

Induced resistance in potato plants by a non-pathogenic *Pseudomonas putida* BTP1 against potato tuber moth (*Phthorimaea operculella* Zeller)

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Abstract: *Pseudomonas putida* strain BTP1 is able to promote induced systemic resistance (ISR) in a wide spectrum of pathosystems. In this study, we investigated induced resistance in potato plants against potato tuber moth (*Phthorimaea operculella* Zeller) by non-pathogenic *P. putida* BTP1. Several physiological indicators in the life cycle of the potato tuber moth, such as survival rate, mean weight of pupae, and sex ratio were studied to assess the protective effect of *P. putida* BTP1. Our results showed that treatment of potato tubers by bacterial suspension of *P. putida* BTP1 caused evident disturbance to the development of *P. operculella* in potato plants. Survival rate of larvae feeding on treated plant leaves and mean weight of pupae decreased significantly. In addition, a clear deviation in the sex ratio in moths, in favor of males, resulted from larvae fed on bacteria-treated plants. This study preliminarily reports the ability of BTP1 to induce resistance in potato plants against potato tuber moth. Consequently, *P. putida* strain BTP1 could be a promising approach for potato tuber moth biocontrol.

1. Introduction

The potato (*Solanum tuberosum* L.) has been considered one of the most important food crops, along with rice, wheat, and maize (Ross, 1986; Douches *et al.*, 2004). Potatoes grow in a variety of geo-environmental conditions. Developing countries cultivate potato to add nutritional balance to their food basket (Douches *et al.*, 2004; Navarre *et al.*, 2009). In Syria, more than 29,000 ha were planted with potato, producing about 609,000 t of tubers in 2005 (Alammouri, 2008). However, severe damage may occur to potato crops at storage periods particularly in developing countries. Within the Lepidoptera order, potato tuber moth *Phthorimaea operculella* (Zeller) belongs to the Gelechiidae family and it has been reported in more than 90 countries, making it a cosmopolitan pest (Visser, 2005; Golizadeh and Esmaeili, 2012). It damages potato throughout the growing season by mining stems, petioles, leaves and tubers by larvae, with the latter considered the typi-

cal damage. The procedure of potato damage begins when larvae penetrate the foliage, including leaves and stems. This insect can infest potato tubers stored and in field or it may develop on plants remaining in the field including tomatoes, aubergine or other solanaceous plants (Gilboa and Podoler, 1995; Coll *et al.*, 2000; Alvarez *et al.*, 2005). Farmers depend broadly on the use of insecticides and other varieties of farming practices (Clough *et al.*, 2008); insecticides are widely used to control this pest. However, insecticides are costly, nonselective, unfriendly to the environment, and affective for only a short period of time (Simmons *et al.*, 2006). Additionally, the phenomenon of resistance to insecticides in Lepidoptera has increased significantly (Gonzalez and Trevathan, 2001). Plant resistance, together with appropriate biological and farming practices in combination with insecticides may provide the best management options (Rondon, 2010).

Plants defeat pathogens through their active defense mechanisms that can be stimulated in some cases by plant growth-promoting rhizobacteria (PGPR) which ultimately reduce disease and render the host plant more resistant to any foreseeable pathogen attacks (Pieterse *et al.*, 2002). Induction of such enhanced defensive capacity is systemic as root

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treatment with a PGPR was shown to trigger protective effects on above-ground plant parts. These reactions are thought to typically result from the activation of latent defense mechanisms that are over-expressed upon subsequent pathogen challenge (Van Loon *et al.*, 1998; Ongena *et al.*, 2002; Bakker *et al.*, 2007). This induced systemic resistance (ISR) can be the basis for integrated plant disease management strategies (Ramamoorthy *et al.*, 2001; Zehnder *et al.*, 2001; Saravanakumar *et al.*, 2007). Induced resistance in plants by non-pathogenic rhizobacteria against pests is a very important additional factor for the protection of agricultural crops. Several studies have indicated the ability of many strains of rhizobacteria (PGPR) to induce systemic resistance against a large number of insect pests (Racke and Sikora, 1992; Zehnder *et al.*, 1997). For example, in cucumber against Striped Cucumber beetle *Acalymm avittatum* (Zehnder *et al.*, 1997, 2001), in cotton against American boll worm *Helicoverpa armigera* (Vijayasamundeeswari *et al.*, 2009), in tomato against whitefly *Bemisia tabaci* (Valenzuela-Soto *et al.*, 2010) and also in cucumber against spider mites *Tetranychus urticae* (Tomczyk, 2006). A non-pathogenic BTP1 showed enhancement of resistance level in many plants including bean, cucumber, and tomato against fungal pathogens (Ongena *et al.*, 2002, 2004; Adam *et al.*, 2008). In a previous study performed *in vitro* on grapevine rootstocks, we demonstrated the influence of *P. putida* BTP1 on reproduction and development of grapevine phylloxera (Adam *et al.*, 2013).

