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ORIGINAL ARTICLE

Effects of *Pseudomonas putida* and Vitazyme® on growth and development of the potato tuber moth

Imad Idris*, Akram Adam, Ali Hashem, George Saour

[1] *Department of Biotechnology, Atomic Energy Commission of Syria. AECS, P. O. Box 6091 Damascus, Syria*

*E-Mail: ***scientific@aec.org.sy***

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The ability of *Pseudomonas* putida strain BTP1 and the biostimulant (Vitazyme®) to protect potato plants from the potato tuber moth *Phthorimaea operculella* (PTM) (Zeller) was investigated. Significant negative effects on survival, pupal weight, and fertility of the insect were observed between treatments and control. The results revealed that the BTP1-treated foliage had significantly the highest negative impact on PTM development and reproduction compared to other treatments. The combination of BTP1 and Vitazym® did not result in a synergistic detrimental effect on potato tuber moth reproduction. However, the biostimulant and BTP1 treatments showed the largest negative effects on PTM reproduction due to the density of hairs and trichomes on the treated foliage. Application of BTP1 and Vitazyme® could be a potential tool to reduce the use of insecticides and enhance integrated pest management against potato tuber moth.

Key words: Biostimulant, leaf hairs, plant resistance, potato tuber moth, Pseudomonas putida

The potato tuber moth (PTM) *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) is a serious insect pest that damages potato crop both in the field and during storage; it is currently present in over 90 countries in Mediterranean basin, tropical and subtropical areas due to climate change (Cho *et al*., 2021). The larvae feed on the leaves, stems, and petioles of potatoes, making tunnels through the tubers (Clough *et al*., 2010; Zhang *et al*., 2021). Chemical insecticides have been used frequently and dominantly to control *P. operculella*. However, insecticides are still a major health and environmental concern. Additionally, PTM is becoming increasingly resistant to the use of these insecticides. An organic biostimulant developed through biotechnology can improve plants' growth rates, nutrient efficiency, or impart tolerance to biotic and abiotic stressors, without having nutritional or insecticidal properties (Dara, 2021; Pereira *et al*. 2021). There are many compositions of biostimulants, but the main components are humic substances and marine algae extracts (carriers), vitamins (ascorbate, vitamins B, and a-tocopherol), C-hydrolysate, mycorrhizal fungi, and other compounds whose proportions vary from manufactures. Although originally developed for tissue culture applications, they have also shown to be effective in enhancing plant growth by increasing nutrient uptake as well as root development without heavy reliance on fertilizers (Richardson *et al*., 2004). The increased efficiency of nutrient uptake improves the health and vigor of the plant and increases production of secondary metabolites such as polyphenols (Sivaramakrishnan *et al*., 1996). Previous studies have demonstrated that mortality of neonate larvae of *P. operculella* on biostimulant-treated foliage differed significantly than those on untreated foliage (Saour, 2010). On the other hand, a very important additional factor for crop protection is the induction of systemic resistance (ISR) by non-pathogenic rhizobacteria (Racke and Sikora, 1992; Zehnder *et al*., 1997). According to several studies, plant growth-promoting rhizobacteria (PGPR) strains cause systemic resistance to several insect pests, (Zehnder *et al*., 1997; Adam *et al*., 2016; Delgado-Ramírez, 2021). In previous studies, *Pseudomonas putida* BTP1 influences the development and reproduction of grapevine phylloxera *Daktulosphaira vitifoliae* (Hemiptera: Phylloxeridae), in addition to potato tuber moth *P. operculella* in potato plants were demonstrated (Adam *et al*., 2013, Adam *et al*., 2016). Therefore, this study aims to determine whether organic biostimulants and *P. putida* strain BTP1 have negative impacts on *P. operculella* growth and development under field conditions.

MATERIALS AND METHODS

Insects

The insects used in this experiment were obtained from a laboratory stock culture, which was replenished with field collections of PTM individuals each year. As described by Saour and Makee (1997), larvae were reared in plastic containers (40 x 25 x 10 cm) on wax-coated potato tubers.

Bacterial strain and biostimulant (Vitazyme®) preparation

A strain of *Psudomonas putida*, BTP1, was originally selected due to its specific properties regarding pyoverdine-mediated iron transport (Jacques *et al*., 1995; Ongena *et al*., 2002). As described previously by Ongena *et al*., 2002 it was maintained and prepared for use in ISR assays. For the bioassays, BTP1 strains were grown on a rotary shaker at 28°C for 24 h in Erlenmeyer flasks (250 ml) containing 100 ml of Casamino Acids medium (CAA). The cells were centrifuged at 16500 g for 15 minutes at 4°C and then rinsed in sterile NaCl (5g l^{-1}). In order to obtain a bacterial suspension of 10^8 CFU ml^{-1}, the final pellet was resuspended in sufficient sterile distilled water.

