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Endophytic Colonization of *Solanum Tuberosum* L. (Solanales: Solanaceae) Plants can Affect the Infestation of Serious Pests

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Abstract: The present study investigates the effects of the fungal entomopathogens *Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea*, following their endophytic colonization of the *Solanum tuberosum* L plants. Our results showed the studied fungal species isolates had no effects in promoting or suppressing the growth of *S. tuberosum* potato but affect the infestation of the serious pests.

***Keywords*:** endophytic entomopathogenic fungi, potato plants, *Beauveria, Metarhizium, Isaria*, serious pests

1. Introduction

In recent years, many studies have concerned the endophytic entomopathogenic fungi for their insect-control, insect-repellent [21][31] or entomopathogenic potentials [4][5][33][34]. Aside from the potential benefits to plant growth, endophytic entomopathogenic fungi may cause increased rates of infection and mortality among feeding insect pests and may also up-regulate systemic plant defenses. Endophytes are microorganisms that spend at least part of their lives in a non-parasitic association with plants [31]. There is accumulated evidence that many entomopathogenic fungi go through an endophytic phase in several plant species. Usually, this endophytic relationship reinforces plants with insecticidal or insect repellent traits, a characteristic that could be exploited for designing environmentally friendly applications for insect control in agriculture. Fungal entomopathogens such as *Beauveria bassiana Balsamo* (Vuillemin) (Hypocreales: Cordycipitaceae), *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) and *Isaria fumosorosea* (Wize) Brown & Smith (Hypocreales: Clavicipitaceae), play important roles in the regulation of insect populations [6][21][31][34]. It has been suggested that entomopathogenic endophytes may be a promising substitute for chemical insecticides and transgenic plants [14]. Moreover, the symbiosis of endophytes with the host plants usually carries no symptoms and, what is more, causes the plant to modify its response to environmental changes [1][7][15][35].

In this light, the present work, whose has the objective is to document the endophytic colonization to of the potato plant. It is a new approach to controlling devastating pests especially considering that insect pests have grown resistant to chemical pesticides, that certain groups of insecticides have been banned and that the public asks for non-chemical plant protection methods. There is, therefore, an increasing interest in the use of endophytic entomopathogenic fungi in biological plant protection.

2. Materials and Methods

2.1 Experimental materials

Local fungal strains of *B. bassiana* (strain H20), *I. fumosorosea* (starin IIBS) and *M. anisopliae* (strain II54) from the Achaia region, Greece, were used. The isolates were kept in Petri dishes on the nutrient SDA medium (Sabouraud Dextrose Agar, OXOID Ltd., Basingstoke Hampshire, U.K.) and were renewed every month [8]. The Petri dishes were kept in continuous darkness, at 25 ± 1 °C and 85 ± 5% relative humidity, to enable the incubation of the fungi. The developed fungi were isolated again to avoid infestation and to achieve clear Conidia were harvested by scraping the surface of the Petri dishes with a sterilized scalpel and by flooding the dishes with a sterile liquid solution of 0.1% Tween 80 (20 mL per plate).

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The conidial suspensions were stirred using a magnetic stirrer (Bande Stirrers magnetic stirrer MS300, Bante Instruments Inc., Sugar land, TX 77479, USA) and filtered twice using a sterile cloth. Suspensions were adjusted according using a Neubauer hemocytometer (TIEFE 0. 100 mm 1/400 9 mm). Conidia suspensions prepared which contained 1×10^8 conidia/mL. The viability of conidia was determined after 24 h. The germination test was run for every stock suspension in order to ensure the constancy of the viability assessments. The average viability of conidia was for *I. fumosorosea* 98.7%, *M. anisopliae* 99.2% and *B. bassiana* 96.9%. Preparation of conidial suspensions and conidial germination took place in a laminar flow chamber (Equip Vertical Air Laminar Flow Cabinet Clean Bench, Mechanical Application Ltd. Athens, Greece).

