



**Acknowledgements:**

The authors gratefully acknowledge the Hellenic Entomological Society for the doctoral scholarship award granted to the first author.



# Evaluation of Entomopathogenic Action of *Beauveria bassiana* Using Two Application Methods: Trunk Inoculation on Kiwi Trees and Seed Coating on Cotton

Vasileios Papantzikos<sup>1</sup>, Spiridon Mantzoukas<sup>2</sup> and Georgios Patakioutas<sup>1</sup>

<sup>1</sup>Department of Agriculture, University of Ioannina, Arta Campus, 47100, Greece

<sup>2</sup>Institute of Mediterranean Forest Ecosystems, Terma Alkmanos, 11528 Ilissia Zografou, Greece

## INTRODUCTION

The insecticidal action of the entomopathogenic fungi (EPF), especially of *Beauveria bassiana*, has been documented for a wide range of sucking pests, and in recent years, its formulated application has been tested in several crops. In this work, two different application methods of *B. bassiana* PPRI 5339 Velifer® formulation were studied over two years.

## METHODS

In one case, *B. bassiana* was applied A) as a coating to the cotton seed *Gossypium hirsutum* (Fig. 1A) and B) via syringe inoculation to the kiwi trunk *Actinidia deliciosa* “Hayward” (Fig. 1B). The sucking insects *Aphis gossypii* on cotton plants (Fig. 3A) and *Halyomorpha halys* on kiwi trees (Fig. 3B) were counted. In addition, the total chlorophyll content (TCHL) and the leaf area were measured during the experiment. For each case, plants without the application of *B. bassiana* were used as a control (C). Asterisks in figures \*, \*\*, \*\*\*, denote statistically significant differences between treatments at probability values of  $p \leq 0.05$ ,  $\leq 0.01$ , and  $\leq 0.001$ , respectively, according to the two-way ANOVA with Tukey’s post hoc test using SPSS v. 25 (IBM-SPSS Statistics, Armonk, NY, USA).

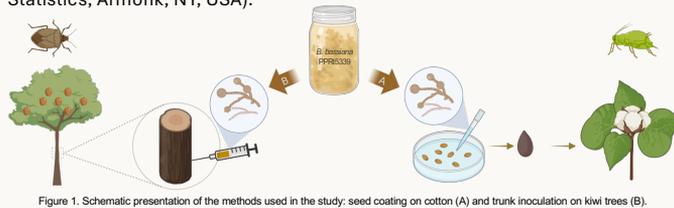


Figure 1. Schematic presentation of the methods used in the study: seed coating on cotton (A) and trunk inoculation on kiwi trees (B).

## RESULTS & DISCUSSION

The average number of *H. halys* in C was higher with a statistically significant difference ( $F=19.88$ ,  $df=3.72$ ,  $p<0.001$ ) with B (Fig. 2ii). The lower *H. halys* number in treatments with *B. bassiana* has also been observed in hazelnut *Corylus avellana* orchards by Ozdemir et al. 2022 [1]. In the case of coated cottonseed, the *A. gossypii* number was lower in the *B. bassiana* treatment (Fig. 2i) with a statistical difference with C ( $F=11.88$ ,  $df=2.51$ ,  $p<0.001$ ). Seed coating is a practice that may ensure higher colonization of the EPF [2], because, after seed planting, the EPF is preserved in the soil [3], at beneficial conditions for their growth, shielding them from UV radiation [4], in a protective environment with moisture and availability of nutrients.