However, to our knowledge no studies have been performed yet to assess the effects of PGPR on *P. operculella* in potato plants. Therefore, the present work aims to demonstrate the protective effect triggered by *P. putida* strain BTP1 against *P. operculella* in potato plants. The larvae survival rate, the mean weight of pupae and the sex ratio were studied as a biometers to detect the induced resistance.

2. Materials and Methods

Establishment of the potato tuber moth colony

Insects used in the experiments were reared on waxed potato slices as described by Rahalakar *et al.* (1985). The experiments were conducted at a constant temperature of 25±1°C with 70±5% RH, and a photoperiod of 12:12 (L:D) h.

Microbial strain and inoculum preparation

Pseudomonas putida strain BTP1, isolated from barley roots, was originally selected for its specific features regarding pyoverdine-mediated iron transport (Jacques *et al.*, 1995; Ongena *et al.*, 2002). It was maintained and prepared for use in the ISR assays as previously described by Ongena *et al.* (2002). For the bioassays, BTP1 strain was grown in Erlenmeyer flasks (250 ml) containing 100 ml of Casamino Acids medium (CAA) for 24 h on a rotary shaker (150 rpm) at 28 °C. Cells were removed by centrifugation at 16500 g for 15 min at 4°C and washed in sterile NaCl (5g l⁻¹). The final pellet was resuspended in an adequate volume of sterile distilled water to obtain a bacterial suspension at 10⁸ CFU ml⁻¹.

Assays for induced resistance

“Draja” 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. The tubers were then planted in 10 L plastic pots containing autoclaved, moistened soil (three tubers/pot) to exclude any microorganism could affect BTP1. The pots were placed in a greenhouse at 25±1°C (day) and 23±1°C (night) with daylight of 16 h and relative humidity of 85-95%. Both control and treated plants were under the same watering and fertilizing conditions during the planting period.

Fresh leaves excised from potato plants (six to seven weeks old) were used for feeding the newly hatched larvae (24 h). For each treatment, 120 larvae in 10 (18 x 12 x 8 cm) plastic boxes (12 larvae/box) were fed on leaves until they reached the pupal stage. The boxes were resealed with parafilm to keep the larvae from escaping, and were then incubated at 25±1°C with daylight of 12 h and relative humidity of 70%. Each four-day-old pupae was weighed and placed separately within a small plastic tube. The pupae were classified into three groups according to their weights: small pupae (<5 mg), medium pupae (6-7 mg) and large pupae (>8 mg) to determine the larger sex. The number of pupae and the number of emerging moths (males or females) were recorded in order to calculate the survival rate of larvae and the sex ratio (the number of male/the number of female). The experiment was repeated three times.

Isolation of bacteria from potato plant leaves

Small leaf samples were taken from different parts of the potato plants treated with *P. putida*

BTP1. The samples were sterilized with sodium hypochlorite solution (5%) for 3 min and washed three times for 3 min. Samples were left to dry on sterile paper. They were then grown on Petri dishes containing the Casamino acid (CAA) medium. The dishes were incubated at $30\pm 1^\circ\text{C}$ for 72 h.

Statistical analysis

Statistical analyses were performed using STATISTIC program version 6 (Statsoft, Inc. 2003) at 5% level ($P = 0.05$). Data were subjected to analysis of variance (ANOVA) for the determination of differences between means. Differences between means of pupal weight were tested for significance using Tukey HSD test. Ratio Analysis test (Z-test) was used to compare the percentages of larval survival rate.

