SOIL BEHAVIOUR OF SOME INDIGENOUS *BEAUVERIA* sp. STRAINS

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Abstract: This research was aimed to study the effect of organic matter content of soil on the *Beauveria* sp. persistence and distribution. Knowing that the behavior of conidia is not only dependent on the type of soil, but also on particularities of the fungal strains themselves (there are differences in fungal behavior between species and even strains), there were tested some indigenous *Beauveria* sp., who's potential for long-term control of soil-dwelling pests is important to be known. In soil enriched with organic fertilizers, conidia vertical movement of strains was measured by recovery of colony-forming units (CFUs). In laboratory experiments it was found that the vertical movement of *B. bassiana* conidia was higher in fertilized soil, where it was registered 74% average CFUs recovery, compared to 68% in the unfertilized soil. *B. brongniartii* viability tests showed 86% average percentage of conidia germination, with a maximum value (91%) in the case of conidia isolated from humus and a minimum value (82%) when conidia was isolated from compost.

Keywords: *Beauveria bassiana, B. brongniartii, persistence, vertical movement*

INTRODUCTION

Fungi are the largest group of microorganisms colonizing the soil environment after bacteria (Tkaczuk et al., 2012). The soil environment is usually the conventional isolation site for entomopathogenic fungi (Keller & Zimmerman, 1989; Hajek, 1997) and several species can be found in both cultivated and natural habitats (Steenberg, 1995; Vanninen, 1996; Bidochka et al., 1998; Klingen et al., 2002, 2003; Meyling & Eilenberg, 2006). Entomopathogenic fungi are among the natural enemies of pests in agroecosystems and the fungi are candidates for conservation biological control in temperate regions. Conservation biological control is a biological control strategy in which farming practices and environmental manipulations are adopted to enhance the living conditions for specific natural enemies of pests (Meyling & Eilenberg, 2007).

The entomopathogenic fungus *Beauveria* sp. is well adapted for soil survival, either as conidia or saprophytic mycelia (Gottwald & Tedders, 1984). The soil provides protection against the harmful effects of extreme temperatures and solar radiation, ensuring longer persistence of *Beauveria* sp. than epigenous habitats. The fungus survives in the soil during periods of adverse environmental conditions or periods of low host densities.

Studies on *Beauveria* behavior in soil focused on the manner in which persistence and long-term efficacy of fungal propagules are affected by different factors in the soil environment: soil type, moisture conditions, temperature, antagonistic organisms (Vanninen et al., 2000), nutritional factors (Jaronski, 2010), direct solar radiation (Gardner et al. 1977), water infiltration value of soil (Storey & Gardner, 1988), rainfall and crop residue (Bruck & Lewis, 2002); intrinsic features (species and strain), cultural conditions, harvest, storage method and formulation of the

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MPCA; the presence and action of organisms that feed on fungi, agricultural practice, including tillage, use of chemical pesticides and fertilizers can also influence the fungal persistence (Scheepmaker & Butt, 2010). There are studies proving that the percentage of viable conidia recovery in soil is influenced by the season and by the conditions (laboratory or field) of carrying out tests (Müller-Kögler & Zimmermann, 1986).

Considering that the use of entomopathogenic fungal inoculants implies their application into the soil, the investigation of environmental competences related to their potential to move into soil is of major importance (Jackson & O'Callahan, 1997; Jackson et al., 2000). It is estimated that lack of studies on the fungal persistence and movement in the field can be considered as a major impediment to their development and regulation in Europe (Swiergiel et al., 2015).

This research was aimed to study the effect of organic matter content of soil on the *Beauveria* sp. persistence and distribution. Knowing that the behavior of conidia is not only dependent on the type of soil, but also on particularities of the fungal strains themselves (there are differences in fungal behavior between species and even strains), there were tested some indigenous *Beauveria* sp., whose potential for long-term control of soil-dwelling pests is important to be known. In soil enriched with organic fertilizers, conidia vertical movement and persistence of strains was measured by recovery of colony-forming units (CFUs).