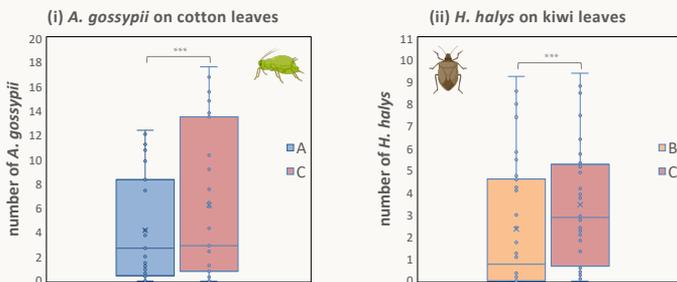


Figure 2. Variation of pest number for each experimental case: (i) *A. gossypii* on cotton leaves and (ii) *H. halys* on kiwi *A. deliciosa* “Hayward” leaves. Definition of treatments: (A) cottonseed coating of *B. bassiana*; (B) kiwi trunk-inoculation of *B. bassiana*; and (C) control.

## CONCLUSIONS

Both A and B treatments with *B. bassiana* reduced the number of *A. gossypii* and *H. halys*, respectively, noting high TCHL and leaf area. This is encouraging in the research of new protocols that may enhance plant health and pest management, given the need for environmentally friendly techniques in the face of climate change.



Figure 3. Pests found on the control treatment leaves for each case: A. *gossypii* on cotton leaves (A) and *H. halys* on kiwi A. leaves (B).

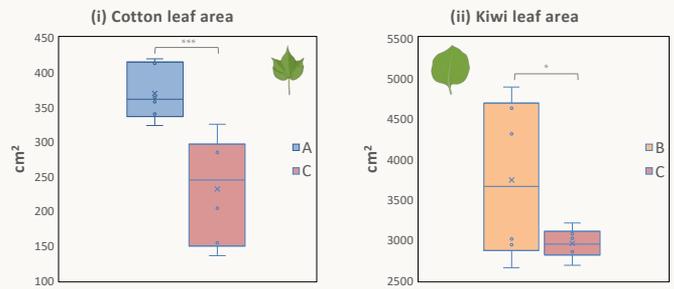


Figure 4. Variation in leaf area ( $cm^2$ ) for each experimental case: (i) *A. gossypii* on cotton leaves and (ii) *H. halys* on kiwi *A. deliciosa* “Hayward” leaves. Definition of treatments: (A) cottonseed coating of *B. bassiana*; (B) kiwi trunk-inoculation of *B. bassiana*; and (C) control.

In the cotton plants treated with *B. bassiana* (A), a larger leaf area was detected with a statistically significant difference ( $F=11.55$ ,  $df=2$ ,  $p<0.001$ ) compared to C (Fig. 4i). The larger leaf area in seed coatings with *B. bassiana* has also been detected in other studies with *Phaseolus vulgaris* L., [5], and *Zea mays* L., [6]. Leaf area was also significant in the case of kiwifruit trunk inoculation (B) compared to the C ( $F=14.41$ ,  $df=3.20$ ,  $p=0.027$ ), noting a significant difference (Fig. 4ii).

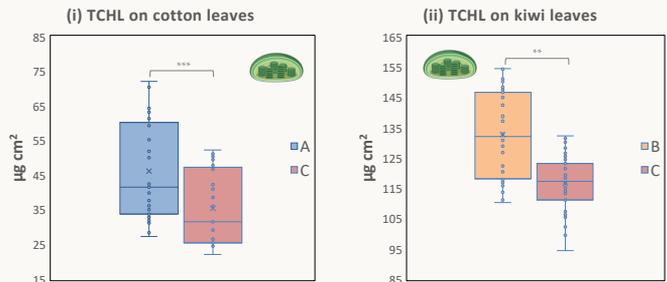


Figure 5. Variation in TCHL ( $\mu g cm^2$ ) for each experimental case: (i) *A. gossypii* on cotton leaves and (ii) *H. halys* on kiwi *A. deliciosa* “Hayward” leaves. Definition of treatments: (A) cottonseed coating of *B. bassiana*; (B) kiwi trunk-inoculation of *B. bassiana*; and (C) control.

