the control. Inoculated plates were incubated at 25 ± 1°C until the fungal growth of the control plates reached the edge of the plate. The reduction in mycelial growth of *P. infestans* was calculated using the formula suggested by Pandy *et al*. [33] as follows: R = C – T / C × 100, whereas: R = Mycelial growth reduction (%) of the pathogen, C = Radial growth of the pathogen in control plates (cm) and T = Radial growth of the pathogen in dual culture plate (cm).

**Effect of Ridomil gold plus on *P. infestans* growth**

The recommended fungicide Ridomil gold plus (Mefenoxam and Copper oxychloride) at the concentrations of 2g/L against the linear mycelial growth of *P. infestans* was evaluated via the poisoned food technique according to **Borum & Sinclair [34]**. The prepared V8-PDA medium was dispersed in 200 ml quantities into 250 ml Erlenmeyer flasks and sterilized by autoclaving at 121°C for 15 min. Fungicide concentration were prepared based on the active ingredients and then added to V8-PDA medium before its solidification to obtain the final concentrations of 2g/L and mixed gently with 0.1% Tween 80 (Sigma) to enhance solubility. Then, 15 ml of fungicide amended V8-PDA medium was poured in sterilized 9 cm Petri plates. The poisoned medium was allowed to solidify. The V8-PDA medium without fungicide was kept as control. Then, 0.5 cm fungal mycelial disc of *P. infestans* was picked from 7-days-old purified culture with the help of a sterilized cork borer and then the disc was inoculated in the center of each plate. Three Petri plates were used as replicates for each treatment as well as untreated control. The inoculated plates were incubated at 27±2°C.The colony diameter (cm) of *P. infestans* was measured when the *P. infestans* growth reached the Petri plate edge of the control. The percent inhibition in linear mycelial growth was calculated using the following formula**:**

$$\textbf{Mycelial growth inhibition (\%)} = [(dc-dt)/dc] \times 100$$

**Where**: dc = Average diameter of *P. infestans* growth in control.
dt = Average diameter of *P. infestans* growth in fungicide treatment.

**Field experiment**

Field experiments were carried out during two successive seasons at Omar Makram Village, El-Tahrir county, El-Behera governorate, to evaluate the protective effect of tested bacterial and fungal isolates viz., *P. fluorescens*, *B. subtilis*, *T. harzianum* and *T. viride*, applied as induction systemic resistance and as antagonists against potato late blight under field conditions. Experiments were conducted under natural infection in plots (4 × 8 m) each comprised of 8 rows ( 32 holes / row and one seed piece was sown in each hole ) in a randomized complete block design with three replicates (plots) for each treatment. Seed tuber (cv. Necola) were cut longitudinally using sterilized knife into pieces with 2-3 sprout per piece. The potato seed pieces have been disinfected before use by deceiving in a solution of sodium hypochlorite solution (10%) for 10 min and rinsing twice with sterile distilled water. Disinfected potato seed pieces was air dried for 24 h under shadow place. Then, seed tuber pieces were planting in loamy clay well-drained soil to a depth of 10 cm. In addition, irrigation and nutrients such as phosphorus, nitrogen and potassium were added to ensure adequate plants nutrition during mid-growth and tuberization as recommended**.**

**Application method**

All treatments with bioagents of *T. harzianum, T. viride*, *B. subtilis* and *P. fluorescens*, were applied individually as foliar application on potato plants which had 4-5 compound leaves as follows:

**Induction of systemic resistance treatment**

Foliar spray of bioagents suspensions on potato plants was carried out once time at 30 days after planting.

**Antagonists treatment**

Foliar spray of bioagents suspensions on potato plants was carried out four times (weekly) beginning at 60 days after planting.

**Induction of systemic resistance + antagonists treatment**

Foliar spray of bioagents suspensions on potato plants was carried out once time at 30 days after planting plus foliar spray of bioagents suspensions four times beginning at 60 days after planting.

