



SHORT NOTE

Pathogenicity of *Beauveria bassiana* against Leafcutter Ants *Atta laevigata* (F. Smith, 1858) (Hymenoptera: Formicidae)

KELLE D.R. PEREIRA, RONNIE V.S. VELOSO, ZAIRA V. CALDEIRA, WILSON F. JÚNIOR, SÁVIO D. MEIRA,
EMILLY G.S. FERREIRA, IVANI T. OLIVEIRA, RICARDO S. DA SILVA, MARCUS A. SOARES

Department of Agronomy, Federal University of Jequitinhonha and Mucuri's Valleys (UFVJM), Diamantina-MG, Brazil

Article History


Edited by

Evandro Nascimento Silva, UEFS, Brazil
Received 27 November 2024
Initial acceptance 01 June 2025
Final acceptance 10 June 2025
Publication date 02 July 2025

Keywords

Entomopathogenic fungus; Lethal time;
Biological control; Saúvas.

Corresponding author

Marcus Alvarenga Soares 
Department of Agronomy, Federal
University of Jequitinhonha and Mucuri
Valleys (UFVJM)
CEP: 39100-000 - Diamantina-MG, Brasil.
E-Mail: marcus.alvarenga@ufvjm.edu.br

Abstract

Leaf-cutter ants are major agricultural pests causing substantial crop losses. Biological control using entomopathogenic fungi offers an eco-friendly alternative to chemical methods. This study evaluated the pathogenicity of *Beauveria bassiana* (ISO77) against *Atta laevigata* (Hymenoptera: Formicidae) and determined lethal times (LT50 and LT99) under laboratory conditions. Ants were exposed to conidial suspensions at 1×10^7 and 1×10^8 conidia·mL⁻¹. Mortality was assessed at 25 ± 2 °C with a 12-hour photoperiod over 10 days. The fungus was pathogenic at both concentrations, with higher conidial levels accelerating mortality. PROBIT analysis indicated LT50 and LT99 values of 120 and 214 hours for 1×10^7 conidia·mL⁻¹, and 93 and 157 hours for 1×10^8 conidia·mL⁻¹, respectively. These results demonstrate the potential of *B. bassiana* ISO77 as a biological control agent for leaf-cutter ants.

Ants are abundant and dominant insects occupying diverse ecological niches, with approximately 15,700 described species and subspecies worldwide (Nogueira et al., 2022; Paramanandham et al., 2022; Schultheiss et al., 2022; Bragança et al., 2023). They play numerous ecological roles, acting as predators, pollinators, scavengers, parasites, and herbivores, and contributing to soil fertility and seed dispersal, as well as serving as indicators of ecological conditions (Swanson et al., 2019; Johari et al., 2021). Leaf-cutter ants behave as herbivores in the Neotropics, cultivating the symbiotic fungus *Leucoagaricus* sp. as their main food source with plant parts (Farias et al., 2018; Cantarelli et al., 2019; Folgarait & Goffré, 2021; Cotazo-Calambas et al., 2022). These ants can extract up to 900 kg of plant biomass per nest annually, causing significant damage to agricultural plantations, pastures, and reforestation areas, including seedling death, plant defoliation, and increased insecticide use

(Garcia et al., 2020; Valencia-Giraldo et al., 2021; Cardoso et al., 2022; Pimentel et al., 2022).

The traditional method for controlling leaf-cutting ants is chemical, but it is often ineffective and environmentally damaging due to non-specificity, toxicity, resistance, persistence, and high operational costs (Vinha et al., 2020; Cardoso et al., 2022; Cotazo-Calambas et al., 2022; Folgarait & Goffré, 2023). The indiscriminate use of insecticides can lead to pest resistance, reduction of natural enemies, contamination of water and soil, and health risks. Thus, there is growing interest in alternative methods that are both economically viable and environmentally safe (Folgarait & Goffré, 2021; Loko et al., 2022).