3. Results

Isolation of bacteria from potato plant leaves

The isolation of bacteria test on treated-potato plant leaves showed no bacterial colonies grew in the Petri dishes, indicating that *P. putida* BTP1 did not migrate through the plant (from the tubers to the leaves). There was no direct contact between bacteria and larvae.

Effect *P. putida* BTP1 on potato plants against potato tuber moth

Larvae survival rate. Induced resistance experiments showed the death of large numbers of larvae of *P. operculella* in different ages of development, particularly in *P. putida* BTP1-treated potato plants (Fig. 1.1). The larval survival rate decreased significantly (35%) when the larvae were fed on the excised leaves from *P. putida* BTP1-treated plants compared with the control plants (Fig. 1.2). Significant differences between BTP1 and control were observed in all experiments.

Effect on pupal weight. The results of three independent experiments showed that there was a negative impact on mean pupal weight in *P. putida* BTP1-treated potato plants. Where mean of pupal weight was 7.77 ± 0.12 mg in the control potato plants, it decreased significantly in *P. putida* BTP1-treated plants to 6.24 ± 0.15 mg (Fig. 2). This implies an approximately 20% weight reduction of pupae in potato plants pre-inoculated with *P. putida* BTP1 as compared with the control. Significant differences

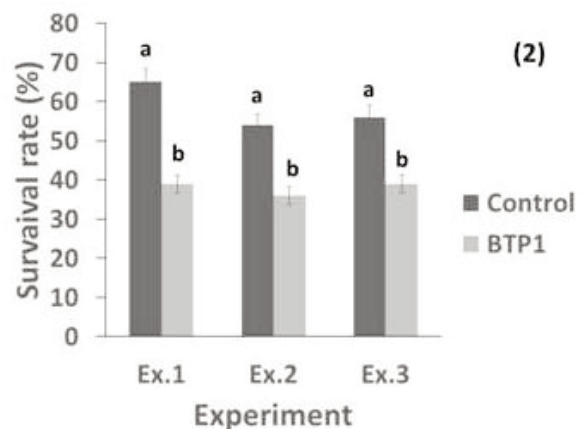
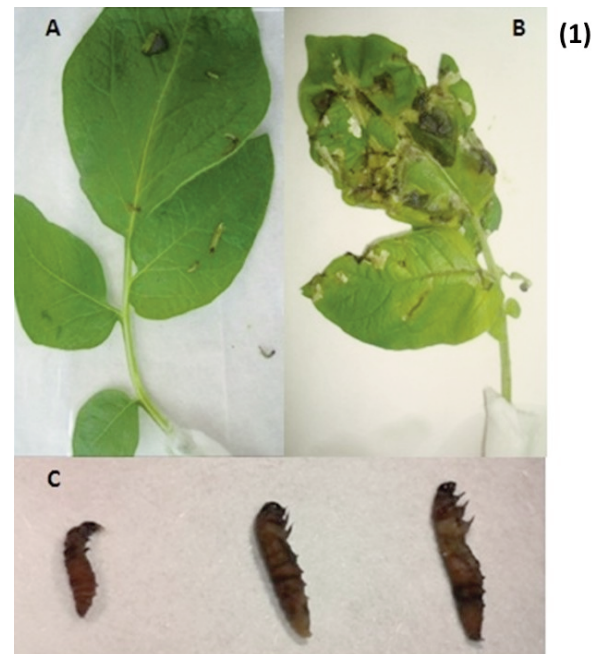


Fig. 1 - Example of potato leaves infested by potato tuber moth showing larvae feeding on *P. putida* BTP1-treated plant leaves (1A), and control plant leaves (1B). (1C): profile of the dead larvae in different stages because of malnutrition. (2): Influence of potato tuber treatment by the bacterial suspension of *P. putida* BTP1 on the survival rate of larvae of the potato tuber moth. Three separate experiments were carried out (120 larvae per treatment and per experiment were used). Data were subjected to ANOVA and the differences between means were tested for significance using Tukey HSD test (values with different letters are significantly different at $P < 0.001$).

between BTP1 and control were observed in all experiments.

