Bioagrica 1 (1) 1-6
Article ID : *bioagrica-2003312112235*

Bioagrica

Journal homepage: http://www.htpub.org/Bioagrica/

Endophytic Characteristic of Entomopathogenic Fungi Beauveria on Bean Plant

Nastaran Arshadi[1], Ezatollah Sedaghatfar[2]✉, Alireza Golnaraghi[3] and Travis Glare[4]

[1] *Department of Plant Pathology, Arak Branch, Islamic Azad University, Arak, Iran*
[2] *Research Center for Applied Plant Sciences, Arak Branch, Islamic Azad University, Arak, Iran*
[3] *Department of Plant Protection, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[4] *Bio-Protection Research Centre, University Lincoln, Lincoln, New Zealand*

Article Information

Article History
Received: 14/09/2019
Accepted: 20/02/2020
Available online: 31/03/2020

Keywords
Endophytic
Beauveria
Bean
Entomopathogen fungi

Abstract

The endophytic effect of entomopathogenic fungi Beauveria on bean plant in laboratory and greenhouse conditions via soil-wetting method is conducted. To investigate the fungus to be endophyte in plant, 2weeks after bean growth, the inoculating of fungus via mentioned method was conducted and then every part of the plant (leaf, root, stem) was cut and each part were put in a Petri dish containing PDA and endophyte fungi growth was observed. The method was capable to make the plant endophytic. This method can protect the plant from pests and diseases attack.

1. Introduction

Entomopathogen fungi are found abundantly in nature and are easy to collect and propagate. They are non-pathogen for plants. In 2001, the telemorph *Beauveria bassiana* was determined as a Cordyceps and in the recent classification in the phyllum Ascomycota, class Pyrenomycetes, order Hypocreales, and family Cordycipitaceae. The telemorph is Cordyceps bassiana and its anamorph is *Beauveria bassiana* (Sung *et al*., 2007).

Endophytic inoculation of B. isolates are conducted with different methods, including soil-wetting, main root and seed-soaking, root, rhizome, and stem-soaking, stem injection, and leaf-spraying (Vega, 2008). These methods were conducted on banana, bean, cacao, coffee, corn, cotton, date, sorghum, tomato, and wheat (Akello *et al.*, 2009; Akello *et al.*, 2007; Brownbridge *et al.*, 2012; Gurulingappa *et al.*, 2010). The long-term systematic infection by endophyte entomopathogen fungi can be a competitive action against plant pathogens. Colonizing the plants by Beauveria, including B. bassiana can have a better effect on defense system, energy metabolite, and plant photosynthesis (Gómez-Vidal *et al*., 2009). Bean plant is one of the vulnerable plants against pests and diseases, and more than 400 pests and 200 pathogens can affect its production and function (Van Schoonhoven and Voysest, 1989). On the other hand, each crop has a variety of pests and diseases that should be controlled in the farmer s point of view. To control the damage of plant disease, sometimes the plants are sprayed chemically several times a year and this causes the high use of poision and contaminates the environment.

✉ *Corresponding author email address: e-sedaghatfar@iau-arak.ac.ir*

The objective of this research is to assess colonizing the plant tissue by B. species after artificial inoculation in labaratory conditions and the endophytic charectritics of B. species on growth and function of the plant.

2. Materials and methods

2.1. Preparing Beauveria species

Isolatesof B. were collected from the soil of different regions in Iran, including: 1-Quchan, 2-Arak, 3-Qom, 4-Astaneh, 5-L, 6-e, 7-salafchegan, 8-Kerman, 9-Mashhad, 10-Robat karim, 11-Isfahan, 12-Shiraz, 13-P, 14-Poldokhtar, 15-Yazd, 16-Yassuj, 17-Shahryar, 18-Farahan, 19-Borojerd, 20-Andimeshk, 21-khoram Abad.

2.2. Isolation the fungi from the soil

To isolat fungus from the soil, soil-baiting method (Zimmermann, 1986) with a slight change was used. And for this method we have used Ephesia larva.