Regarding TCHL, the higher the amount in leaves, the more beneficial it is for the plant, because it is involved in various metabolic processes [7]. As shown in both figures (Fig. 5i and 5ii), on the one hand, the pest presence in both cotton plants and kiwi can reduce TCHL in the leaf’s tissues of the control as a result of the sucking damage caused by *A. gossypii* and *H. halys* respectively. On the other hand, the presence of *B. bassiana* in the plant tissues of both coated cottonseed (A) ( $F=13.22$ ,  $df=2$ ,  $p<0.001$ ) and trunk-inoculated kiwi (B) ( $F=29.11$ ,  $df=3.58$ ,  $p=0.009$ ) assists the plants by limiting the pests’ dispersion and, the leaf area remains intact presenting less sucking damage. The previous reasons may shape the environment for the greater TCHL in the EPF treatments. This observation comes in agreement with the work of Geroh et al. 2014 [8], where the leaf TCHL in the treated okra *Abelmoschus esculentus* plots with *B. bassiana* was enhanced due to the EPF pathogenic action on the *Tetranychus urticae*, and because of the beneficial effect in plant’s metabolism.

## RESOURCES

- Ozdemir, I.O.; Yildirim, E.; Uluca, M.; Tuncer, C. Efficacy of Native *Beauveria bassiana* and *B. pseudobassiana* Isolates Against Invasive Brown Marmorated Stink Bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae). *BioRxiv*. <https://doi.org/10.1101/2022.04.19.491994>.
- Ponada-Vergara, C.; Lopez, K.; Abuduen, M.; Vial, S.; Reales, M. Root Colonization by Fungal Entomopathogen Systemically Primes Belowground Plant Defense against Cabbage Root Fly. *Journal of Fungi* 2022, 8, 869. <https://doi.org/10.3390/jf8090869>.
- Gentile-Lischi, F.; Russo, F.; Campos, M.; Quevedo-Munaga, E. Effects of Soil Treatments with Entomopathogenic Fungi on Soil Dwellers Non-Target Arthropods at a Commercial Olive Orchard. *Biological Control* 2019, 159, 239–244. <https://doi.org/10.1016/j.biocontrol.2019.07.001>.
- Kaiser, D.; Bacher, S.; Weber-Schiffano, L.; Grieswanger, G. Efficacy of Natural Substances to Protect *Beauveria bassiana* Conidia from UV Radiation. *Pest Manag Sci* 2019, 75, 556. <https://doi.org/10.1002/ps.5209>.
- Alarifi, A.; Wajidi, T.; Elm, A.A.; Taha, H.; Alyani, M.; Hammad, R.N.S. Entomopathogenic Fungus *Beauveria bassiana* Accelerates Growth of Common Bean (*Phaseolus vulgaris* L.). *Chemical and Biological Technologies in Agriculture* 2019, 6, 1–6. <https://doi.org/10.1186/s13030-019-0145-4>.
- KuchipudiPrasadPrabhakaravally, L.; Ferreira Gomes, P.; Gomes Flores, R.; Rodrigues Padua, M.C.; Elk-Ramou, M.J. Effect of *Beauveria bassiana*-Seed Treatment on *Zea mays* L. Response against *Sitona* (Fugate). *Applied Sciences* (Switzerland) 2021, 11, 2267. <https://doi.org/10.3390/app11102267>.
- Martins, T.; Barro, A.N.; Rossi, E.; Antunes, L. Enhancing Health Benefits through Chlorophyll and Chlorophyll-Rich Agro-Food: A Comprehensive Review. *Molecules* 2023, 28, <https://doi.org/10.3390/molecules28144034>.
- Geroh, M.; Gopal, R.; Kanika, T. *Beauveria bassiana* (Basmati) Vulliamii (Strain ITCC-4958) as Acaricide against *Tetranychus urticae* Koch (Acari: Tetranychidae). *Indian J Agric Res* 2014, 48, 384–388. <https://doi.org/10.15693/IJARR.2014.4810315>.