MATERIALS AND METHODS

Laboratory tests of *Beauveria bassiana* conidial suspensions in soil enriched with pig manure fertilizer

Conidia suspension (4.4×10^2 conidia/ml) was prepared from *B. bassiana* (local strain BbS107) from RDIPP collection of entomopathogenic microorganisms (Andrei et al., 2012). The experiment was conducted in pots of vegetation with a capacity of 10L containing soil enriched with pig manure fertilizer, maintained at constant humidity of 60% of the field capacity. The pig manure fertilizer was applied at corresponding dose of 60 t/ha and had the following physicochemical properties:

- Soluble forms (aqueous extract 1:5) - 34.7% moisture, 37.1% organic matter, pH 7.6, $N\text{-}NH_4$ (ppm 154.35), $N\text{-}NO_3$ (ppm 260), HCO_3 (ppm 2.145), Cl^- (ppm 1240.9), Na^+ (ppm 155), K^+ (ppm 2.500), Ca^{++} (ppm 100), Mg^{++} (ppm 87).
- Total forms of macro- and microelements – (%): N 1.34 , P 0.18 , K 0.25, Na 0.075, Ca 0.55, Mg 0.67; (ppm): Zn 439.2 , Cu 55, Mn 475, Pb 45, Cd 1, Cr 50, CO 20.

Two variants were tested: (I) unfertilized soil treated with *B. bassiana* conidial suspensions, and (II) fertilized soil treated with *B. bassiana* conidial suspensions.

Soil sampling was performed each thirty days during four months, after conidia application (T_0-T_{120}). Soil was sampled using a sampling tube, at selected depths: 0-5cm, and 5-10cm respectively. The isolation of *Beauveria* conidia, 1g of soil was diluted in 200ml sterile water and stirred for 15 minutes. After 10 minutes of settle for particle deposition the operation was repeated three times, the remaining particles were dispersed finally in sterilized water. Decimal dilutions were made to get about 25 fungal colonies; the soil suspension was plated on PDA media. Colony forming units were counted after 3 weeks of incubation at 26 ± 1ºC, in the dark.

Laboratory tests of *Beauveria brongniartii* granulated formulation on organic fertilizers

A patented Romanian *B. brongniartii* strain (Fătu et al., 2014), BbgMm1a/09, was used in the tests. The *B. brongniartii* strain was cultivated in culture bags, each containing 500g of barley and 400ml distilled water (54 days, 25ºC, dark), resulting experimental granulated bioinsecticide.

Four days after sterilization, the organic fertilizers were inoculated with 10g of bioinsecticide (~3.4 x 10^{10} CFUs/g bioinsecticide) for each bag. Prior to inoculation, five grams of substrate were used to determine ph and moisture, estimated after heating at 105ºC until constant weight (Table 1).

Tabel 1. Moisture and pH values of experimental organic substrates

Native soil		Compost	Manure	Humus
Water content (%)				
R.1	6.6	30	24.1	27.6
R.2	6.9	32.4	30.2	31.0
R.3	6.1	40.0	27.6	27
R.4	14.5	38.1	31.4	31.9
R.5	13.3	38.5	26.4	-
Soil pH				
7.1		6.0	6.4	3.92

Further, the bags were left partially open, in growth rooms with a variable temperature (25-30ºC), in the dark, during 30 days. Saprophytic fungal development was evaluated for each type of substrate; CFUs recovery was evaluated monthly, during 3 months. For this, one gram soil sample were taken from 0-5 cm depth, randomly in four different points. One gram from the mixed samples was suspended in 9ml sterile water containing 0.01% Tween80, and vortexed for 1 minute. From this suspension 0.5ml of were used for a plate dilution series in Petri dishes containing semi-selective PDAY Dodine culture medium (Dodine Supelco, 0.1g/l, penicillin-G Sigma 0.4g/l, streptomycin sulfate Sigma Ald. 1g/l, Difco yeast extract 1g/l, PDA Fluka 39g/l). The Petri dishes were incubated at 25°C for 5 days, in the dark. Native soil was considered as control. The presence of *B. brongniartii* was confirmed primarily based on red pigmentation of culture medium; the identification was done on PDA culture medium, by plating technique.

Field persistence of *Beauveria brongniartii* granulated formulation

The experiments were set up in two forest nurseries, Zavoi-Siret (NFA Branch Botosani) and Izvor (NFA Branch Suceava), in north and north-east of Romania, located at an altitude of 274 and 468m respectively. Application of biological insecticide BioMelCon was described by Fătu et al. (2015). Briefly, the product was incorporated in the first 10cm of soil, in doses between 50-200 kg/ha/year. At Izvor nursery, BioMelCon was administrated in three doses (variants): V_1 (100 kg/ha/year), V_2 (150 kg/ha/year), V_3 (200 kg/ha/year). At Zavoi Siret nursery BioMelCon was applied in three doses (variants): V_1 (50kg/ha/year), V_2 (75kg/ha/year), V_3 (100kg/ha/year). Recovery of CFUs at different times after treatment application was performed.