Biological control with entomopathogenic fungi, such as *Beauveria bassiana* (Balsamo) (Cordycipitaceae), offers a promising alternative due to its epizootic potential and ability to transmit between individuals via direct contact (Yasin et al.,



2017; Rojas et al., 2018). Additionally, *B. bassiana* exhibits high genetic variability, enabling it to control a broad range of pests and persist in the environment as a saprophyte (Deka et al., 2021; Elkhateeb et al., 2021; Mota Filho et al., 2021; Stefanelli et al., 2021; Yucel, 2021; Swathy et al., 2024). This study aimed to evaluate the pathogenicity of *B. bassiana* for controlling *Atta laevigata* (F. Smith, 1858) (Hymenoptera: Formicidae) and to determine lethal times, contributing to the development of improved control strategies.

The experiment was conducted at the Plant Pathology and Biological Control Laboratories of the Department of Agronomy at the JK Campus, University of Jequitinhonha and Mucuri's Valleys (UFVJM), Diamantina, Brazil. Leaf-cutting ants were collected from nests in Augusto de Lima, MG, transported to the laboratory, and maintained for bioassays. The ISO77 isolate of *B. bassiana* was obtained from the commercial product Boveril WP® (Koppert Biological Systems, Piracicaba, MG, Brazil) and stored on inclined PDA at 5 °C. It was revitalized by transferring it to Petri dishes containing Sabouraud Dextrose Agar and incubated at 25 ± 2 °C for 13 days with a 12-hour photoperiod. Morphological identification was confirmed by microscopic examination using Amann's Blue solution.

Following Cardoso et al. (2022), conidial suspensions were prepared by transferring 6 mm discs from the revitalized colony to PDA dishes, incubated for 13 days at 25 ± 2 °C.

Conidia were scraped, suspended in sterile water with 0.01% Tween 80, and standardized using a Neubauer chamber to concentrations of 1.0×10^7 and 1.0×10^8 conidia·mL⁻¹.

Ants were exposed to three treatments: control (sterile water + 0.01% Tween 80), 1.0×10^7 conidia·mL⁻¹, and 1.0×10^8 conidia·mL⁻¹. A total of 150 ants were used, with five replicates of 10 ants per treatment. Each ant was individually placed in a Petri dish containing moistened filter paper, 1 mL of the respective conidial suspension, and a wad of cotton moistened with a 10% honey solution. The dishes were maintained at 25 ± 2 °C with a 12-hour photoperiod and monitored every 24 hours for 10 days. Dead ants, with or without visible fungal outgrowth, were transferred to new dishes with moist cotton to confirm death due to fungal infection by observing typical mycosis. The methodology was adapted from Stefanelli et al. (2021).

Mortality was assessed at 24-hour intervals up to 240 hours post-exposure. Confirmed mortality data were subjected to PROBIT analysis to estimate LT50 and LT99 (Qiu et al., 2014; Cruz-Cruz et al., 2020). Ants in the control group exhibited a mortality rate of 5% after 240 hours. At a concentration of 1.0×10^7 conidia·mL⁻¹, the mortality curve was sigmoidal, with LT50 at 120 hours and LT99 at 214 hours. In contrast, at 1.0×10^8 conidia·mL⁻¹, LT50 was achieved in 93 hours and LT99 in 157 hours (Table 1), demonstrating that higher concentrations accelerated mortality (Fig 1).

Table 1. Lethal time (LT50 and LT99), confidence interval, sample size used for LT estimation (N), Chi-square, degrees of freedom (Df), p-value, intercept (H), and slope, for the concentrations of 1.0×10^7 and 1.0×10^8 conidia·mL⁻¹ of *Beauveria bassiana*

Treatment	Time	Confidence Interval (95%)	N	Chi-square	Df	p-value	H	slope
1.0×10^7	LT50 (hours)	120 (108 – 138)	27	35.1	25	0.0868	1.40	0.0484
	LT99 (hours)	214 (181 – 291)	27	35.1	25	0.0868	1.40	0.0484
1.0×10^8	LT50 (hours)	93 (86 – 103)	20	18.1	18	0.447	1.00	0.0717
	LT99 (hours)	157 (138 – 197)	20	18.1	18	0.447	1.00	0.0717

N represents the sample size used for LT estimation (N = Df + 2). Chi-square is the test used to verify the fit of the data to the PROBIT model. Df stands for degrees of freedom. H refers to the intercept. Slope represents the inclination.