The potato plants we used, were pre-germinated in 2×2cm pots (one seed per pot at a depth of about 1cm) with Pindstrum plus peat substrate and they were then transplanted into three-liter pots with Pindstrum plus type peat substrate (Fig. 1). Sterile sprayers were used for each entomopathogenic microorganism. After spraying with the fungal suspensions, the plants were covered for 24 hours with large diameter black bags to maintain high moisture on the surface of the plant [19][20]. The experiments were performed during a period of approximately three months and the duration was 21 days each. The natural infestations were checked every 15 days.

Figure 1. Potato plants with endophytic entomopathogenic fungi

2.2 Investigate procedure for endophytic stage of the entomopathogenic fungi

To investigate the presence of endophytic stage entomopathogenic fungi, randomized *S. tuberosum*. Samples of *S. tuberosum* leaves were cut into 1cm diameter and 0.5cm thick discs in a laminar flow chamber. The samples were surface sterilized by immersion in 96% ethanol solution for one minute, in 6% sodium hypochlorite solution for five minutes and finally, in 96% ethanol solution for thirty seconds [19][20]. Then, sterile leaf samples were inoculated into SDA substrate using a sterile metal hook. The cultures on the SDA substrate samples were incubated in the dark at 25°C ± 2 and 80% humidity. Control of growth lasted fourteen days. The germination of fungal conidia on the potato leaves was evaluated using an optical microscope (40x). The number of leaves with fungal growth was calculated using the following formula: number of potato leaves that presented fungal growth / total number of samples [19][20][28]. For each fungus, eight samples from different areas of the leaf were taken, which were then placed on the SDA growth substrate and grown in the dark at 25°C ± 2 and in 80% humidity. At the end of the experiment, measured the height of the potato plants (distance from the ground to the apical part of the stem) and the number of leaves.

2.3 Identification and classification procedure of insects at the experimental cultivation of potato

The identification and classification of insects found to infect the experimental *S. tuberosum* plant organisms was carried out at the Patras Plant Protection Department. Insect identification and classification was made using a stereomicroscope ZEISS Stemi 508 (Carl Zeiss Microscopy GmbH, Jena, Germany) at 2x magnification. The genitalia slides of the Lepidoptera were examined using a stereomicroscope ZEISS Stemi 508 (Carl Zeiss Microscopy GmbH, Jena, Germany) and images were taken with a Nikon D-90 camera.

2.4 Statistical analysis

All statistical analyses were conducted using the SPSS v.23 (IBM-SPSS Statistics, Armonk, NY, USA). For growth measurements, colonization percentage one-way ANOVA was performed. Bonferroni's post-hoc test was used to compare means of treatments.

3. Results

The entomopathogenic fungi were isolated from potato leaves once again. Mycelium began to appear 6 days later and had developed completely after 11 days at 25°C ± 2 and at 95% of humidity. Successful re-isolations of the three fungi were obtained from both leaves and stems of plants from corresponding treatments. Decline of colonization was observed after 14 days, in leaves of plants treated with endophytes (F = 3.156, df = 2. 220, P = 0.133).

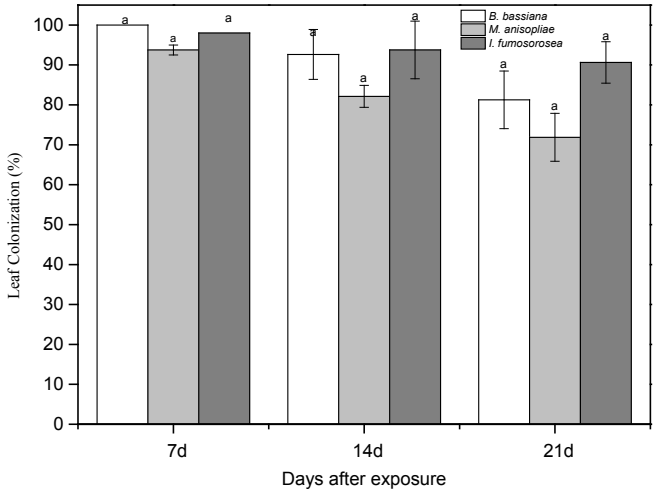

Figure 2. Mean (±sd; n=24) colonization of potato leaf parts by *B. bassiana, M. anisopliae and I. fumosorosea* at 7 days, 14 days, and 21 days after exposure. Mean ± sd values with the same superscript letter are not different in a significant way (Bonferroni's test: P<0.05)