RESULTS AND DISCUSSIONS

The vertical movement of BbS107 strain applied as conidial suspension in soil enriched with pig manure fertilizer was measured by recovery of colony-forming units, CFUs (figure 1). Compared with levels recorded at the time of *B. bassiana* conidia application (T_0), CFUs recovery in soil revealed a decrease of conidia at T_{30} in both experimental variants; the decrease was higher at a depth of 5-10cm, registering 30% fewer CFUs in fertilized soil (II) and 23% fewer CFUs in unfertilized soil (I). The smallest percentage decrease (19%) of CFUs number was registered in fertilized soil, at a depth of 0-5cm. In the next 30 days after treatment, the percentage of conidia recovery began to rise; at the time T_{60} it was registered a CFUs increase of 2% in unfertilized soil, an increase of 9-11% in the fertilized soil respectively. The CFUs recovery percentage was 1.22 times higher at the depth of 0-5cm compared to the CFUs number at 5-10cm depth. The increasing trend of the CFUs percentage continued at the next observation interval (T_{90}), registering increases in the CFUs number with 8-16% in unfertilized soil (I, 0-5cm), with 31-49% of those in fertilized soil (II, 0-5cm) respectively. In both types of soils, in the period between 90-120 days, the CFUs recovery percentage, at 5-10cm depth, was approximately 2 times lower than at 0-5cm depth.

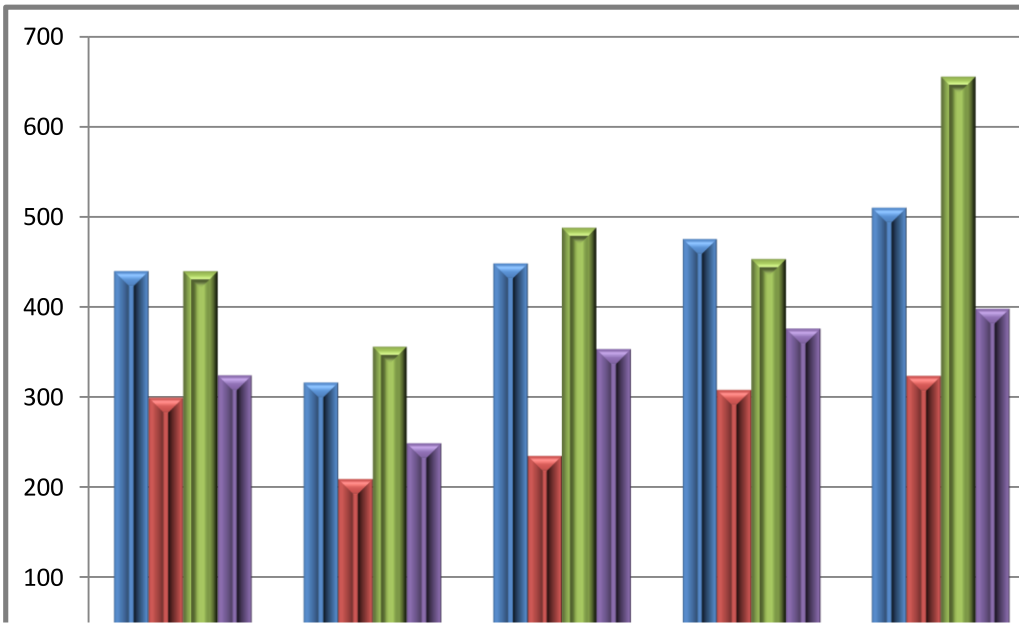

Figure 1. BbS107 colony forming units (CFUs) counted in a period of 120 days in unfertilized soil (I) and fertilized soil (II), at 0-10 cm depth

Analyzing the dynamics of the BbS107 CFUs recovery percentage over a period of 90 days of *B. bassiana* incubation in two types of soil, it was found that vertical movement of conidia was higher in fertilized soil, where it was registered 74% average CFUs recovery, compared to 68% in unfertilized soil (figure 2).