**Data collection and analyses**

**Disease assessment**

Late blight disease severity (%) was recorded up to 90 days of planting by the scale from 0 to 4 according to Cohen *et al*. [35] based on the infected leaf area as follows:

0 = No leaf lesions.
1 = 25 % or less.
2 = 26 to 50.
3 = 51 to 75.
4 = 76 to 100 % infected leaf area.

**Effect on *P. infestans* sporulation**

Sporangia of *P.infestans* / $cm^2$ were counted in potato leaves after 80 days of planting. Leaves of each treatment were detached gently at the early morning and immersed in screw cap jars containing 10 ml of distilled water. Sporangia were released from lesions using a brush, then they were counted using hemocytometer slide. Area of lesions were detected by placing the cut lesion on millimeters quarter paper.

**Biochemical studies**

Determination of chitinase and *β*.1, 3-glucanase enzyme activities were carried out at 80 days after planting. Potato leaves were collected and to extract the enzyme, plant leaves (g) were homogenized with 0.2 M Tris HCl buffer (pH 7.8) at 0°C containing 14 m M *β*- mercaptoethanol at the rate of 1/3 w/v. The extracts were obtained by filtering off the debris with a clean cloth and centrifuging at 3,000 rpm for 15 min. The supernatants were recovered and kept in a tube in an ice bath until assayed. The supernatant was used to determine the activity of chitinase and β-1,3-glucanase enzymes [36] by using UV spectrophotometer. The determination of chitinase enzyme was carried out using colloidal chitin as substrate and dinitrosalicylic acid (DNS) as reagent to measure reducing sugars according the method described by Monreal and Reese [37]. Chitinase activity was expressed as mM N-acetyl glucose amine equivalent released/ gram fresh weight/ 60 minutes at 450nm. *β*.1,3-glucanase was assayed based on the method described by Miller [38]. Laminarin (Sigma) was used as a substrate and dinitrosalicylic acid (DNS) as reagent to measure reducing sugar. *β*.1,3-glucanase activity was expressed as mM glucose equivalent released/ gram fresh weight/ 60 minutes at 500nm.

**Potato tuber yield**

Effect of biocontrol agents application on potato tuber yield under field conditions was studied. Therefore, potato tuber were harvested after 120 days of planting. Tuber yield per each treatment was recorded and the average of the tuber yield (metric ton / hectare) was calculated for each treatment.

**Statistical analysis**

Tukey test for multiple comparisons among means was utilized [39].

## RESULTS

**Laboratory experiments**

**Antagonistic effect of bioagents against *Phytophthora infestans in vitro***

The *in vitro* antagonistic effect of bioagents as well as the suppressive effect of the fungicide Ridomil gold plus against the linear mycelial growth of *P. infestans* are shown in Table 1. Ridomil gold plus was completely inhibiting the growth of *P. infestans* at the concentration of 2.0g/L. All antagonists significantly reduced the radial mycelial growth of *P. infestans* compared to the control without the antagonists. Among them the antagonists *B. subtilis* and *T. harzianum*, caused the maximum growth reduction of *P. infestans*,

followed by *Trichoderma viride* and *P. fluorescens*, where the reduction values being 84.4, 83.3, 75.1 and 77.6.

**Table 1: Reduction caused by some biocontrol agents and Ridomil gold plus against linear mycelial growth of *Phytophthora infestans* via the daul culture technique**.

| Bioagent | Linear mycelial growth (mm) and reduction (%) | |
|---|---|---|
| | Growth (mm) | Reduction (%) |
| ***Trichoderma harzianum*** | 15.0 c[(1)] | 83.3 |
| ***Trichoderma viride*** | 22.4 b | 75.1 |
| ***Bacillus subtilis*** | 14.0 c | 84.4 |
| ***Pseudomonas fluorescens*** | 20.2 b | 77.6 |
| **Ridomil gold plus at 2.0g/L** | 00.0 d | 100 |
| **Control** | 90.0 a | 0.0 |

1- Figures with the same letter are not significantly different ( P= 0.05).