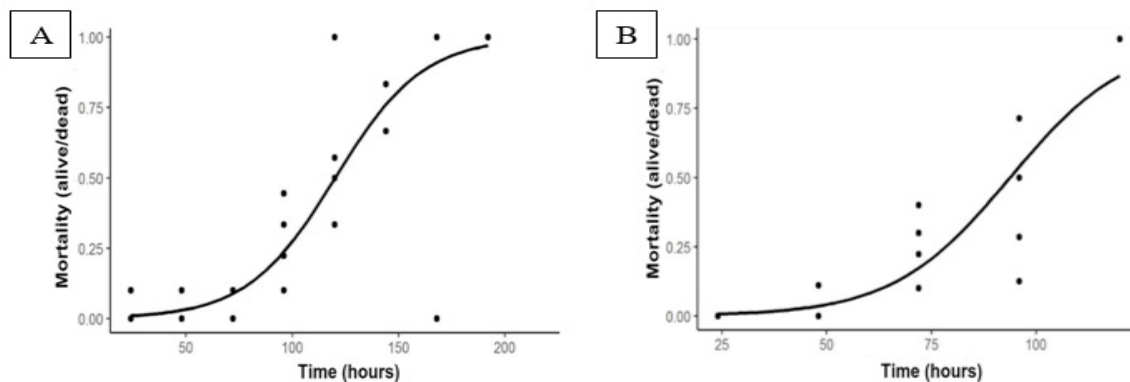


Fig 1. Mortality (dead/alive) of leafcutter ants subjected to concentrations of 1.0×10^7 and 1.0×10^8 conidia·mL⁻¹ of *Beauveria bassiana* assessed over 240 hours (10 days).

A - Mortality (dead/alive) of leafcutter ants subjected to the concentration of 1.0×10^7 conidia·mL⁻¹.

B - Mortality (dead/alive) of leafcutter ants subjected to the concentration of 1.0×10^8 conidia·mL⁻¹.

The progressive increase in mortality among ants exposed to *B. bassiana* confirmed its pathogenic effect, as evidenced by fungal extrusion from cadavers (Fig 2). From the first day of exposure, contaminated ants exhibited behavioral changes, such as increased agitation and leg grooming. Such grooming behavior functions as a social immunity strategy to remove fungal contaminants and prevent pathogen spread within the colony (Tranter & Hughes, 2015; Gandra et al., 2016; Cotazo-Calambas et al., 2022; Goes et al., 2022; Folgarait & Goffré, 2023; Liu et al., 2019; Ma et al., 2023).

The shortest lethal times observed at higher conidial concentrations align with previous findings, such as *Metarhizium anisopliae* applied to *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae), achieving 100% mortality within 216 hours (Quesada-Moraga et al., 2006).

The efficacy of *B. bassiana* is linked to conidial adhesion to the insect cuticle, germination, proliferation within the host, and the production of toxic compounds (Folgarait & Goffré, 2021; Leal et al., 2024; Liu et al., 2023).

The ISO77 isolate was pathogenic to *A. laevigata* at both tested concentrations. The increase in conidial concentration accelerated insect mortality, indicating that the conidial dose influences the mortality rate and enhances spore spread through sporulation (Portilla et al., 2017; Loko et al., 2022). The concentration of 1.0×10^8 conidia·mL⁻¹ proved most suitable for controlling these ants, demonstrating greater efficiency regarding lethal time. Therefore, *B. bassiana* ISO77 is a promising biological control agent against leaf-cutting ants under laboratory conditions and thus warrants further field trials for validation.



Fig 2. Ant without fungal extrusion and ant with characteristic fungal extrusion, evidencing the growth of *Beauveria bassiana* after exposure to concentrations of 1.0×10^7 and 1.0×10^8 conidia·mL⁻¹.

A - Ant from control killed without fungus extrusion.

B - Ant with characteristic fungal extrusion after exposure to concentrations of 1.0×10^7 and 1.0×10^8 conidia·mL⁻¹ of *Beauveria bassiana*.

Acknowledgments

The authors wish to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Financial Code 001, the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), and the Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM).

Authors' Contribution

KDRP: Conceptualization, Methodology, Validation-Verification, Investigation, Original Draft Writing, Review and Editing, Visualization, Supervision, Project Administration.

RVSV: Software, Formal Analysis, and Data Curation.

ZVC: Review and Editing.

WFJ: Conceptualization.

SDM: Investigation.