The largest number of conidia was found in all types of soil, 30 days after inoculation, registering $2.22x10^2$ conidia/g in native soil (figure 3), 2.92x10 conidia/g in manure (figure 4) $2.07x10^2$ conidia/g in compost (figure 5) and $5.62x10^2$ conidia/g in humus (figure 6).

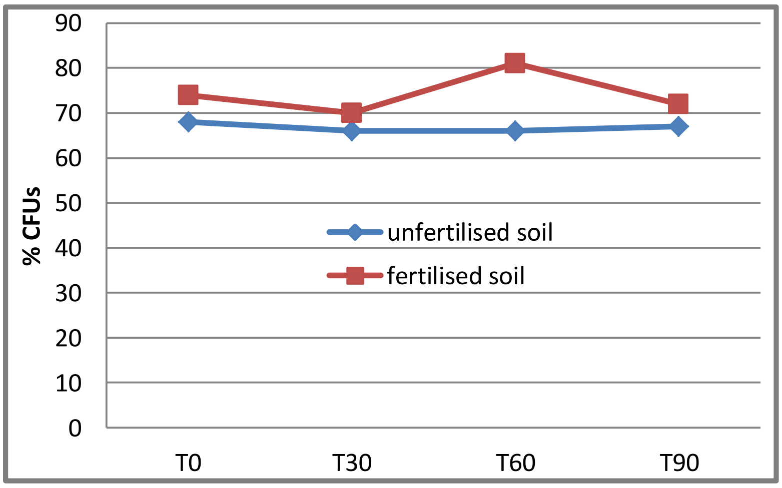

Figure 2. Recovery of BbS107colony forming units (CFUs) at 5-10 cm depth

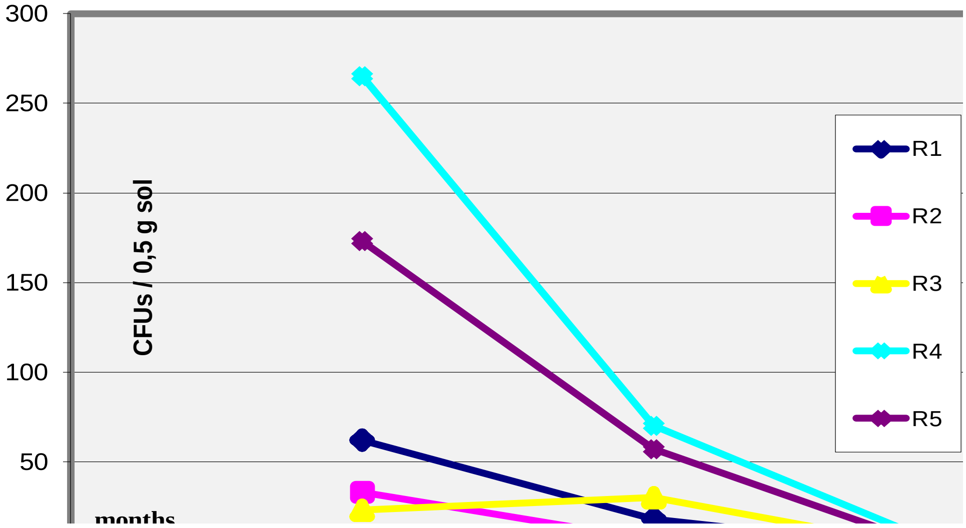

Figure 3. BbgMm1a/09 persistence conidia in native soil

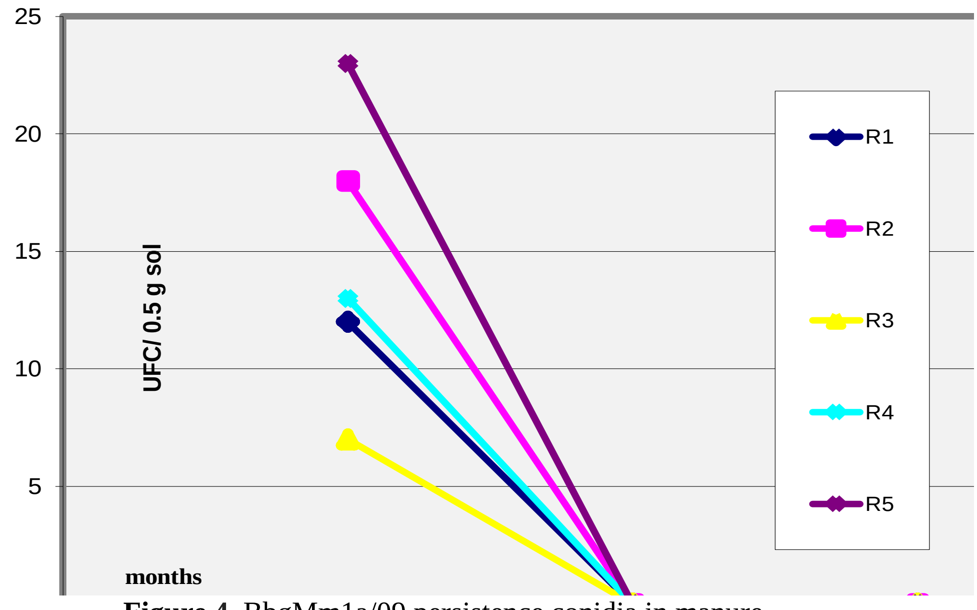

Figure 4. BbgMm1a/09 persistence conidia in manure

In the next 60 days after inoculation, the percentage of conidia recovery decreased by 67% in native soil, by 75% in compost and by 97% in humus. No conidia have been identified in manure fertilizer. In the next 90 days after inoculation, they have been found very low percentage of conidia, 0.48% in the native soil and only 0.04% in humus respectively. Conidia viability tests showed 86% average percentage of conidia germination, with a maximum value (91%) in the case of BbgMm1a/09 conidia isolated from humus and a minimum value (82%) when conidia was isolated from compost.