**Field experiments**

**Effect of foliar spray with bioagents on the severity of late blight**

Effect of foliar spray with bioagents viz., *B. subtilis, P. fluorescens, , T. harzianum* and *T. viride*, applied as induction systemic resistance and as antagonists on potato late blight severity (%) under field conditions are shown in Table 2. Results indicate that all treatments significantly reduced the severity of late blight during two growing seasons. The most effective treatment is the combination between induction systemic resistance treatment and antagonists treatment. Antagonists applied as induction systemic resistance reduced late blight severity by the range of 44.4 to 73.1%, while antagonists treatment reduced late blight severity by the range of 41.7 to 66.7%, but the combined treatments caused reduction by the range of 55.6 to 86.6%, during the two growing seasons. The highest reduction in late blight severity was obtained with each of *T. harzianum* and *B. subtilis* when applied as combined treatments, which reduced disease severity by 80.8 & 84.6 and 80.6 & 86.1 %, during the two growing seasons, respectively. It was followed by each of *T. viride* and *P. fluorescens* when applied as combined treatments, which reduced disease severity by 61.5 and 55.5 %, during the two growing seasons, respectively. Meanwhile, single treatments of bioagents showed moderate effect.

**Table 2: Effect of foliar spray with microorganisms applied as induction of systemic resistance and as antagonists or both of them on late blight severity of potato under field conditions**.

| Treatment[(1)] | Late blight disease severity [(2)] | | | |
|---|---|---|---|---|
| | First growing season | | Second growing season | |
| | Severity (%) | Reduction (%) | Severity (%) | Reduction (%) |
| **Induction of systemic resistance treatment** | | | | |
| ***Trichoderma harzianum*** | 0.8 cd [(3)] | 69.2 | 1.1 c | 69.4 |
| ***Trichoderma viride*** | 1.2 bc | 53.8 | 2.0 b | 44.4 |
| ***Bacillus subtilis*** | 0.7 de | 73.1 | 1.0 c | 69.4 |
| ***Pseudomonas fluorescens*** | 1.4 b | 46.2 | 1.8 b | 50.0 |
| **Antagonists treatment** | | | | |
| ***Trichoderma harzianum*** | 1.0 bcd | 61.5 | 1.2 c | 66.7 |
| ***Trichoderma viride*** | 1.3 bc | 50.0 | 2.1 b | 41.7 |
| ***Bacillus subtilis*** | 0.9 bcd | 65.4 | 1.2 c | 66.7 |
| ***Pseudomonas fluorescens*** | 1.3 b | 50.0 | 2.0 b | 44.4 |
| **Induction of systemic resistance treatment + Antagonists treatment** | | | | |
| ***Trichoderma harzianum*** | 0.5 e | 80.8 | 0.7 cd | 80.6 |
| ***Trichoderma viride*** | 1.0 bcd | 61.5 | 1.6 b | 55.6 |
| ***Bacillus subtilis*** | 0.4 e | 84.6 | 0.5 d | 86.1 |
| ***Pseudomonas fluorescens*** | 1.0 bcd | 61.5 | 1.6 b | 55.6 |
| **Ridomil gold plus ( 2g/L)** | 0.4 e | 84.6 | 0.7 cd | 80.6 |
| **Control** | 2.6 a | - | 3.6 a | - |

1-Foliar spraying with bioagents was carried out once time when applied as induction of systemic resistance treatment at 30 days of planting and four times when applied as antagonists treatment at 60 days of planting.
2-Late blight severity were determined according to the scale from 0 to 4 suggested by **Cohen *et al*. (1991).**
3- Figures with the same letter are not significantly different ( P= 0.05).