With regard to pupae weight, we observed that the pupae, which were classified into three groups according to their weight (small, < 5 mg; medium, pupae 6-7 mg; large > 8 mg), in control plants were mostly large pupae (approximately 61%) while the

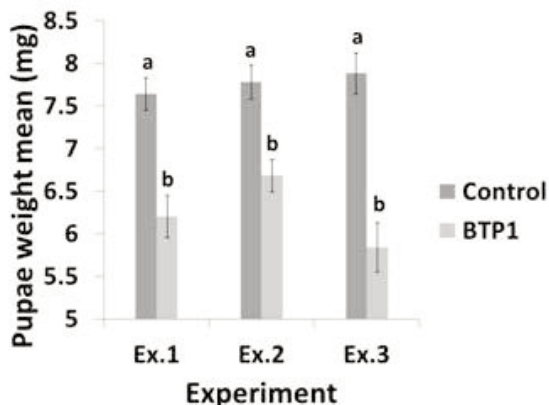


Fig. 2 - Influence of potato tuber treatment with bacterial suspension of *P. putida* BTP1 on mean of pupal weight of the potato tuber moth. Three separate experiments were carried out. Each column represents the weight mean of 25 pupae. Data were subjected to ANOVA and the differences between means were tested for significance using Tukey HSD test (values with different letters are significantly different at $P < 0.001$).

small pupae was almost absent (3%) (Fig. 3). In contrast, in BTP1-treated plants, the percentage of the large pupae decreased significantly to reach (36%), while the percentage of the small pupae increased significantly to reach (27%) (Fig. 3).

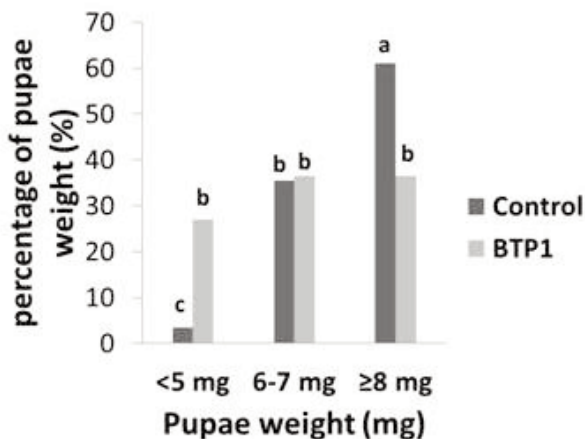


Fig. 3 - Influence of potato tuber treatment with bacterial suspension of *P. putida* BTP1 on percentages of pupal weight of the potato tuber moth (75 pupae/treatment). Data were subjected to test ratio analysis (Z-test) to determine the significant differences at $P < 0.05$ (values with different letters are significantly different).

Sex ratio of *P. operculella*

The sex ratio of moths (number of males/number of females) emerged from control (180 moths) and *P.*

putida BTP1-treated potato plants (91 moths) was calculated. A clear deviation was observed in the sex ratio in favor of males from feeding on BTP1-treated plants: 1.84:1, compared to 1:1 in control plants.

4. Discussion and Conclusions

Our study has shown that the larval survival rate and the mean of pupal weight were significantly decreased in BTP1-treated potato plants compared to control. These results are consistent with previous studies conducted on whitefly, which showed a significant decrease in survival rate (number of nymphs which are able to develop and reach the adult stage) in tomato plants treated by rhizobacteria (Valenzuela-Soto *et al.*, 2010). In addition, similar results were found in PGPR-treated cotton bolls with mortality of larval, malformation of pupal and adult with decreased adult emergence of American bollworm *H. armigera* (Vijayasamundeeswari *et al.*, 2009). Moreover, changes in dietary behavior of the rice leaf roller (*Cnaphalocrocis medinalis*) was observed, and there was a decrease in the larval and pupal weight in treated rice leaves by rhizobacteria (Radjacommare, 2002). The reduction of larval survival rate and pupal weight in BTP1-treated potato could be attributed to the inability of larvae to feed on treated plant leaves. It is well known that the growth of phytophagous larvae is affected indirectly by chemical or physical conditions or even both which characterize their host plants. For instance, PGPR-treated plants may have a decrease in essential nutrients or have compounds that inhibit growth, or both (Reese and Field, 1986; Bong and Sikowski, 1991; Yaman *et al.*, 1999).