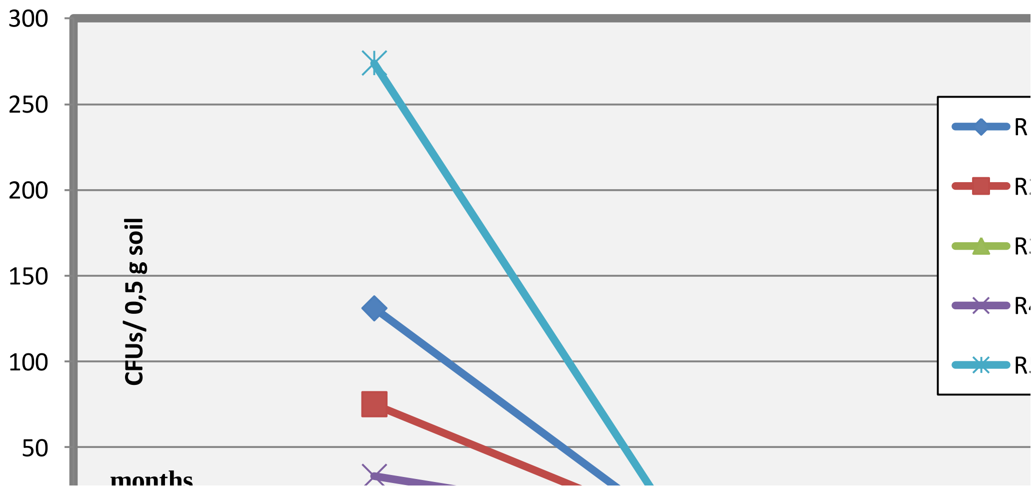

Figure 5. BbgMm1a/09 persistence conidia in compost

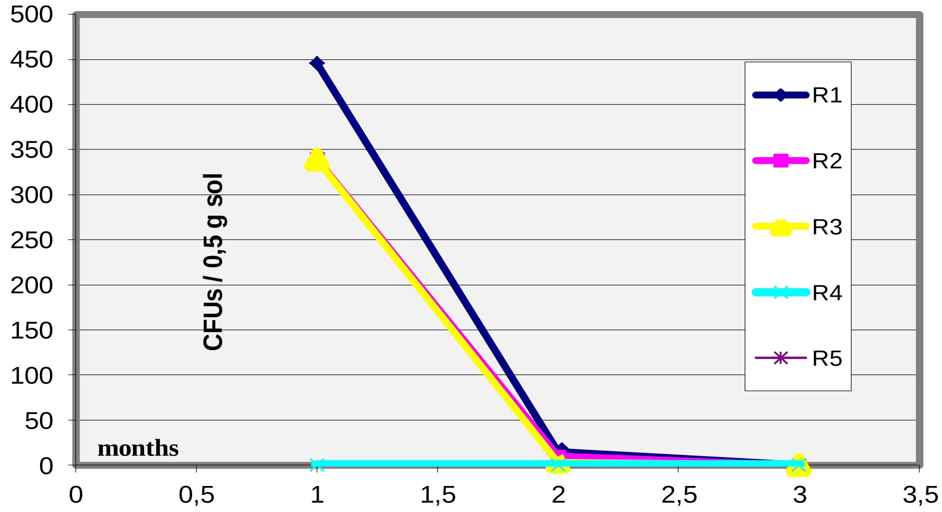

Figure 6. BbgMm1a/09 persistence conidia in humus

Field persistence of *B. brongniartii* revealed that at Izvor nursery, the UFCs recovery percent registered the following values: 100 days after treatment application: 0.52% (V_1) and 2.68% (V_3); three years after treatment application: 0.01% (V_2), five years after treatment application: 0.67% (V_1). At Zavoi Siret nursery, the UFCs recovery percent registered the following values after 130 days: 0.39% (V_1) and 0.30% (V_3); four years after treatment application, it was registered a strong increase of CFUs by 1.03 times higher for V_1, 20.3 times higher for V_2 and 39.6 times higher for V_3, which proves a high reproductive potential for *B. brongniartii*. Five years after treatment application, a UFCs average percentage of 0.015 % was recovered.

Various situations regarding the density of conidia applied on soil surface or incorporated into soil are reported in the literature - from high densities one year after application up to complete disappearance of applied *B. bassiana* after 6 months (Storey et al., 1989). Recent studies on the short- and long- term persistence of the commercial strain of *B. bassiana* revealed that fungal density decreased to 25% at 49 days after application and to 0.4% after 55 weeks (Świergiel et al., 2015). A decrease acceptable in terms of efficiency of biological treatment can be desirable, according to some authors which consider the rapid reduction in *B. bassiana* density in cultivated soils to be an advantage, in relation to damage for non-target organisms (Scheepmaker & Butt, 2010). In greenhouse experiments, the number of viable conidia in soil decreased by a factor of more than 10 during less than one year (Müller-Kögler & Stein, 1970). Müller-Kögler and Zimmermann (1986) reported a similar decrease of about 10^2 during one year.

Studies on the spatial-temporal abundance and distribution of fungal entomopathogens in different cropping systems have revealed the opportunity to prospect the population dynamics and ecological niches of specific fungal taxa (Meyling et al., 2011).