2.3. Suspensions Preparation

According to Sedehi *et al*. (2014); first, a loop of the conidia is taken and put in 1 ml of Triton 100x 0.1% suspensions and was mixed by shaking for 10 seconds and 100 microliter of the suspension was taken with a sampler and poured in NA medium. Then, it was laid in the incubator for 24 hours in 25 c temperature. Then, the single germinated spore was transferred into the Petri dish containing PDA medium. After 4 weeks the fungus growth in sterile condition, they were scratched from the medium and were put in 10 ml Triton 100 x 0.1% suspension and were mixed by shaking for 1 min. Tiffany was used to separate hyphae and conidium suspension. The number of conidia was increased to 1*10 conidia/ml and globule counting slide was used to count conidia.

2.4. Greenhouse Investigation

In this experiment, pots containing cocopeat soil put in greenhouse temperature at 25°C. Before planting the bean seeds in the pots, the seeds were first soaked in 0.5 hypochlorite for 2 min. Then, they were washed in Ethanol 70% for 2 min, and finally were completely washed with setron strilled water and then dried. 3 bean seeds were planted in each pot in the depth of 10 cm of soil with 3 replications. Every 2-3 day, they were irrigated with distilled water. When the plant was in trifoliate stage (14 days post-growth), inoculation of fungi onto the soil was conducted.

In this method, 10 ml of fungus suspension was poured on the soil of each pot with a scaled cylinder. Two weeks after inoculation, the height of the plant was measured. Then, seprating the plant with root from the soil, washing, and measuring the wet weight of the plant were conducted (Parsa *et al*., 2013).

2.5. Laboratory and endophytic investigations

To investigate the fungi being endophyte inside the plant, each part of the plant(leaf, root, stem)was first cut for 1 cm and was sterilled in 3 steps. In the first step, each part was soaked in sodium chloride 0.5 for 2 min, and in the second step in ethanol 70% for 2 min, and in the final step distilled water was used to steril the plant. Four pieces of each part of the plant were put in Petri dishes containing PDA medium with 2 mg/l tetracycline, streptomycine, and penicilium. Finally, the Petri dishes were sealed with parafilm and were put in the ancubator in 25 c temperature. After 20 days the Petri dishes were observed and the leaf, root, and stem containing endophyte were taken and transfered into a new Petri dish. Finally, they were observed under microscope (Parsa *et al*., 2013).

2.6. Statistic analysis

This experiment was carried out in completely randomized plot with 22 samples in 3 replications. The height of the bush, the wet and dry weight of the roots and shoots were considered as the indicator for the amount of function of Beauveria isolates.

To calculate the wet and dry weight of the roots and shoots, and the height of the bushes in conidium-soaked suspension experiment, remained shoots of the bushes were first separated from the soil and crown and after measuring the height, wet weight of the shoots was measured. Shoots were put in paper bags and the name of each treatment and replication number were written. The roots of the remained bushes were separated from the shoots, their wet weight was measured, and the roots were put in paper bags and the name of treatment and the replication number were written on each. In order to measure the dry weight, bags containing shoots and roots were put in the oven for 3 days in 45 celsius for seedlings to be dried completely. After this period of time, dried shoots and roots weight were measured and recorded (Parsa *et al*., 2013).

3. Results

Fungal entomopathogens Beauveria are capable to make the bean plant endophyte via this method (figure 1).