**Effect of foliar spray with bioagents on *P. infestans* sporulation**

Effect of foliar spray with bioagents viz., *B. subtilis, P. fluorescens, , T. harzianum* and *T. viride*, applied as induction systemic resistance and as antagonists on the average number of *P.infestans* sporangia/ $cm^2$ lesion in treated potato plants under field conditions are shown in Table 3. Results indicate that all treatments significantly reduced the average number of sporangia/ $cm^2$ lesion in treated plants. The most effective treatment is the combination between induction systemic resistance treatment and antagonists treatment. After 80 days of planting, antagonists applied as induction systemic resistance reduced the number of *P.infestans* sporangia/ $cm^2$ lesion by the range of 60.0 to 92.0%, while antagonists treatments reduced the number of sporangia/ $cm^2$ lesion by the range of 60.0 to 88.0%, but the combined treatments caused reduction by the range of 65.0 to 96.0%.

**Table 3: Reduction in average number of *P.infestans* sporangia/ $cm^2$ lesion in treated potato plants with microorganisms applied as induction of systemic resistance and as antagonists or both of them under field conditions.**

| Treatment [1] | Reduction (%) in sporangia/ $cm^2$ lesion | | |
|---|---|---|---|
| | Days after planting | | |
| | 40 | 60 | 80 |
| **Induction of systemic resistance treatment** | | | |
| ***Trichoderma harzianum*** | 92.0 b | 88.0 c | 85.0 c |
| ***Trichoderma viride*** | 70.0 d | 64.0 ef | 60.0 f |
| ***Bacillus subtilis*** | 95.0 a | 93.0 ab | 92.0 a |
| ***Pseudomonas fluorescens*** | 70.0 d | 66.0 e | 63.0 e |
| **Antagonists treatment** | | | |
| ***Trichoderma harzianum*** | 90.0 b | 90.0 b | 85.0 c |
| ***Trichoderma viride*** | 72.0 d | 63.0 ef | 60.0 f |
| ***Bacillus subtilis*** | 92.0 b | 90.0 b | 88.0 bc |
| ***Pseudomonas fluorescens*** | 72.0 d | 70.0 e | 70.0 d |
| **Induction of systemic resistance treatment + Antagonists treatment** | | | |
| ***Trichoderma harzianum*** | 94.0 a | 91.0 b | 90.0 b |
| ***Trichoderma viride*** | 75.0 d | 68.0 d | 65.0 e |
| ***Bacillus subtilis*** | 97.0 a | 96.0 a | 96.0 a |
| ***Pseudomonas fluorescens*** | 75.0 d | 77.0 d | 70.0 d |
| **Ridomile plus ( 2.0g/ L)** | 88.0 c | 88.0 c | 90.0 b |
| **Control** | 0.0 e | 0.0 g | 0.0 g |

1-Foliar spraying with bioagents was carried out once time when applied as induction of systemic resistance treatment at 30 days of planting and four times when applied as antagonists treatment at 60 days of planting.

The highest reduction in the number of sporangia was obtained with each of *T. harzianum* and *B. subtilis*, when applied as combined treatments, which reduced the sporulation by 90.0 and 96.0 % at 80 days after planting. Single treatments with each of *T. harzianum* and *B. subtilis* applied as induction resistance treatment or antagonists treatment reduced the sporulation more than 85.0 %.

**Effect of foliar spray with bioagents on defence enzymes activity**

Chitinase and *ß*-1,3-glucanase activities of potato plants in response to foliar spray with bioagents *viz*., *B. subtilis, P. fluorescens, , T. harzianum* and *T. viride*, were recorded. Results in Table (4) indicate that all bioagents stimulated the activities of both enzymes. The most effective treatments were the combined application between induction systemic resistance treatment and antagonists treatment. It increased the chitinase and *ß*-1,3-glucanase by the range of 87.0 to 139.0 and 78.6 to 142.9 %, respectively. High increase was also observed with individual treatments of induction systemic resistance or antagonists, which increased the chitinase and *ß*-1,3-glucanase by the range of 52.2 to 130.0 and 85.7 to 135.7 %, respectively. Foliar spray with each of *T. harzianum* and *B. subtilis*, when applied as combined treatments, increased chitinase and *ß*-1,3-glucanase activities by 117.4 &139.0 and 114.4 & 142.9 %, respectively.