On the other hand, treatment of potato tubers with *P. putida* BTP1 also caused a clear deviation in sex ratio in favor of males. Quezada-Garcia *et al.* (2014) proved that nutritional variation causes differential mortality to the larger sex and the most sensitive to nutritional stress (female) in spruce budworm (*Choristo neural fumiferana* (Clemens); Lepidoptera). In contrast, House *et al.* (2011) demonstrated that offspring mortality in the dung beetle (*Onthopha gustaurus*; Coleoptera) vitally depends on the amount of resources that females have provisionally. In addition, they also showed that males have greater nutritional demands than females during development, which ultimately leads to higher mortality in the male population (the larger sex and the

most sensitive to nutritional stress) (House et al., 2011; Quezada-Garcia et al., 2014). These findings are consistent with our results which showed that females were larger (16.74%) than males.

In conclusion, understanding the mechanisms of induced defense by *P. putida* BTP1 is very important to enhance the resistance in potato plants. The current study provides evidence that *P. putida* strain BTP1 has a protective effect in potato plants against potato tuber moth. Based on similar studies that illustrated that the accumulation of some toxic phenolic compounds in the cells of resistant plants led to an increase in the death rate in insects, we believe that the treatment of potato tubers with *P. Putida* BTP1 leads to secondary metabolic changes in treated plant cells which elicit the production of defense compounds (Lattanzio et al., 2000; Zehnder et al., 2001; Arimura et al., 2005; Melvin and Muthukumar, 2008).

This study preliminarily reports the ability of *P. putida* strain BTP1 to induce resistance in potato plants against potato tuber moth. This bacterial strain could be a promising agent for potato tuber moth biocontrol. However, the controlled environment (plastic pots, sterile soil, and humidity) may lead to different results compared to farm applications due to competitors, T/HR condition, dispersion of inoculum, etc. Consequently, more research is needed to determine the mechanisms of defense induced in potato plants.

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References

ADAM A., IDRIS I., AYYOUBI Z., 2013 - In vitro *Pseudomonas putida* BTP1-induced systemic resistance in grapevine rootstocks against phylloxera (*Daktulosphaera vitifoliae*). - Adv. Hort. Sci., 27(4): 137-142.

ADAM A., ONGENA M., DUBY F., DOMMES J., THONART P., 2008 - Systemic resistance and lipooxygenase-related defence response induced in tomato by *Pseudomonas putida* strain BTP1. - BMC Plant Biology, 8: 113.

ALAMMOURI N., 2008 - Agricultural commodity outlook of Syrian potato. - Ministry of Agriculture and Agrarian Reform, NAPC National agricultural policy center, Syria, pp. 23.

ALVAREZ J.M., DOTSETH E., NOLTE P., 2005 - Potato tuberworm a threat for Idaho potatoes. - University of Idaho, College of Agricultural and Life Sciences, Moscow, ID, USA, CIS 1125: 1-4.

ARIMURA G.-I., KOST C., BOLAND W., 2005 - Herbivore-induced, indirect plant defences. - Biochim. Biophys. Acta, 1734(2): 91-111.

BAKKER P.A.H.M., PIETERSE C.M.J., VAN LOON L.C., 2007 - Induced Systemic resistance by fluorescent *Pseudomonas* spp. - Phytopathology, 97(2): 239-243.

BONG C.F.J., SIKOROWSKI P.P., 1991 - Effects of cytoplasmic polyhedrosis virus and bacterial contamination on growth and development of corn earworm, *Heliothis zea* (Boddie). - Journal of Invertebrate Pathology, 57: 406-412.

CLOUGH G., DEBANO S., RONDON S., DAVID N., HAMM P., 2008 - Use of cultural and chemical practices to reduce tuber damage from the potato tuberworm in the Columbia Basin. - HortScience, 43: 1159-1160.