Potato tubers were washed in sterile water, dipped separately in a suspension of *P. putida* strain BTP1 for 30 min, and air-dried, while control tubers were treated with sterile water.

Vitall Earth Resources (Gladewater, Texas, USA) the organic biostimulant (Vitazyme®) were used for this experiment. Among the biological stimulants included in Vitazyme® are sea algae (kelp) and fish soluble solids, natural humic acids, natural chelates, compost percolates, sequestrates, digested multifermented cereal grain extracts, vitamins, and growth regulators. The organic biostimulant-treated rows were sprayed to runoff, with a

0.75% Vitazyme solution diluted in water, directly on the seed pieces at planting. Thirty days after planting, a second application (0.75% Vitazyme solution/row) was performed to the leaves and soil.

Potato planting and design treatments

Seed tubers (commercial Draja cultivar) were planted at a depth of 8-10 cm with a spacing of 25 cm between plants and 70 cm between rows. In this trial, 500 m^2 of land was cultivated arranged in eight rows (25 m long) with two rows for each of the treatments (BTP1, Vitazym®, BTP1 with BTP1) and two rows for control, separated by an alley of 1.5 meters to prevent row interspersion. The rows contained 100 potatoes and were fertilized appropriately (120 kg K_2O, 120 kg P_2O_5, 120 kg N / h). Weekly irrigation was provided.

Approximately 800 leaves were excised from potato plants (six to seven weeks old) from the third, fully expanded leaf of similar size. To remove all impurities, the leaves were thoroughly washed with distilled water and shook to remove excess water. Each leaflet was placed individually on filter paper (Grade 1, Whatman, England), moistened with distilled water, and placed in a plastic Petri dish with an ID of 85 mm. As part of each treatment, 200 larvae (aged ≤ 24 h) were placed in 20 plastic boxes (18 x 12 x 8 cm), fed 200 leaves until they reached the pupa stage. Four days after placement, the larvae were gently prodded with a camel-hair brush; if no reaction was evident, the neonate was declared dead. Survivor larvae were carefully placed in transparent plastic boxes (4 x 3 x 2 cm), and fresh leaves were presented every four days, resealed with parafilm to prevent larvae escape.

After 7 days, the survival rate of larvae was calculated based on the number of larvae (aged ≤ 24 hours). Every tow-day-old pupa (31 pupae per treatment) was weighed and placed in a plastic tube. Based on the survival larvae rate, the pupae and adult moths' survival rates were estimated, and the mortality rate was calculated. Newly emerged females (0-18 hours) (n = 25) were paired with 1-day-old normal males in 350 ml transparent plastic boxes with a filter paper oviposition site and a 10% sucrose solution as food sources. In the experiment, males and females were kept together until death. Eggs were removed daily, counted, and left to hatch for determining fertility (percentage egg hatch). Light and relative humidity were held at 25°C and 70 %, respectively.

Forty-five days after planting, the number of hairs and trichomes per unit leaf area (1 cm^2) on the adaxial leaflet surface for and treatments and control were counted using a binocular microscope at 15x magnification (Kyowa Optical, Japan). Leaflets (n= 40 leaflet/plant) from the third-last fully expanded leaf were randomly picked from potato plants and subjected to count procedure. For each treatment, the experiment was repeated three times.

Statistical analysis

Statistical significance is defined as $P < 0.05$. Stat View statistics software (version 5.0; SAS Institute, Cary, North Carolina, USA) (Landau *et al*., 1999) was utilized to perform all statistics analyses. ANOVA-Tukey HSD test was used to determine the statistical significance of the mean of fertility females, number of hairs trichomes per unit leaf area (1 cm^2), and percentage difference between mortality and pupae weight. Schneider-Orelli (Kroschel and Koch, 1996) formula used to calculate PTM mortality parameters was introduced as follows: % Effectiveness = % Mortality - % Mortality in control/100 - Mortality in control, and

% Decrease = ((Control – Treatment) / Control) *100

RESULTS

Table 1 shows that *P. operculella* larval, pupal and adult mortality rates were highest on *P. putida* BTP1-treated potato leaves compared to other treatments (df= 4, f = 1064.177; P < 0.0001; df= 4, f = 60.162; P < 0.0001; df= 4, f = 132.195; P < 0.0001). Despite the fact that BTP1, Vitazym® and BTP1+ Vitazym® treatments significantly differ from control and fertilizer treatments, there was a synergistic effect observed in adult's mortality when the BTP1 and Vitazym® treatments were combined.

The pupal weight and female fertility differed significantly between treatments and control across all experiments (df= 4, f = 155.166; P < 0.0001; df= 4, f = 198.158; P < 0.0001) (Table 2). Results showed that BTP1 treatment negatively affected mean pupal weight and female fertility. The mean pupal weight and female fertility decreased by 21% and 19% compared to the control (8.98, 7.08 mg and 99.24, 79.56 eggs, for control and BTP1 treatments, respectively) (Table 2).