The growth of potato leaves ranged from 20cm (*I. fumosorosea, B. bassiana*) to 22cm (Control) (Figure 5) and the plant's height, from 18.9cm (*M. anisopliae*) to 26cm (*B.bassiana*) (Figure 3). From the above results, there appear to be statistically significant differences between plant heights (F = 4.902, df = 3.140, P = 0.024) while there are no statistically significant differences in the number of leaves between colonized plants (F = 1.759, df = 3.140, P = 0.670).

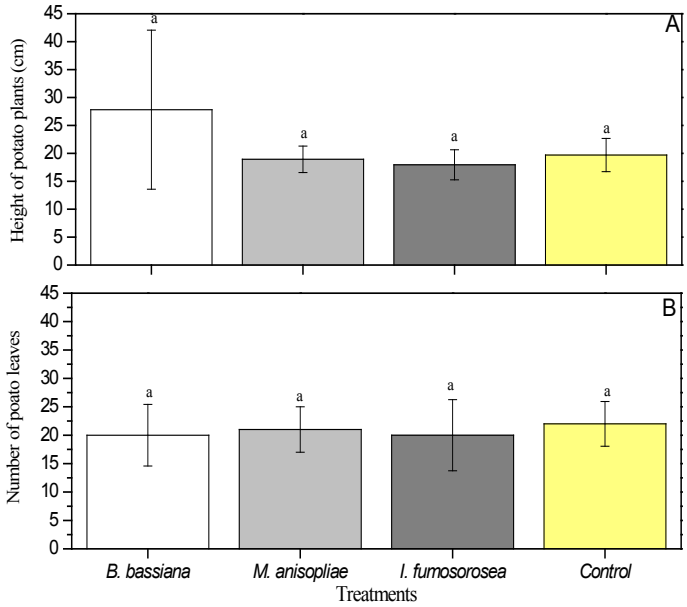

Figure 3. *S. tuberosum* growth in terms of A) height of potato plants and B) number of potato leaves. Mean ± sd values with the same superscript letter are not different in a significant way (Bonferroni's test: P<0.05)

The average number of pests per potato plant sprayed with the entomopathogenic fungi *B. bassiana* in four of the five samples the larvae of *T. vaporarorium* were found, the maximum mean number of larvae was recorded at the 4th sampling (20 larvae per plant). Also, larvae of *P. operculla* were found in four of the five samples, the maximum mean number of larvae being recorded in the 4th sampling (2.33 larvae per plant). The average number of enemies per potato plant sprayed with the entomopathogenic fungi *M. anisopliae.* Specifically, in all the samples, *T. vaporarorium* nymphs were

found, the maximum mean number of nymphs of the hemisphere was recorded at the 4th sampling (25 nymphs per plant). The average number of pests per potato plant sprayed with the entomopathogenic fungi *I. fumosorosea* in two of the five samples the larvae of *T. vaporarorium* were found, the maximum mean number of larvae was recorded at the 5th sampling (24 larvae per plant). Also, larvae of P. operculla were found in two of the five samples, the maximum average number of larvae being recorded in the 4th sampling (1.67 larvae per plant). Finally, the average number of insects per plant in the control were found to contain *T. vaporarorium* the maximum average number being recorded at the 5th sampling (67 larvae per plant). Also, larvae of *P. operculla* were establish in four of the five samples, the maximum mean number of larvae being recorded in the 3rd and 5th sampling (36 larvae per plant). Aphids of *M. euphorbiae* species were recorded. The maximum mean number of aphid larvae was for *M. euphorbiae* 13 larvae per plant at 3rd sampling. Potato plants were naturally infected by insects during the experiment.