COLL M., GAVISH S., DORI I., 2000 - Population biology of the potato tuber moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae) in two potato cropping systems in Israel. - Bul. Entomol. Res., 90: 309-315.

DOUCHES D.S., PETT W., SANTOS F., COOMBS J., GRAFIUS E., LI W., METRY E.A., NASR EL-DIN T., MADKOUR M., 2004 - Field and storage testing Bt potatoes for resistance to potato tuberworm (Lepidoptera: Gelechiidae). - J. Econ. Entomol., 97: 1425-1431.

GILBOA S., PODOLER H., 1995 - Presence-absence sequential sampling for potato tuberworm (Lepidoptera: Gelechiidae) on processing tomatoes: selection of sample sites according to predictable seasonal trends. - J. Econ. Entomol., 88: 1332-1336.

GOLIZADEH A., ESMAEILI N., 2012 - Comparative life history and fecundity of *Phthorimaea operculella* (Lepidoptera: Gelechiidae) on leaves and tubers of different potato cultivars. - J. Econ. Entomol., 105: 1809-1815.

GONZALEZ M.S., TREVATHAN L.E., 2001 - Effect of biological and chemical seed treatment on seedling disease of soft red winter wheat in Mississippi. - Seed Sci. Technol., 29(2): 413-427.

HOUSE C.M., SIMMONS L.W., KOTIAHO J.S., TOMKINS J.L., HUNT J., 2011 - Sex ratio bias in the dung beetle *Onthophagus taurus*: adaptive allocation or sex-specific offspring mortality? - Evol. Ecol., 25: 363-372.

JACQUES P., ONGENA M., GWOSE I., SEINSCH D., SCHRODER H., DELFOSSE P., THONART P., TARAZ K., BUDZIKIEWICZ H., 1995 - Structure and characterization of isopyoverdin from *Pseudomonas putida* BTP1 and its relation to the biogenetic pathway leading to pyoverdines. - Z. Naturforsch, 50: 622-629.