Our results show that on the adaxial leaflet surface of the potato's plants treated with BTP1, Vitazym® and BTP1+ Vitazym®, the number of hairs and trichomes density/cm^2 on potato leaflet increased and differed significantly from that of control and fertilizer. The highest increase was observed in BTP1 treatments (df= 4, f = 202.704; P < 0.0001) (Fig. 1).

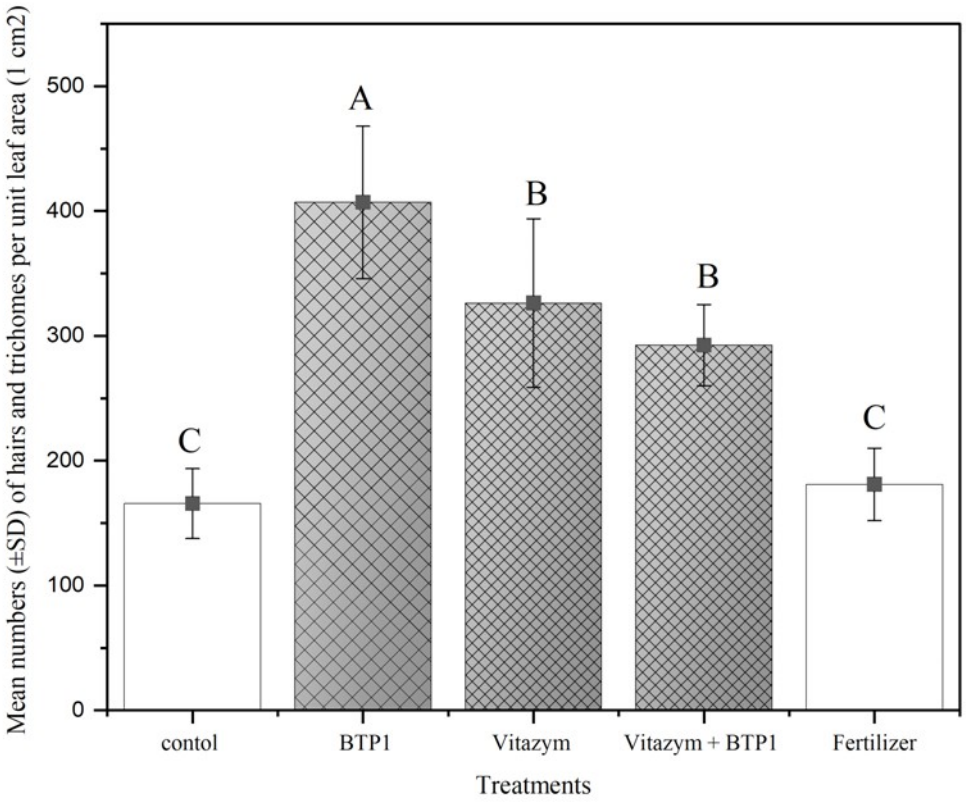

Figure. 1. Mean number (±SD) of hairs and trichomes per unit leaf area (1 cm^2) on the adaxial leaflet surface of plant potato sprayed with an organic bio- stimulant (Vitazym®) and *P. putida* BTP1under field conditions (Means followed by different capital letters are significantly different at P < 0.05 (Tukey HSD test))

Table 1: Mean percentages (±SD) of larval, nymphs, and adults mortality of potato tuber moth fed on foliage of potato plants sprayed with an organic biostimulant (Vitazym®) and *P. putida* BTP1 under field conditions

Mortality %	**control**	**BTP1**	**Vitazym®**	**Vitazym® + BTP1**	**Fertilizer**	**F- value**	***P<***
Larvae	**19.3±2.7 E**	**54.5 ± 1.7 A**	**33.5± 2.2 C**	**49.9 ± 1.6 B**	**23.35 ± 2.23 D**	**1064.74**	**0.0001**
Nymph	**30.8±2.9 B**	**43.7 ± 3.7 A**	**40.5± 3.6 A**	**41.7 ± 2.3 A**	**33.00 ± 2.7 B**	**60.162**	**0.0001**
Adults	**49.3±1.2 D**	**63.3 ± 2.4 A**	**60.2± 2.6 B**	**65.3 ± 2.4 A**	**56.5 ± 3.1 C**	**132.195**	**0.0001**

Effectiveness %

Mortality %	BTP1	Vitazym®	Vitazym® + BTP1	Fertilizer
Larvae	**35**	**14**	**30**	**4**
Nymph	**13**	**9**	**11**	**2**
Adults	**14**	**10**	**16**	**7**

Means within rows marked with the same letter are not significantly different at P < 0.05 (Tukey HSD test).