**Effect of foliar spray with bioagents on tuber yield**

Results in Table (5) indicate that all treatments significantly increase the potato yield during the two

ISSN: 0975-8585

growing seasons. The most effective treatments were the combined application between induction systemic resistance treatment and antagonists treatment. The highest increase in tuber yield was obtained with each of *T. harzianum* and *B. subtilis*, when applied as combined treatments, which increase potato yield by 55.0 and 53.6 & 51.8 % during the two growing seasons respectively. Single treatments of bioagents showed moderate effect.

**Table 4: Chitinase and *β*.1, 3-glucanase activities in potato plants treated with microorganisms applied as induction of systemic resistance and as antagonists or both of them under field conditions.**

| Treatment[1] | Defence enzyme activities[2] | | | |
|---|---|---|---|---|
| | Chitinase | Increase (%) | *β*.1, 3-glucanase | Increase (%) |
| **Induction of systemic resistance treatment** | | | | |
| ***Trichoderma harzianum*** | 5.0 ab [3] | 117.4 | 2.9 b | 107.1 |
| ***Trichoderma viride*** | 4.0 b | 073.9 | 2.6 bc | 085.7 |
| ***Bacillus subtilis*** | 5.3 a | 130.0 | 3.3 a | 135.7 |
| ***Pseudomonas fluorescens*** | 4.0 b | 073.9 | 2.4 c | 071.4 |
| **Antagonists treatment** | | | | |
| ***Trichoderma harzianum*** | 4.5 b | 095.7 | 2.8 c | 100.0 |
| ***Trichoderma viride*** | 3.5 c | 052.2 | 2.7 c | 092.9 |
| ***Bacillus subtilis*** | 4.8 b | 108.7 | 3.2 a | 128.6 |
| ***Pseudomonas fluorescens*** | 4.0 bc | 073.9 | 2.4 c | 071.4 |
| **Induction of systemic resistance treatment + Antagonists treatment** | | | | |
| ***Trichoderma harzianum*** | 5.0 ab | 117.4 | 3.0 a | 114.3 |
| ***Trichoderma viride*** | 4.3 b | 087.0 | 2.5 c | 078.6 |
| ***Bacillus subtilis*** | 5.5 a | 139.0 | 3.4 a | 142.9 |
| ***Pseudomonas fluorescens*** | 4.0 b | 073.9 | 2.5 c | 078.6 |
| **Ridomile plus ( 2.0g/ L)** | 3.0 c | 030.4 | 2.0 d | 042.9 |
| **Control** | 2.3 d | - | 1.4 e | - |

1-Foliar spraying with bioagents was carried out once time when applied as induction of systemic resistance treatment at 30 days of planting and four times when applied as antagonists treatment at 60 days of planting.
2- Chitinase activity was expressed as mM N-acetyl glucose amine equivalent released/ gram fresh weight/ 60 minutes and *β*.1,3-glucanase activity was expressed as mM glucose equivalent released/ gram fresh weight/ 60 minutes.
3- Figures with the same letter are not significantly different ( P= 0.05).

**Table 5: Tuber yield of potato plants treated with microorganisms applied as induction of systemic resistance and as antagonists or both of them under field conditions.**

