Ethiopian Journal of Environmental Studies & Management 17(1): 86 – 94, 2024
ISSN:1998-0507 doi: https://ejesm.org/doi/v17i1.7
Submitted: January 29, 2024 Accepted: March 18, 2024

LABORATORY EVALUATION OF THE INSECTICIDAL POTENTIALS OF DIFFERENT ISOLATES OF *Beauveria bassiana* (Balsamo) VUILLEMIN ON *Zonocerus variegatus* (Orthoptera: Acridoidea)

NWOGWUGWU, J.O., *AYANDOKUN, A.E., OSUNLAJA, O.A. AND ALAMU, O.T.
Department of Forest Conservation and Protection, Forestry Research Institute of Nigeria, Ibadan, Nigeria
*Corresponding author: gbadeayans@yahoo.com

Abstract
The grasshopper, Zonocerus variegatus, is a crop pest in Africa that causes significant damage to crops and forest tree seedlings. The application of synthetic chemicals as management technique in the control of Z. variegatus has deleterious effects on the ecosystem. Therefore, it becomes pivotal to explore other better and safer strategy in the management of this pest. To this end, this study evaluated some isolates of entomopathogenic fungi, Beauveria bassiana, as an alternative in controlling Z. variegatus under laboratory conditions. Synthetic insecticide DDVP (2, 3-dichlorovinyl dimethyl phosphate) and sterile distilled water were used as positive and negative controls, respectively. Contact toxicity and food bioassay methods were employed in the experiments. Mortality counts of the insects were recorded daily for 5 days. The result of the contact application showed that isolate Bb 362 caused significantly higher mortalities of 63% and 90% at 1- and 5-days post application, respectively. Isolates Bb 115 and Bb 11 caused 40% and 55% insect mortalities, respectively, at 5 days post-application. Application of DDVP (2,3-dichlorovinyl dimethyl phosphate) caused 100% insect mortality within 24 hours post application, while food treated with fungal suspensions recorded low insect mortalities after 5 days post application. The study conclusively established that contact toxicity of Beauveria bassiana isolate Bb 362 showed promising potential in Z. variegatus management and can be used as an effective biocontrol agent.

Key Word: *Entomopathogenic fungi, Beauveria bassiana, Zonocerus variegatus, Mortality, Biocontrol agent*

Introduction

Grasshoppers have posed a significant threat to agriculture since the dawn of civilization (Long *et al*., 2019). They are often grouped together with locusts due to their similarities and the nature of damage they cause. Populations of locusts and grasshoppers can rapidly reach harmful levels, causing extensive damage in a short period. In cases of severe infestation, food security becomes a concern, posing a threat to the livelihoods of rural communities in affected areas (Belayneh, 2005).

The Acridoidea family comprises over 500 species known to have caused damage on pasture and crops, resulting in financial losses in the past (Long *et al*., 2019). Notably are the significant outbreaks of various grasshopper species in the United States between 1986 and 1988, affecting 8.2 million hectares and resulting in substantial financial losses totalling $75 million (Lockwood *et al*., 2000). Additionally, in 2010, 2.4 million hectares in Wyoming were invaded by grasshoppers, causing financial losses of $7.4 million (McNary *et al*., 2011). In another study, Branson *et al*. (2006) reported that in North America, grasshoppers annually destroy 21-23% of rangeland and vegetation, resulting in monetary losses of $1.25 billion.

Zonocerus variegatus is recognised as a pest that affects numerous crops in West and Central Africa (Kekeunou *et al*., 2006). The earliest recorded instance of crop damage by grasshoppers in southern Nigeria dates to 1910 (Chiffaud, 1990). Furthermore, this insect has been implicated in the transmission of various plant diseases, such as *Okra mosaic viruses* in Ivory Coast, *Cowpea mosaic viruses* in Nigeria (De Gregorio 1989), and bacterial burn of cassava in Nigeria (De Gregorio, 1989; De Visscher, 1990; Chiffaud, 1990). It is also recognized as a pest and vector of rice diseases in different countries across Africa and worldwide (Nwilene, 2009).