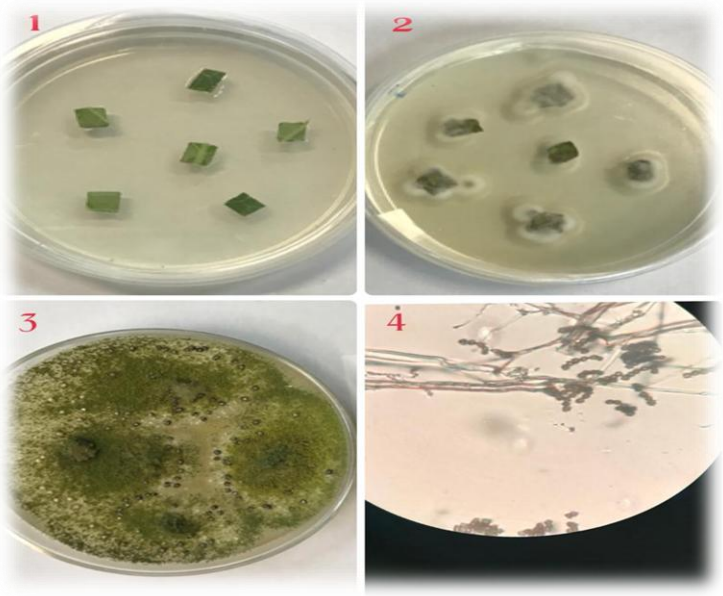

Figure 1. 1-Culturing leaf cuts on medium, 2-emerging of Beauveria after cultivation each part of the plant on PDA medium, 3- fungus growth after 20 days, 4- microscopic observation.

The objective of this experiment was to determine the most efficient Beauveria isolation on increasing the height and other parts of the plant in comparison with control. Results obtained from analyzing seedlings variances show that there is a significant difference of 99% between treatments (Figure 1).

By comparing seedlings height via soil-wetting method with Beauveria in figure 2, it is observed that isolates 14, 15, 16 had height 30, 28.25, 27.5 cm respectively and the wet weight of seedlings roots in figure 3, it is observed that isolates 5, 16, 4, were 1.73, 1.62, 1.38 g respectively and wet weight of the seedlings stem and leaf in figure 4, it is observed that the isolates 17, 16, 15 were 4.94, 4.70, 4.36 g respectively and the dry weight of seedlings roots with Beauveria in figure 5, it is observed that isolates 18, 20, 5, were 0.74, 0.11, 0.11 g respectively and the dry weight of seedlings stem and leaf in figure 6, it is observed that isolates 5, 6, 17, were 0.57, 0.47, 0.47 g respectively have the most effect on the each part of the plant and other isolations have less effect.

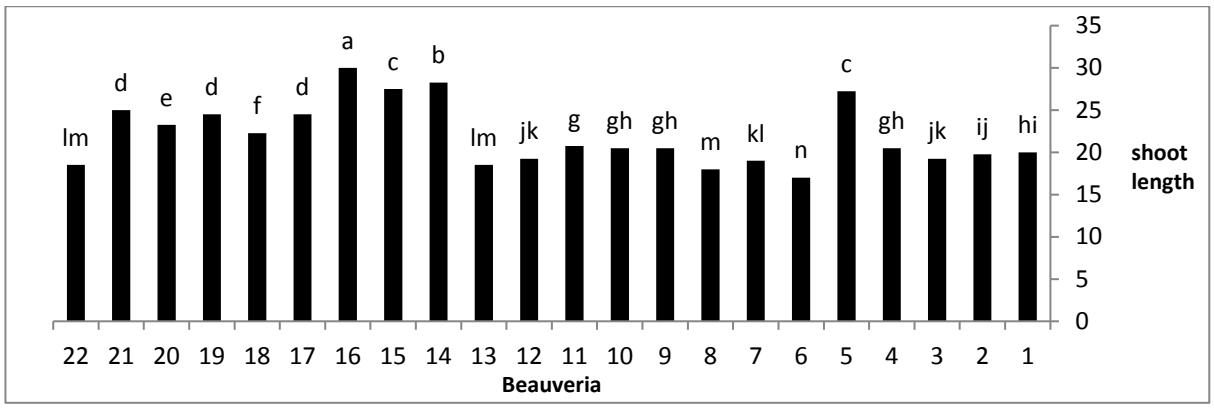

Figure 2. The comparison of the average effect of endophytic colonization of Beauveria isolates by soil-wetting on the bean seedlings height based on Duncan mulitiple-domain test.

Effect of Beauveria isolates via soil-wetting on root wet weight shows isolates 5 and 16 had the highest endophytic colonization on bean seedlings roots. However isolates 4 and 14 had appropriate colonization and effect on root wet weight respectively at statistics levels; c and d (figure 3).