- LATTANZIO V., ARPAIA S., CARDINALI A., VENERE D.D., LINSALATA V., 2000 - *Role of endogenous flavonoids in resistance mechanism of vigna to Aphids*. - J. Agric. Food Chem., 48(11): 5316-5320.
- MELVIN J.M., MUTHUKUMARAN N., 2008 - *Role of Certain Elicitors on the Chemical Induction of Resistance in Tomato against the Leaf Caterpillar Spodoptera litura Fab.* - Not. Bot. Hort. Agrobot. Cluj, 36(2): 71-75.
- NAVARRE D.A., GOYER A., SHAKYA R., 2009 - *Nutritional value of potatoes: vitamin, phytonutrient and mineral content*, pp. 395-324. - In: SINGH J., and L. KAUR (eds.) *Advances in potato chemistry and technology*. Elsevier Inc., Amsterdam, The Netherlands, pp. 508.
- ONGENA M., DUBY F., ROSSIGNOL F., FAUCONNIER M.L., DOMMES J., THONART P., 2004 - *Stimulation of the lipoxygenase pathway is associated with systemic resistance induced in bean by a nonpathogenic Pseudomonas strain*. - Mol. Plant Microbe Interact., 17(9): 1009-1018.
- ONGENA M., GIGER A., JACQUES P., DOMMES J., THONART P., 2002 - *Study of bacterial determinants involved in the induction of systemic resistance in bean by Pseudomonas putida BTP1*. - Eur. J. Plant Pathol., 108(3): 187-196.
- PIETERSE C.M.J., VAN WEES S.C.M., TON J., VAN PELT J.A., VAN LOON L.C., 2002 - *Signaling in rhizobacteria-induced systemic resistance in Arabidopsis thaliana*. - Plant Biology, 4(5): 535-544.
- QUEZADA-GARCIA R., PURESWARAN D., BAUCE E., 2014 - *Nutritional stress causes male-biased sex ratios in eastern spruce budworm (Lepidoptera: Tortricidae)*. - Can. Entomol., 146: 219-223.
- RACKE J., SIKORA R.A., 1992 - *Isolation, formulation and antagonistic activity of rhizobacteria towards the potato cyst nematode, Globodera pallida*. - Soil Biol. Biochem., 24: 521-526.
- RADJACOMMARE R., NANDAKUMAR R., KANDAN A., SURESH S., BHARATHI M., RAGUCHANDER T., SAMIYAPPAN R., 2002 - *Pseudomonas fluorescens based bioformulation for the management of sheath blight and leafhopper in rice*. - Crop Prot., 21: 671-677.
- RAHALAKAR G.W., HARWALKAR M.R., RANANAVARE H.D., TAMHANKAR A.J., SHANTHRAM K., 1985 - *Rhynchophorus ferrugineus*, pp. 279-286. - In: SINGH P., and R.F. MOORE (eds.) *Handbook of insect rearing*. Elsevier, New York, USA, pp. 514.
- RAMAMOORTHY V., VISWANATHAN R., RAGUCHANDER T., PRAKASAM V., SAMIYAPPAN R., 2001 - *Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases*. - Crop Prot., 20(1): 1-11.
- REESE J.C., FIELD M.D., 1986 - *Defense against insect attack in susceptible plants: Black cutworm (Lepidoptera: Noctuidae) growth on corn seedlings and artificial diet*. - Ann. Entomol. Soc. Am., 79(2): 372-376.
- RONDON S.I., 2010 - *The potato tuberworm: A literature review of Its biology, ecology, and control*. - Am. J. Potato Res., 87: 149-166.
- ROSS H., 1986 - *Potato breeding. Problems and perspectives*. - Verlag Paul Parey, Berlin, Germany, 123.
- SARAVANAKUMAR D., MUTHUMEENA K., LAVANYA N., SURESH S., RAJENDRAN L., RAGUCHANDER T., SAMIYAPPAN R., 2007 - *Pseudomonas-induced defence molecules in rice plants against leafhopper (Cnaphalocrocis medinalis) pest*. - Pest Manag. Sci., 63(7): 714-721.
- SIMMONS A.T., NICOL H.I., GURR G.M., 2006 - *Resistance of wild lycopersion species to the potato moth Phthorimaea operculella (Zeller) (Lepidoptera: Gelechiidae)*. - Aust. J. Entomol., 45(1): 81-86.
- TOMCZYK A., 2006 - *Increasing cucumber resistance to spider mites by biotic plant resistance inducers*. - Biological Lett., 43(2): 381-387.
- VALENZUELA-SOTO J.H., ESTRADA-HERNANDEZ M.G., IBARRA-LACLETTE E., DELANO-FRIER J.P., 2010 - *Inoculation of tomato plants (Solanum lycopersicum) with growth-promoting Bacillus subtilis retards whitefly Bemisia tabaci development*. - Planta, 231: 397-410.
- VAN LOON L., BAKKER P., PIETERSE C., 1998 - *Systemic resistance induced by rhizosphere bacteria*. - Annu. Rev. Phytopatol., 36: 453-483.
- VIJAYASAMUNDEESWARI A., LADHALAKSHMI D., SANKARALINGAM A., SAMIYAPPAN R., 2009 - *Plant growth promoting rhizobacteria of cotton affecting the developmental stages of Helicoverpa armigera*. - J. Plant Res., 49(3): 239-243.
- VISSER D., 2005 - *Guide to potato pests and their natural enemies in South Africa*. - Arc- Roodeplaat Vegetable and Ornamental Plant Institute, Pretoria, South Africa.
- YAMAN M., DEMIRBAG Z., BELDUZ A.O., 1999 - *Investigations on the bacterial flora as a potential biocontrol agent of chestnut weevil, Curculio elephas (Coleoptera: Curculionidae) in Turkey*. - African Journal of Mycology Biotechnology, 54: 625-630.
- ZEHNDER G., KLOEPPER J., YAO C., WEI G., 1997 - *Induction of systemic resistance in cucumber against cucumber beetles (Coleoptera: Chrysomelidae) by plant growth-promoting rhizobacteria*. - J. Econ. Entomol., 90(2): 391-396.
- ZEHNDER G.W., MURPHY J.F., SIKORA E.J., KLOEPPER J.W., 2001 - *Application of rhizobacteria for induced resistance*. - Eur. J. Plant Pathol., 107(1): 39-50.