EGSF: Investigation.

ITO: Resources, Review and Editing, and Supervision.

RSS: Resources, Review and Editing, and Supervision.

MAS: Conceptualization, Resources, Original Draft Writing, Review and Editing, and Supervision.

References

- Bragança, M.A.L., Souza, M.L.O., Oliveira, R.J. & Samuels, R.I. (2023). Phorid fly parasitism rates vary between individual colonies of the leaf-cutting ant *Atta laevigata*. *Agricultural and Forest Entomology*, 25: 558-570. <https://doi.org/10.1111/afe.12575>
- Cantarelli, E.B., Costa, E.C., Pezzutti, R.V., Zanetti, R. & Fleck, M.D. (2019). Damage by *Acromyrmex* spp. to an initial *Pinus taeda* L. planting. *Floresta Ambiente*, 26: e20160060. <https://doi.org/10.1590/2179-8087.006016>
- Cardoso, S.R.S., Rodrigues, A., Forti, L.C. & Nagamoto, N.S. (2022). Pathogenicity of filamentous fungi towards *Atta sexdens rubropilosa* (Hymenoptera: Formicidae). *International Journal of Tropical Insect Science*, 42: 1215-1223. <https://doi.org/10.1007/s42690-021-00640-2>
- Cotazo-Calambas, K., Niño-Castro, A., Valência-Giraldo, S.M., Gómez-Días, J.S. & Montoya-Lerma, J. (2022). Behavioral response of the leaf-cutting ant *Atta cephalotes* (Hymenoptera: Formicidae) to *Trichoderma* sp. *Journal of Insect Behavior*, 35: 92-102. <https://doi.org/10.1007/s10905-022-09800-9>