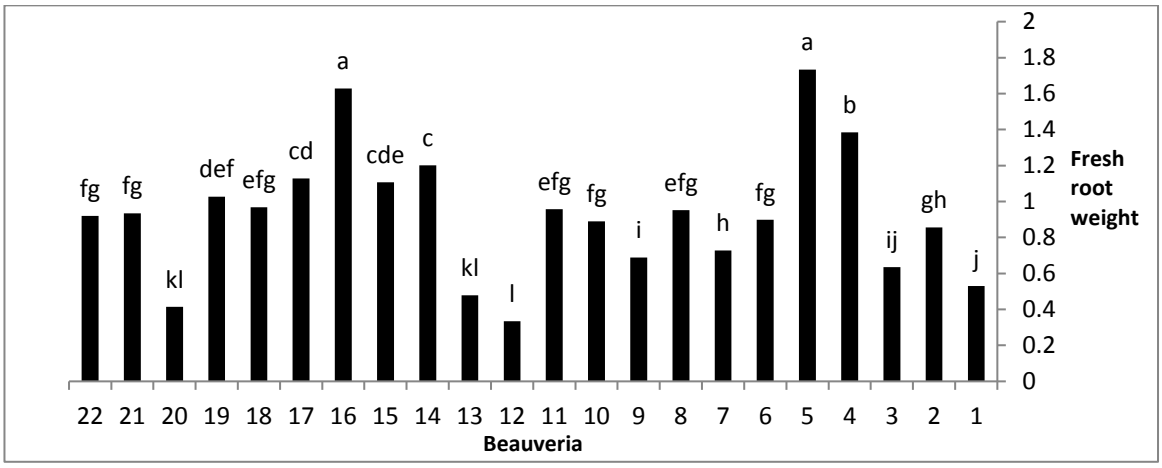

Figure 3. The comparison of the average effect of endophytic colonization of Beauveria isolates via soil-wetting on roots wet weight of bean seedlings based on Duncan multi-domain test.

Endophytic colonization effects of Beauveria isolates in soil-wetting method on bean seedlings shoot wet weight (stem and leaf) nearly shows any significant differences between isolates however the isolates 5, 15, 16 and 17 have more wet weight than the others (figure 4).

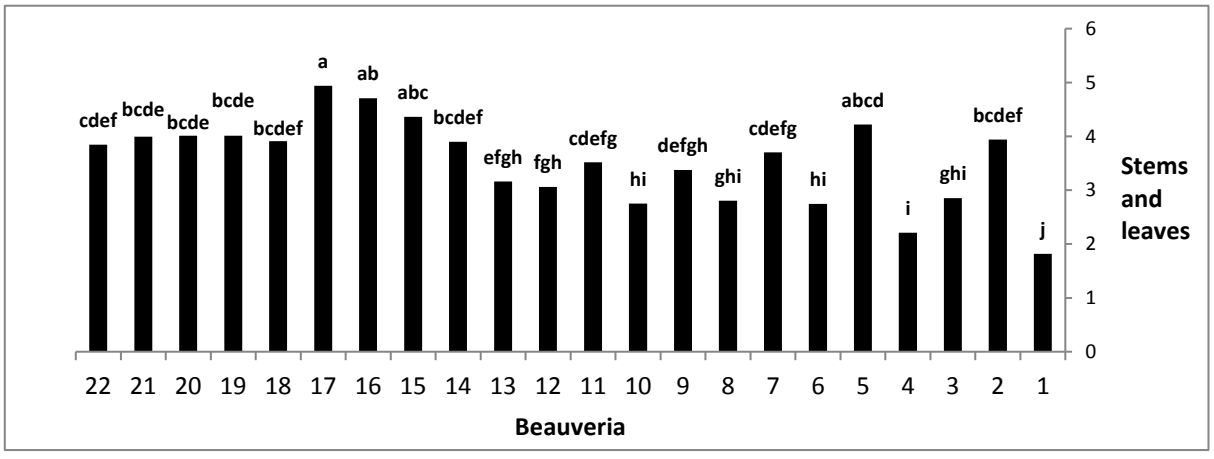

Figure 4. The comparison of the average effect of endophytic colonization of Beauveria isolates by soil-wetting method on the wet weight of bean seedlings stem and leaf based on Duncan multi-domain test.