There are studies that demonstrated the *Beauveria* conidia longevity in soil is dependent on the season. Müller-Kögler and Zimmermann (1986) presented the findings of some winter and summer experiments on the *B. bassiana* viability in artificially contaminated soil. The authors registered during the winter field experiment a decrease in the number of conidia from about 10^6

at the beginning to about 10^4 or 10^3 after 1 year according to soil depth; in the same period, during the summer field experiment the corresponding values were 10^7 at the start to about 10^5 conidia/g dry soils. It is also estimated that the conidia viability is influenced by some toxic compounds in soils. Laboratory experiments with *B. bassiana* strain Bb-Pb2 have proved that onion's leaf litters, soil containing of decomposed onion, and the mixtures of both media reduced abilities of growth, conidia production and conidia germination on PDA media (Sudirman et al., 2008).

Some experiments focused on type of soil influence on the vertical movement of some commercially formulated *B. bassiana* conidia showed that in 4 different types of soil, characterized by different proportions of sand, clay and silt, conidia were mechanically retained in the surface layer, the recovery percentage of CFUs at 10-15 cm depths being very small (Storey & Gardner, 1988).

CONCLUSIONS

Analyzing the density dynamics of conidia applied on soil surface or incorporated into soil over a period of 90 days, it was found that:

- CFUs recovery percentage of *B. bassiana* BbS107 conidial suspension was 1.08 times higher in fertilized soil compared with the unfertilized soil.
- CFUs recovery percentage of *B. brongniartii* BbgMm1a/09 granulated product was very low (< 0.5%) both in native soil and in organic fertilizers tested in laboratory conditions.
- Under field conditions, four years after BbgMm1a/09 granulated product application, it was registered a strong increase of CFUs recovered from plots treated (>35% at an application rate of 200 kg product/ha/year).
- *B. bassiana* local strain BbS107 and *B. brongniartii* local strain BbgMm1a/09 proved a reproductive potential witch allowed soil colonization and the control of soil-dwelling pests.

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