Zonocerus variegatus exhibits high population abundance and fluctuates depending on vegetation cover type, particularly in regions where *Chromolaena odorata* is more prevalent (Kekeunou *et al*., 2007). It is a dominant species among grasshoppers in many farmlands in Nigeria and throughout Africa (Oku *et al*., 2011). During outbreaks, grasshoppers can spread wide and inflict catastrophic damage on grassland, forage, cereal crops, vegetables, and orchard (Lockwood *et al*., 2002).

Various strategies have been employed to manage *Z. variegatus*, with chemical methods consistently prominent. In the past, success in controlling grasshoppers primarily relied on widespread use of broad-spectrum and often cumulative applications of chemical pesticides (Long *et al*., 2019). However, the extensive use of chemical pesticides has given rise to several environmental issues, including environmental contamination, the development of insect resistance, and detrimental effects on human health (Gyawali, 2018). The development of resistance to various chemical insecticides has led to the repeated application of heavy doses, resulting in chemical accumulation in agricultural fields (Ray *et al*., 2016). The detrimental consequences of pesticides in the ecosystem have underscored the need for more environmentally safe, cost-effective, and reliable pest control strategies (Skrzecz *et al*., 2020).

Integrated Pest Management (IPM) in agriculture is a holistic approach to managing pest populations in crop production systems, emphasizing pest management rather than outright elimination. This approach integrates various techniques, including the use of resistant varieties, cultural manipulations, trap cropping, and natural enemies as biological control agents (Skrzecz *et al*., 2020). The application of entomopathogenic microorganisms for insect pests control offers an excellent alternative to chemicals due to its ready availability, harmlessness to beneficial insects, and absence of chemical residues in agricultural ecosystems (Skrzecz *et al*., 2020). Organisms such as *Bacillus* spp.,

Enterobacter spp., *Pseudomonas* spp. *Paenibacillus* spp., and fungi such as *Beauveria* spp., *Metarhizium* spp., *Paecilomyces* spp., *Isaria* spp., *Lecanicillium* spp., and *Hirsutella* spp. have been identified as potential candidates for biological control measures (Skrzecz *et al*., 2020; Sukovata *et al*., 2020).

The fungus, *Beauveria bassiana* (Bals.) Vuill. (Ascomycota: Cordycipitaceae) has demonstrated dual protective potential against *Rhizoctonia solani* and *Pythium myriotylum* in tomato seedlings treated with *B. bassiana* (Tumialis *et al*., 2018; Sukovata and Jaworski, 2010) and has proven effective against various pests. These include *Helicoverpa armigera* in broad bean (Batta, 2007), *Helicoverpa zea* in cotton and tomato (Bukhari *et al*., 2011), *Sesamia calamistis* (Li, 1988), *Ostrinia nubilalis* (Feng *et al*., 1994) and *Spodoptera frugiperda* (Kuzhuppillymyal-Prabhakarankutty *et al*., 2021) infesting corn plants, as well as the Pine-Tree Lappet Moth, *Dendrolimus pini* (Kovac *et al*., 2020). However, there is a need to explore the potential of different isolates of *B. bassiana* against other pests to expand the knowledge about the application of this important entomopathogenic fungus. Therefore, this study aimed to assess the effectiveness of three isolates of *B. bassiana* in the control of *Z. variegatus* under laboratory bioassay conditions.

Materials and Methods

Insect Collection and Preparation

The unsexed third nymphal stage of *Z. variegatus* was collected from the Forestry Research Institute of Nigeria arboretum Ibadan in January 2021. The insects were handpicked, collected into Kilner jars, and brought to the laboratory. They were emptied into rearing cages and provided with fresh leaves of *Gmelina arborea* as food. The insects were allowed to stay in the rearing cages for 48 hours, after which healthy ones were picked for the experiment. Twenty nymphal instars were picked into each Kilner jar, and the mouth of the jars was covered with a screwed lid fixed with a metal wire mesh for proper aerations.