Insects were identified as:

1. *Phthorinaea operculla* (Fig. 4)

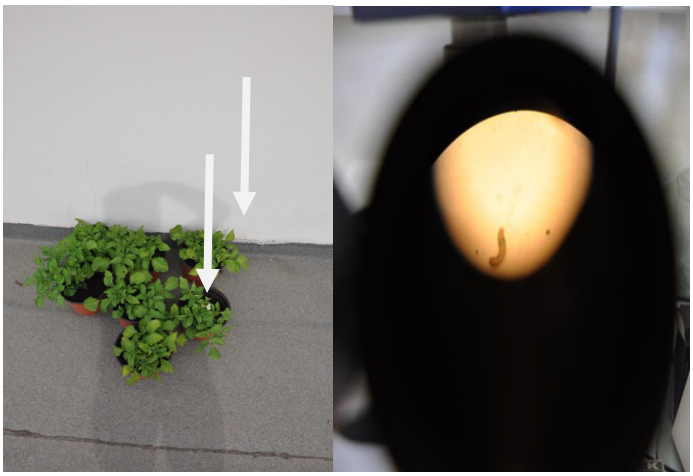

Figure 4. Potato plants infested by *Phthorinaea operculla* in natural conditions (left) and insect larvae during stereoscopic identification (right)

2. *Trialeurodes vaporariorum* (Fig. 5):

Figure 5. Nymphs of *Trialeurodes vaporariorum* hemiptera in natural infestation of potato plants

Figure 6. ***Macrosiphum euphorbiae*** **aphids in natural infestation of potato plants**

4. Discussion

Following research on different kinds of grass, endophytes seem to negatively affect herbivores through an number of mechanisms, ranging from antixenosis and/or antibiosis to the plant generating secondary compounds and/or to the endophytes producing secondary metabolites [14][19][20][31]. Infection by endophytes is conditional upon the genetic and environmental make-up of the insect population [3][30][32]. The host plant odor or taste come from nutrients and odd compounds that are transformed into complex sensorial inputs in herbivore insects [16][27]. These inputs are interpreted by the insect's central nervous system to determine whether a given plant is a suitable host [12][13][18][20].

Established endophytes should not normally influence the physiology and growth of the plant [9][29]. Occasionally, endophytes may enhance host resistance to stressful environmental conditions [35], such as drought and lack of nutrients [15], or strengthen host defense against biotic threats [1][2][14]. Endophytic entomopathogenic fungi may increase plant growth in a way that allows plants to better tolerate insect herbivory and compensate for lost biomass [21]. Posada and Vega [25] found that the presence of entomopathogenic fungi had a positive impact on all growth parameters of coffee seedlings, whereas [11][22][23][35] observed that *B. bassiana* in tomato and cotton plants contributed to a substantial increase in the height of these crops. Castillo Lopez and Sword [17] noted that inoculation with *B. bassiana* and *Purpureocillium lilacinum* (Thom) Samson (Hypocreales: Ophiocordycipitaceae) produced an enhancement of certain growth parameters in cotton plants, such as dry weight and size of the reproductive structures. Greenfield et al. [10]observed increased growth of cassava plants, post inoculation with *B. bassiana* and *M. anisopliae*. Qayyum et al. [26] who inoculated two different strains of *B. bassiana* in tomato plants noted that one strain favored plant growth, while the other delayed plant growth and development and caused a reduction in the size of the fruits. *B. bassiana* benefited all growth parameters of *Glycine max* (L.) Merr. [29]. There was a decrease in the number of *P. operculla* larvae compared to control plants as well as for *T. vaporarorium* in potato cultivation. Our results indicate that the three fungal isolates used in this study had no role in suppressing or promoting the growth of *S. tuberosum.*

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