- Cruz-Cruz, D., Gómez-Ruiz, J., Barrera, J.F., Sánchez-Peña, S.R. & Mora, J.F.V. (2020). Pathogenicity of *Beauveria bassiana* against immature stages of *Diaphorina citri* under laboratory conditions. *BioOne*, 45: 609-620. <https://doi.org/10.3958/059.045.0303>
- Deka, B., Baruah, C. & Babu, A. (2021) Entomopathogenic microorganisms: their role in insect pest management. *Egyptian Journal of Biological Pest Control*, 31: 121. <https://doi.org/10.1186/s41938-021-00466-7>
- Elkhateeb, W.A., Mousa, K.M., Elnahas, M.O. & Daba, G.M. (2021). Fungi against insects and contrariwise as biological control models. *Egyptian Journal of Biological Pest Control*, 31: 13. <https://doi.org/10.1186/s41938-020-00360-8>
- Farias, R.P., Costa, L.E.N., Barros, I.C.L., Oliveira, A.F.M. & Mehlreter, K. (2018). Selective fern herbivory by leaf-cutter ants of *Atta cephalotes* (L.) in Brazil. *Brazilian Journal of Botany*, 41: 923-929. <https://doi.org/10.1007/s40415-018-0499-z>
- Folgarait, P.J. & Goffré, D. (2021). Biological control of leaf-cutter ants using pathogenic fungi: experimental laboratory and field studies. *Entomologia Experimentalis et Applicata*, 169: 813-824. <https://doi.org/10.1111/eea.13078>
- Folgarait, P.J. & Goffré, D. (2021). *Conidiobolus lunulus*, a newly discovered entomophthoralean species, pathogenic and specific to leaf-cutter ants. *Journal of Invertebrate Pathology*, 186: 107685. <https://doi.org/10.1016/j.jip.2021.107685>
- Folgarait, P.J. & Goffré, D. (2023). Control of pest ants by pathogenic fungi: state of the art. *Frontiers in Fungal Biology*, 4. <https://doi.org/10.3389/ffunb.2023.1199110>
- Gandra, L.C., Amaral, K.D., Couceiro, J.C., Della Lucia, T.M.C. & Guedes, R.N.C. (2016). Mechanism of leaf-cutting ant colony suppression by fipronil used in attractive toxic baits. *Pest Management Science*, 72: 1475-1481. <https://doi.org/10.1002/ps.4239>
- Garcia, J.M., Bordignon, A.M., Gonzaga, G.S. & Torezan, J.M.D. (2020). Tree seedling responses to leaf-cutting ants herbivory in Atlantic Forest restoration sites. *Biotropica*, 52: 789-1006. <https://doi.org/10.1111/btp.12808>
- Goes, A.C., Kooij, P.W., Culot, L., Bueno, O.C. & Rodrigues, A. (2022) Distinct and enhanced hygienic responses of a leaf-cutting ant toward repeated fungi exposures. *Ecology and Evolution*, 12: e9112. <https://doi.org/10.1002/ece3.9112>
- Johari, A., Hermanto, M.A. & Wulandari, T. (2021). Ant diversity inhabited oil palm plantations in a peatland in Sumatra, Indonesia. *Nusantara Bioscience*, 13: 158-163. <https://doi.org/10.13057/nusbiosci/n130204>
- Leal, L.J.A., De la Pena, A.H., Fletes, I.O., López, P.A., Villa-Ruano, N. & Romero-Arenas, O. (2024). Virulence Bioassay of Entomopathogenic Fungi against Adults of *Atta mexicana* under Controlled Conditions. *Applied Sciences*, 14: 3039. <https://doi.org/10.3390/app14073039>
- Liu, H., Lia, Y., Zhang, K., Huang, Z., Wang, S., Liu, C. & Nível, N. (2023). *Akanthomyces dipterigenus* acts as a potential biocontrol agent by causing mortality and inhibiting population growth of *Bemisia tabaci*. *Biological Control*, 186: 105332. <https://doi.org/10.1016/j.biocontrol.2023.105332>
- Liu, L., Zhao, X., Tang, Q., Lei, C. & Huang, Q. (2019). The Mechanisms of Social Immunity Against Fungal Infections in Eusocial Insects. *Toxins*, 11: 244. <https://doi.org/10.3390/toxinas11050244>
- Loko, Y.L.E., Toffa J., Badá B., Dassou, G.A., Zanzana K., Gavodo, D., Adikpeto, J. & Tamo, M. (2022). Potential of *Metarhizium anisopliae* and *Beauveria bassiana* to control *Dinoderus porcellus* (Coleoptera: Bostrychidae) infesting yam chips. *Journal of Asia-Pacific Entomology*, 25: 101885. <https://doi.org/10.1016/j.aspen.2022.101885>
- Ma, M., Luo, J., Li, C., Eleftherianos, I., Zhang, W. & Xu, L. (2023). A life-and-death struggle: interaction of insects with entomopathogenic fungi across various infection stages. *Frontiers in Immunology*, 14: 1329843. <https://doi.org/10.3389/fimmu.2023.1329843>
- Mota Filho, T.M.M., Stefanelli, L.E.P., Camargo, R.S., Matos, C.A.O. & Forti, L.C. (2021). Biological Control in leaf-cutting ants, *Atta sexdens* (Hymenoptera: Formicidae), using pathogenic fungi. *Revista Árvore*, 45. <https://doi.org/10.1590/1806-908820210000016>
- Nogueira, B.R., Oliveira, A.A., Silva, J.P. & Bueno, O.C. (2022). Collection and Long-Term Maintenance of Leaf-Cutting Ants (*Atta*) in Laboratory Conditions. *Biology*, 186. <https://doi.org/10.3791/64154>
- Paramanandham, J., Rangeela, P., Ross, PR & Sathiskumar, S. (2022). Pitfall Traps: A Novel Method to Study Approaching Time of Ant Species. *Entomology and Applied Science Letters*, 9: 57-64. <https://doi.org/10.51847/8qtB3V60K0>
- Pimentel, FA, Bailes, O., Pereira, RC & Viana-Bailez, AM. (2022). Phorid parasitoids of the leaf-cutting ant *Atta laevigata* in the Atlantic Forest: occurrence, parasitism rate, and host size. *Entomologia Experimentalis et Applicata*, 170: 495-504. <https://doi.org/10.1111/eea.13170>
- Portilla, M., Snodgrass, G. & Luttrell, R. (2017). Lethal and Sub-Lethal Effects of *Beauveria bassiana* (Cordycipitaceae) Strain NI8 on *Chrysoperla rufilabris* (Neuroptera: Chrysopidae). *Florida Entomologist*, 100: 627-633. <https://doi.org/10.1653/024.100.0321>
- Qiu, H.L., Lu, L., Zhang, C.Y. & He, Y. (2014). Pathogenicity of individual isolates of entomopathogenic fungi affects feeding preference of red imported fire ants *Solenopsis invicta*. *Biocontrol Science and Technology*, 24: 1286-1296. <https://doi.org/10.1080/09583157.2014.933313>