Preparation of Fungi Biocontrol Organisms

The three isolates of *B. bassiana* designated as Bb 11, Bb 115, and Bb 362 were sourced from the microbial collections at IITA Benin. To maintain these cultures, they were sub-cultured on freshly prepared Sabouraud Dextrose Agar (SDA) and incubated for seven days at 25^{o}C to produce sufficient fungal colonies. The SDA medium was prepared by dissolving 65 grams of SDA powder in one liter of distilled water in an Erlenmeyer flask, followed by steaming for 30 minutes to achieve homogeneity, and then autoclaving for 15 minutes at 121°C. To prevent bacterial growth, chloramphenicol powder (0.05g) was added directly into the autoclaved medium at 45^{o}C and gently agitated to ensure uniformity. The medium was subsequently dispensed into 9 cm Petri dishes under a lamina air-flow safety cabinet.

Mycelia fragments and conidia from the newly established cultures were extracted by dislodging them using a sterile scalpel and adding 20 ml sterile distilled water (SDW) containing 0.05% Tween 80 suspension. This mixture was then sieved through 90µm mesh. The concentration of conidia in each suspension was determined using a haemocytometer and a compound microscope at 400x magnification. The concentration was adjusted to 10^{-9} conidia/ml for use in this experimental

study. The viability of the conidia was assessed based on the rate of conidia germination, following the method described by Oliveira *et al*. (2015).

Contact Toxicity Bioassay

The treatments consisted of the suspensions of *Beauveria bassiana* isolates, Bb 11, Bb 115, and Bb 362, DDVP (2,3-dichlorovinyl dimethyl phosphate), a synthetic insecticide serving as the control, and water (a negative control). DDVP was prepared at a rate of 500 ml/ha in accordance with the manufacturer's instructions. In the contact toxicity experiment, twenty insects were put in Kilner jars representing the different treatments. Each insect received two drops of the *B. bassiana* isolates suspension, applied topically on the pronotum. The treatments were replicated five times and arranged in a Completely Randomized Design under standard laboratory conditions. The insects were provided with fresh leaves of *Gmelina arborea* as food daily.

Food Bioassay

In the food bioassay experiment, leaves of *G. arborea* were treated with the same fungal suspension, DDVP, and distilled water as described in the contact toxicity experiment. The insects were allowed to feed on the treated leaves inside the Kilner jars, and new treated leaves were provided daily replacing the old ones daily throughout the experiment.

Mortality of the nymphs in both experiments was recorded at 24-hour intervals for 5 days. Dead grasshopper cardavars were surface sterilized through successive dipping in a 0.5% Sodium hypochlorite (NaOCl) solution for 1 minute, followed by rinsing in sterile distilled water for 1 minute, following the procedure outlined by Lacey and Brooks (1997). Subsequently, they were placed in a sterile petri plate (60 mm in diameter) with a cotton swab moistened with sterile distilled water and then incubated at an ambient temperature of 25ºC. Mycosis was confirmed by microscopic examination of dead grasshoppers.

Data Analysis

Data on the percentage mortality of grasshoppers were analyzed using ANOVA, and treatment means were separated using Tukey's test ($p<0.05$). The standard error of the means (SEM) was also calculated and added to the mean mortality.

Result and Discussion

The Mortality Rate of Z. variegatus in Contact Toxicity Bioassay

In the first 24 hours following the application of DDVP, a 100% mortality rate of *Z. variegatus* was recorded. This observation is consistent with the findings of Farenhorst and Knols (2007), who noted that many available commercial entomopathogenic products are still considered ineffective and less efficient when compared to synthetic insecticides.