Endophytic effects of Beauveria colonization in soil-wetting method shows isolate 18 had the best effec and enhanced the root dry weight of bean seedlings with significant difference the isolates 4, 5, 6, 16 and 20 had good effect on root dry weight at statistical levels (figure 5).

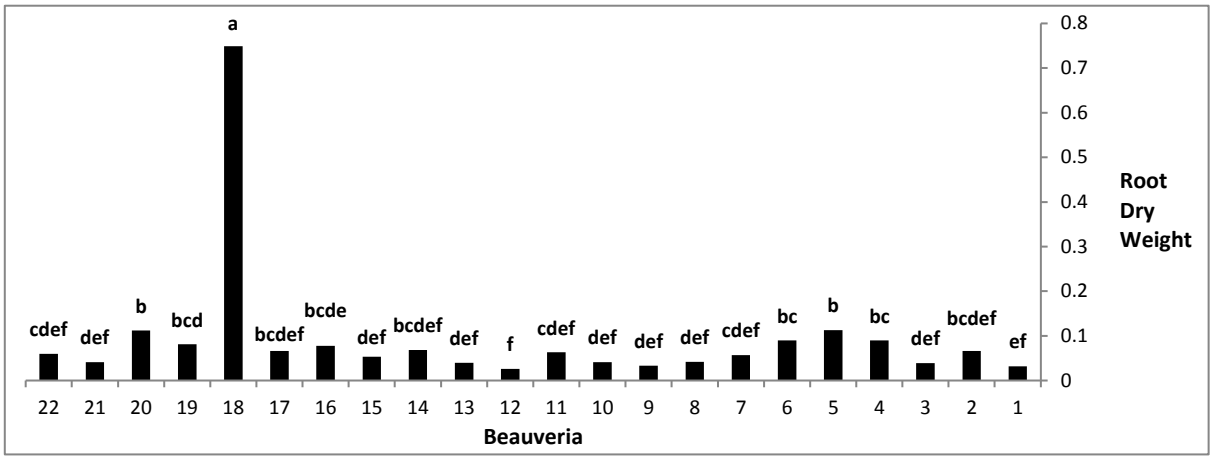

Figure 5. The comparison of the average effect of endophytic colonization of Beauveria isolates by soil-wetting method on the root dry weight of bean seedlings based on Duncan multi-domain test.

Endophytic colonization of Beauveria isolates by soil-wetting method, significantly affected dry weight of bean seedlings shoots, In the way that isolate 5 had best effect and isolates 6 and 17 were placed at the next statistic level (b) of the bean shoot dry weight. Other isolates had not significantly differences in the endophytic effect (figure 6).

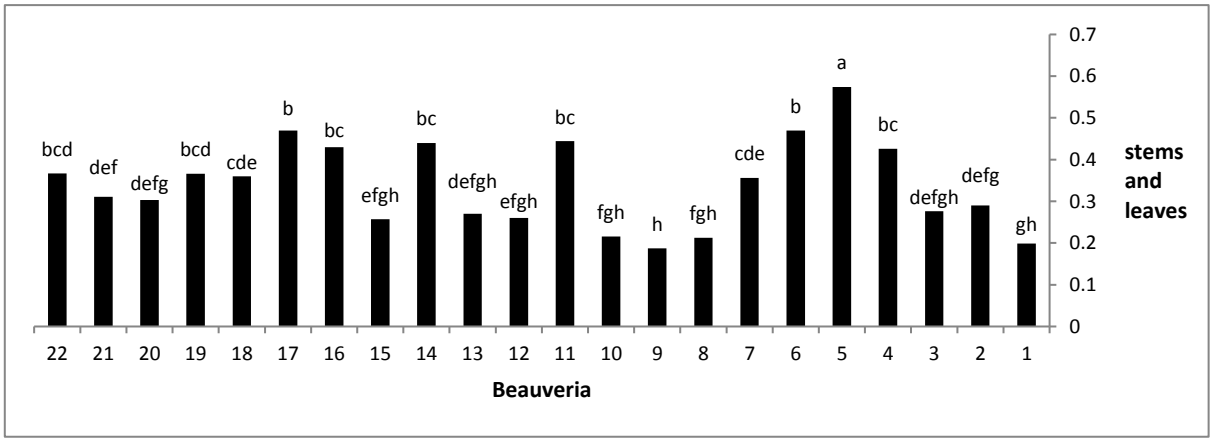

Figure 6. The comparison of average effect of endophytic colonization of Beauveria isolates by soil-wetting method on dry weight of bean seedlings stem and leaf based on Duncan test.