- Quesada-Moraga, E., Ruiz-García, A. & Santiago-Álvarez, C. (2006). Laboratory Evaluation of Entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium anisopliae* Against Puparia and Adults of *Ceratitis capitata* (Diptera: Tephritidae), *Journal of Economic Entomology*, 99: 1955-1966. <https://doi.org/10.1093/jee/99.6.1955>
- Rojas, M.G., Elliott, R.B. & Morales-Ramos, J.A. (2018). Mortality of *Solenopsis invicta* Workers (Hymenoptera: Formicidae) After Indirect Exposure to Spores of Three Entomopathogenic Fungi. *Journal of Insect Science*, 18: 20. <https://doi.org/10.1093/jisesa/iey050>
- Schultheiss, P., Nooten, S.S., Wang, R., Wong, M.K.L., Brassard, F. & Guénard, B. (2022). The abundance, biomass, and distribution of ants on Earth. *PNAS USA*, 119: e2201550119. <https://doi.org/10.1073/pnas.2201550119>
- Stefanelli, L.E.P., Mota Filho, T.M.M., Camargo, R.S., Matos, C.A.O. & Forti, L.C. (2021). Effects of entomopathogenic fungi on individuals as well as groups of workers and immatures of *Atta sexdens rubropilosa* leaf-cutting ants. *Insects*, 12: 10. <https://doi.org/10.3390/insects12010010>
- Swanson, A.C., Shwendenmann, L., Allen, M.F., Aronson, E.L., Artavia-León, A., Dierick, D., Fernández-Bou, A.S., Harmon, T.C., Cruz, C.M., Oberbauer, S.F., Pinto-Tomás, A.A., Rundel, P.W. & Zelikova, T.J. (2019). Welcome to the *Atta* world: A framework for understanding the effects of leaf-cutter ants on ecosystem functions. *Functional Ecology*, 33: 1386-1399. <https://doi.org/10.1111/1365-2435.13319>
- Swathy, K., Parmar, M.K. & Vivekanandhan, P. (2024). Biocontrol efficacy of entomopathogenic fungi *Beauveria bassiana* conidia against agricultural insect pests. *Environmental Quality Management*, 1088-1913: 1520-6483. <https://doi.org/10.1002/tqem.22174>
- Tranter, C. & Hughes, W.O.H. (2015). Acid, silk and grooming: alternative strategies in social immunity in ants? *Behavioral Ecology and Sociobiology*, 69: 1687-1699. <https://doi.org/10.1007/s00265-015-1980-3>
- Valencia-Giraldo, S.M., Niño-Castro A., López-Peña, A., Trejos-Vidal, D., Correa-Bueno, O. & Montoya-Lerma, J. (2021). Immunity and survival response of *Atta cephalotes* (Hymenoptera: Myrmicinae) workers to *Metarhizium anisopliae* infection: Potential role of their associated microbiota. *Plos One*, 16: e0247545. <https://doi.org/10.1371/journal.pone.0247545>
- Vinha, G.L., Cruz, R.A., Lucia, T.M.C.D., Wilcken, C.F., Silva, E.D., Lemes, P.G. & Zanuncio, J.C. (2020). Leaf-cutting ants in commercial forest plantations of Brazil: biological aspects and control methods. *Southern Forests*, 82: 95-103. <https://doi.org/10.2989/20702620.2019.1639596>
- Yasin, M., Wakil, W., Ghazanfar, M.U., Qayyum, M.A., Tahir, M. & Bedford, G.O. (2017). Virulence of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against red palm weevil, *Rhynchophorus ferrugineus* (Olivier). *Entomological Research*, 49: 3-12. <https://doi.org/10.1111/1748-5967.12260>
- Yucel, C. (2021). Effects of local isolates of *Beauveria bassiana* (Balsamo) Vuillemin on the two-spotted spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae). *Egyptian Journal of Biological Pest Control*, 31: 63. <https://doi.org/10.1186/s41938-021-00409-2>