The spore suspensions of the three isolates of *B. bassiana* caused significant mortality of *Z. variegatus* at varying degrees throughout the experiment. In contrast, the lowest mortality rate was recorded on insects treated with water. Generally, the mortality rates of *Z. variegatus* increased with exposure time to different treatments. Between 24 and 120 hours after application, isolate Bb 362 caused significantly higher mortality rates of *Z. variegatus* compared to isolates Bb 11 and Bb 115. There was no significant difference in insect mortality caused by isolates Bb 11 and Bb 115 between 24 and 120 hours after treatment (Table 1). This

study demonstrates that all three isolates of the fungus *B. bassiana* (Bb 11, Bb 115, Bb 362) possess insecticidal potential against the third nymphal stage of *Z. variegatus*. These findings are consistent with the report of Jaber and Ownley (2018), which reported the insecticidal potential of *B. bassiana* as mycopesticides and commercial endophytic fungi. The fungal growth observed in insect cadavers when cultured in a petri dish confirmed that the mortality of *Z. variegatus* was due to the application *B. bassiana* spore suspension. The whitish colony growth represents the fungal conidia of *B. bassiana*, resulting from mycelium penetration into the insect's cuticle. Although all three fungal isolates caused insect mortality within the first day of application, their virulence varied from one isolate to another, with the highest virulence observed in Bb 362. This variation could be attributed to differences in their capacity to produce enzymes and metabolites responsible for insect mortality. Additionally, it is possible that the conidia of Bb 362 are more adapted to the cuticle of *Z. variegatus* nymphs, subsequently penetrating the insect haemolymph after cuticle degradation. This observation is supported by previous study indicating that strains of entomopathogenic fungi belonging to the same species can exhibit varying responses in terms of the number of hosts infected, infestation and germination rates, and optimal development temperature (Shahid *et al*., 2012).

Fungal isolate Bb 362 exhibited the highest mortality rate in grasshopper nymphs within the first day of application, while isolates Bb 11 and Bb 115 required more time to achieve significant mortality. This suggests that isolates Bb 11 and Bb 115 had lower insecticidal potency against *Z. variegatus* compared to Bb 362. The reduced performance of isolates Bb 11 and Bb 115 may be attributed to factors such as the production of mucus substance, conidial viability, and the duration of contact affecting the penetration of the insect cuticle (Holder and Keyhani, 2005).

Furthermore, the study revealed that isolate Bb 362 may have evolved to produce more Orthoptera-specific cuticle-degrading proteases (Dias *et al*., 2008) and mechanisms to evade phagocytic haemocyte cells circulating in the haemolymph of *Z. variegatus* (Bidochka *et al*., 2010).

The Mortality Rate of Z. variegatus in Food Bioassay

In the food bioassay experiment, the application of the synthetic insecticide (DDVP) resulted in a 100% mortality on the target organisms within the first 24 hours (Table 2). This mortality rate was significantly higher ($P<0.05$) than those observed in the other treatment groups. However, no significant differences were observed among the three isolates of the fungus between 24 and 120 hours. Notably, isolate Bb 11 caused a 40% insect mortality at 120 hours post-application. In the feeding bioassay, the application of fungal spore suspension on the leaf did not cause significant insect mortality, except in the case of isolate Bb 11 spore suspension. This observation agrees with the findings reported by Mannino *et al*. (2019), which indicates that fungi can infect and colonize insects to a certain extent when ingested. However, the effectiveness of this infection depends on the specific fungal species.

The Bb 11 fungal isolate took a longer time to cause 40% nymph mortality. This

delay could be attributed to the inability of the fungus to establish effective contact and penetrate the insect cuticle. This finding supports the report of Schabel (1976) that infection resulting from fungal ingestion is a rare route for entomopathogenic fungi.