| Treatment[1] | Potato yield (metric ton / hectare) | | | |
|---|---|---|---|---|
| | First growing season | | Second growing season | |
| | Yield | Increase % | Yield | Increase % |
| **Induction of systemic resistance treatment** | | | | |
| ***Trichoderma harzianum*** | 32.1 bc [2] | 35.0 | 35.7 b | 33.9 |
| ***Trichoderma viride*** | 28.5 d | 20.0 | 31.4 d | 17.9 |
| ***Bacillus subtilis*** | 33.3 b | 40.0 | 33.3 c | 25.0 |
| ***Pseudomonas fluorescens*** | 29.7 d | 25.0 | 30.9 d | 16.1 |
| **Antagonists treatment** | | | | |
| ***Trichoderma harzianum*** | 31.6 bc | 33.0 | 35.9 b | 34.8 |
| ***Trichoderma viride*** | 29.0 d | 25.0 | 32.1 cd | 20.3 |
| ***Bacillus subtilis*** | 33.8 b | 42.0 | 32.1 cd | 20.3 |
| ***Pseudomonas fluorescens*** | 28.6 d | 20.0 | 29.5 e | 10.7 |
| **Induction of systemic resistance treatment + Antagonists treatment** | | | | |
| ***Trichoderma harzianum*** | 36.9 a | 55.0 | 40.9 a | 53.6 |
| ***Trichoderma viride*** | 29.8 d | 25.0 | 33.8 c | 26.8 |
| ***Bacillus subtilis*** | 36.9 a | 55.0 | 40.5 a | 51.8 |
| ***Pseudomonas fluorescens*** | 36.2 de | 25.0 | 33.8 c | 26.8 |
| **Ridomile plus ( 2.0g/ L)** | 32.1 bc | 35.0 | 33.3 c | 25.0 |
| **Control** | 23.8 e | - | 26.6 f | - |

1-Foliar spraying with bioagents was carried out once time when applied as induction of systemic resistance treatment at 30 days of planting and four times when applied as antagonists treatment at 60 days of planting.
2- Figures with the same letter are not significantly different ( P= 0.05).

ISSN: 0975-8585

## DISCUSSION

Late blight caused by *Phytophthora infestans* is the major disease problem of potato causing large economic losses. The disease usually infects leaves, stems and tubers and is responsible for infection by secondary invaders such as *Erwinaia carotovora*, *Pseudomonas solanacearum* and *Fusarium* spp. [40]. There are relatively few reports of biological control as a potentially successful alternative for management of highly destructive epidemics such as potato late blight. Two factors, probably among others, that make biocontrol difficult to this disease are rapid establishment of infection and explosive disease development. It is reasonable to assume that many attempts to use biocontrol for potato late blight have been un-successful and this may be the reason why the litirature in this field is so scarce. In the present study we investigate the efficiency of four microorganisms viz., *B. subtilis*, *P. fluorescens*, *T. harzianum* and *T. viride*, applied either as elicite systemic resistance and as antagonists or both of them against potato late blight. In the *in vitro* tests, *T. harzianum* and *B. subtilis* antagonists highly restricted the growth of the late blight pathogen by 83.3 and 84.4 %, respectively over the control in agar assays. While *T. viride* and *P. fluorescens* restricted the growth of *P. infestans* by 75.1 to 77.6 %, respectively. These findings are in harmony with those obtained by other researchers. Zegeye *et al*. [41] reported that, *in vitro* antagonism test carried out between *T. viride* and *P. infestans,* showed a radial growth inhibition of the pathogen by 36.7% and a complete overgrowth of *T. viride* on *P. infestans* later, whereas *P. fluorescens* inhibited the radial growth of the pathogen by 88%. Chowdappa *et al*. [26] found that both isolates of *B. subtilis* OTPB1 and *T. harzianum* OTPB3 inhibited mycelium growth of *A. solani* and *P. infestans* under *in vitro* conditions. Lamsal *et al*. [17] found that *Bacillus* spp. isolate AB15was the most effective, inhibiting mycelial growth of the pathogen by more than 80% *in vitro*. Yuan-Hang *et al*. [19] stated that the supernatant of these two isolates of *Trichoderma* spp. inhibited mycelium growth and sporangium germination of *P. infestans*. In the present study in field experiment, it was obseved that, the highest severity of late blight at up to 90 days of planting were found in control and the lowest was found in bioagents applications. Neither antagonistic bacteria nor antagonistic fungi appeared to give effective protection against late blight. Successful use of biological control as blight control agents depends upon the method of application. The integration between induction of systemic resistance treatment and antagonists treatment by each of bioagents showed a stronger effect in reducing the severity of late blight than the single application by each treatment. Bacteria with antagonistic activity toward *P. infestans* are found mainly in the genera *Pseudomonas* and *Bacillus* [42, 29, 15, 21]. *B. subtilis* produces three groups of lipopeptides: iturins, agrastatins/ plipastatins and surfactins that synergize each other to inhibit mycelial growth and germ tubes [43]. *Trichoderma* strains exert biocontrol against fungal phytopathogens either indirectly, by competing for nutrients and space; by modifying the environmental conditions or promoting plant growth, defensive mechanisms and antibiosis, or directly, by mechanisms such as mycoparasitism [44]. A biosurfactant, massetolide A, obtained from *Pseudomonas fluorescens*, also adversely affected zoospore behaviour [45]. One of the main modes of action of biosurfactants on zoosporic plant pathogens is the destabilisation of membranes, which causes lysis of the zoospore, concomitant with minor reduction in mycelial growth rate with no effect on the rate of sporangia production [46]. These findings has also been supported by other researchers. Arora [13] found that, a prophylactic spray of *T. viride* at concentrations of $10^8$ cfu/mL before inoculation with the pathogen, restricted development of late blight between 1.5 and 14.0 percent compared to 61.6 and 88.8 percent in control without the antagonists. Zegeye *et al*. [41] found that *T. viride* and *P. fluorescens* significantly reduced the late blight incidence compared to the untreated check. *T. viride* was found to be more efficient than *P. fluorescens* and mixed culture. Lamsal *et al*. [17] found that *Bacillus* spp. AB15was the most effective in suppressing disease by 74% and capable of enhancing different growth parameters (shoot/root length, fresh biomass, dry matter, and chlorophyll content) compared with control plants under greenhouse conditions. Yuan-Hang *et al*. [19] indicated from greenhouse and field experiments that *Trichoderma* isolates R-5 and T-15 reduced the disease incidence by 72.4% and 70.0%, respectively. In the current study, the defense related enzymes such as chitinase and *β*.1, 3-glucanase were significantly higher in bioagents treated potato plants, as compared to untreated control, which may be one of the factors accounting for significant reduction in lesion size caused by *P. infestans*. Also the average number of *P. infestans* sporangia / $cm^2$ of potato leaves was lower in bioagents treated potato plants, as compared to untreated control. *T. harzianum* and *B. subtilis* were reported to induce growth promotion and systemic resistance to many soil and seed borne foliar diseases of various vegetable crops including tomato and potato [20, 21]. This findings is also accordance with the findings of Chowdappa *et al*. (2013) [26], they found that both isolates *Bacillus subtilis* OTPB1 and *Trichoderma harzianum* OTPB3 induction of systemic resistance in tomato seedlings against early and late blight through induction of growth hormones and defense enzymes.