4. Discussion

Greenhouse research results show that isolate 16 by soil-wetting method had the most effect on the height of seedling, and was in a higher statistic group in comparison with control. Accordingly, it can be said that these isolates were the seedlings growth stimulus. Isolates 5 and 16 had the most effect on the root wet weight. Isolate 18 had the most effect on the root dry weight. Isolate 17 had the most effect on the weight of the seedlings stem and leaf wet weight. Isolate 5 had the most effect on dry weight of stem and leaf. Consequently, among all Beauveria isolates, isolates 10, 5, 16, 17, 18, had

the best effect rather than other isolates. Totaly we can select these best Beauveria isolates for biological control.

Acknowledgements

The research was carry out by providing the materials and instruments by Research Center for Applied Plant Sciences (http://rcaps.iau-arak.ac.ir), in Arak Branch, Islamic Azad University and by assistance and cooperation of researchers in the microbial and molecular laboratories in the center. So we appreciate from the center staff and the researchers.

References

Akello, J., Dubois, T., Coyne, D. & Kyamanywa, S. (2009). The effects of *Beauveria bassiana* dose and exposure duration on colonization and growth of tissue cultured banana (Musa sp.) plants. *Biological control* 49(1): 6-10.

Akello, J., Dubois, T., Gold, C. S., Coyne, D., Nakavuma, J. & Paparu, P. (2007). *Beauveria bassiana* (Balsamo) Vuillemin as an endophyte in tissue culture banana (Musa spp.). *Journal of invertebrate pathology* 96(1): 34-42.

Brownbridge, M., Reay, S. D., Nelson, T. L. & Glare, T. R. (2012). Persistence of *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte following inoculation of radiata pine seed and seedlings. *Biological control* 61(3): 194-200.

Gómez-Vidal, S., Salinas, J., Tena, M. & Lopez-Llorca, L. V. (2009). Proteomic analysis of date palm (Phoenix dactylifera L.) responses to endophytic colonization by entomopathogenic fungi. *Electrophoresis* 30(17): 2996-3005.

Gurulingappa, P., Sword, G. A., Murdoch, G. & McGee, P. A. (2010). Colonization of crop plants by fungal entomopathogens and their effects on two insect pests when in planta. *Biological control* 55(1): 34-41.

Parsa, S., Ortiz, V. & Vega, F. (2013).Establishing fungal entomopathogens as endophytes: towards endophytic biological control. JoVE J Vis Exp 74: 1–5.

Sedehi, A., Sedaghatfar, E. & Modarres-Najafabadi, S. (2014). Studies on effect of the *Beauveria bassiana* on eggs and larvae of Plodia interpunctella. *Canadian Journal of Basic and Applied Sciences*: 40-45.

Sung, G.-H., Hywel-Jones, N. L., Sung, J.-M., Luangsa-ard, J. J., Shrestha, B. & Spatafora, J. W. (2007). Phylogenetic classification of Cordyceps and the clavicipitaceous fungi. *Studies in mycology* 57: 5-59.

Van Schoonhoven, A. & Voysest, O. (1989). Common beans in Latin America and their constraints. *Bean Production problems in tropics. Centro Internacional de Agrícultura Tropical. Cali, Colombia*: 33-57.

Vega, F. E. (2008). Insect pathology and fungal endophytes. *Journal of invertebrate pathology* 98(3): 277-279.

Zimmermann, G. (1986). The 'Galleria bait method'for detection of entomopathogenic fungi in soil. *Journal of applied Entomology* 102(1-5): 213-215.