Table 2: Means (±SD) of weight nymph and female fertility of potato tuber moth fed on foliage of potato plants sprayed with an organic bio- stimulant (Vitazym®) and *P. putida* BTP1 under field conditions

Means	Control	BTP1	Vitazym®	Vitazym® + BTP1	Fertilizer	F- value	P<
Nymph weight	8.98±0.33 A	7.08±0.32 E	8.38± 0.23 D	8.58 ± 0.26 C	8.7 ± 0.34 B	155.166	0.0001
Fertility per female	99.24±3.4 A	79.56 ± 3.7 E	84.68± 3.6D	93.25 ± 4.3 C	97.23± 3.7 B	198.958	0.0001

Decrease %

Means	BTP1	Vitazym®	Vitazym® + BTP1	Fertilizer
Nymph weight	21.16	6.68	4.45	3.12
Fertility per female	19.83	14.67	6.04	2.03

Means within rows marked with the same letter are not significantly different at P < 0.05 (Tukey HSD test).

DISCUSSION

Plants treated with PGPR may have a decreased level of necessary nutrients, or they may have compounds that inhibit growth (Reese and Field, 1986; Bong and Sikorowski, 1991; Yaman *et al*.,1999). Our results are consistent with previous studies on whiteflies of tomato *Lycopersicon esculantum* plants, rice leaf rollers *Cnaphalocrocis medinalis* in rice, and American bollworms *Helicoverpa armigera* in cotton (Valenzuela-Soto *et al*., 2010; Commare, 2002; Vijayasamundeeswari *et al*., 2009).

Although *P*. *putida* strain BTP1 and the biostimulant (Vitazym®) have different mechanisms of action, they both affect potato tuber moth development (Saour, 2010; Adam *et al*., 2016). Previously, organic biostimulants were reported to improve plant health and vigor by increasing the number, thickness, and diameter of xylem cells (Berlyn and Sivaramakrishnan, 1996). Mostafa *et al*., (2021) found that bio-based stimulator compounds (BSTC) significantly reduced the infestation of *Liriomyza trifolii* on plants by closing the tunnel end, resulting in the larvae's suffocation and death.

PTM neonate larvae mortality confined to biostimulant-treated potato foliage may be due to thicker cell walls (leaf toughness), stronger vascular tissues, and hardness (due to localized amorphous silica), all of which affect the wear and tear of larvae mandibles (Zalucki *et al*., 2002; Idris *et al*., 2023). In L2, L3 and L4 larval stages, the treatment of potato tubers with BTP1 causes secondary metabolic changes in treated plant cells which elicit the production of defense compounds, and the accumulation of some toxic phenolic compounds in resistant plant cells leads to an increase in the death rate (Lattanzio *et al*., 2000; Zehnder *et al*., 2001; Arimura *et al*., 2005; Joe and Muthukumaran, 2008).

Several studies have demonstrated that foliar glandular trichomes play a significant role in protecting Solanum species from insect pests through behavioral or physical barriers (Zalucki *et al*., 2002; Horgan *et al*., 2007; Peiffer *et al*., 2009). In one study, removing glandular trichomes from wild potato foliage, *Solanum berthaultii* Hawkes, resulted in increased larval mobility, more leaf feeding, shorter larval development and larger pupae (Malakar and Tingey, 2000). In addition, factors such as light intensity, temperature, day length, and application of chemical elicitors have been reported to affect the amount of trichomes per unit area in *Lycopersicon*, *Solanum, Ulmus*, and *Madia* species (Duffey, 1986; Simmons *et al*.,2003; Boughton *et al*., 2005; Bosu and Wagner, 2014; Gonzáles *et al*., 2008). Saour (2010) reported that the higher density of hairs and trichomes on the foliage of biostimulant-treated plants is responsible for the pseudo-resistance phenomenon. However, *P. putida* strain BTP1 is able to promote induced systemic resistance (ISR) in a wide spectrum of pathosystems, including potato plants (Adam *et al*., 2016). Consequently, biostimulant and BTP1 treatments had the largest negative effects on PTM, as

their foliage contained more hairs and trichomes. Finally, our results were obtained from excised leaves experiments and further experiments in planta leaves are needed.

CONCLUSION

In this study, *P. operculella* reproduction on potato leaves treated with four treatments under field conditions was investigated. By applying Vitazym® and BTP1 separately, PTM development decreased, while plant growth and nutrient efficiency were improved. BTP1 induces resistance in potato plants against PTM more effectively than the biostimulant (Vitazym®) and the combination between BTP1 and Vitazym® did not result in a synergistic effect on PTM reproduction.

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CONFLICT OF INTERESTS

The authors declare that they have no potential conflicts of interest.

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