Alizadeh *et al*. [47] found that both *T. harzianum* and *Pseudomonas* sp., isolated from the rhizosphere of cucumber, elicit induced resistance in cucumber against *Botrytis cinerea*.

In the current study, foliar spray of all bioagents suspensions to potato plants, resulted in remarkable increase in tuber yield. Several strains of *B. subtilis* and *T. harzianum* have the ability to promote crop growth and yield, through increased uptake of nutrients stimulated by growth promoting factors such as IAA and GA3 and decreased level of ethylene owing to colonization of root [21, 48, 49, 11]. The increase in IAA and GA3 levels is one of the direct mechanisms by which biocontrol agents promote shoot and root growth and leaf area in tomato plants. These hormones are believed to further stimulate uptake of more nutrients in the soil [50], transduce signals among plant organs and integrate them to produce adequate defense responses to biotic or abiotic stresses [51]. They also colonize the plant roots, provide protection against certain soil borne fungal pathogens as well as stimulate growth and crop yield by hormonal stimulation through induction of host resistance by elicitation [48]. It could be suggested from the present study that, potato plants treated with each of *B. subtilis* and *T. harzianum*, applied as integrated management of induction of systemic resistance treatment and as antagonists treatment can retard the incidence of late blight, defense enzymes *viz.*, chitinase and *β*.1, 3-glucanase activities and increase the yield of tuber.

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