Conclusion

This study highlights the potential of entomopathogenic fungi, *B. bassiana*, as a promising and environmentally friendly alternative for managing *Z. variegatus*, a significant agricultural pest. While chemical insecticide demonstrated rapid initial effectiveness, our focus shifted to the potential of *B. bassiana* isolates, which exhibited varying levels of virulence. Notably, Bb 362 stood out as the most potent, emphasizing the importance of isolate-specific characteristics in pest management. The findings of the study suggest that topical application of fungal formulations may be more efficient than oral ingestion, in line with observations that fungi can infect insects to some extents when consumed. Additionally, the delayed impact of certain isolates, such as Bb 11 may be attributable to their reduced ability to penetrate the insect cuticle. This research contributes to the understanding of sustainable pest management practices and underscores the potential of entomopathogenic fungi as valuable components of integrated pest management strategies. Further investigation into the specific mechanisms and attributes of these fungi is essential for optimizing their use in agricultural pest control.

Table 1: Insecticidal efficacy of topical application of *Beauveria bassiana* isolates on *Zonocerus variegatus*

Treatment	24 hours	48 hours	72 hours	96 hours	120 hours
Bb 11	21.67±6.01c	23.33±4.41c	35.00±5.77c	48.33±7.26b	55.00±7.64b
Bb 115	11.67±9.28c	13.33±8.82cd	23.33±11.6cd	26.67±11.67bc	40.00±11.55b
Bb 362	63.33±8.82b	71.67±4.41b	73.33±3.33b	81.67±3.33a	90.00+5.77a
DDVP	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00+0.00a	100.00±0.00a
H_2O	1.67±1.67c	5.00±2.89d	6.67±4.41d	6.67±4.41c	8.33±4.41c

Means with the same alphabet in the column are not significantly different (Tukey's HSD, P<0.05).

Table 2: Insecticidal potential of leaf application of *Beauveria bassiana* isolates on *Zonocerus variegatus*

Treatment	24 hours	48 hours	72 hours	96 hours	120 hours
Bb 11	6.67±6.67b	23.33±20.89b	31.67±24.55b	36.67±27.28b	40.00±27.83b
Bb 115	0.00±0.00b	1.67±1.67b	5.00±0.00b	6.67±1.67b	6.67±1.67b
Bb 362	0.00±0.00b	0.00±0.00b	1.67±1.67b	6.67±1.67b	6.67±1.67b
DDVP	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a
H_2O	0.00+0.00b	0.00+0.00b	3.33+1.67b	3.33+1.67b	3.33+1.67b

Means with the same alphabet in the column are not significantly different (Tukey's HSD, P<0.05).

Acknowledgement

Authors would like to thank IITA, Benin, for the provision of *Beauveria bassiana* isolates used for this study.

References

Batta, Y. (2007). Control of postharvest diseases of fruit with an invert emulsion formulation of *Trichoderma harzianum* Rifai. *Postharvest Biology and Technology*, 43(1): 143-150.

Belayneh, Y.T. (2005). Acridid pest management in the developing world: a challenge to the rural population, a dilemma to the international community. *Journal of Orthoptera Research*, 14: 187-195.

Bidochka, M.J., Clark, D.C., Lewis, M.W. and Keyhani, N.O. (2010). Could insect phagocytic avoidance by entomogenous fungi have evolved via selection against soil amoeboid predators? *Microbiology,* 156: 2164-2171.

Branson, D.H., Joern, A. and Sword, G.A. (2006). Sustainable management of insect herbivores in grassland ecosystems: new perspectives in grasshopper control. *BioScience*, 56: 743-755.

Bukhari, T., Takken, W. and Koenraadt, C.J. (2011). Development of *Metarhizium anisopliae* and *Beauveria bassiana* formulations for control of malaria mosquito larvae. *Parasites Vectors*, 4: 23. https://doi.org/10.1186/1756-3305-4-23

Chiffaud, J.M. (1990). Le croquet puant *Zonocerus variegatus* (Linne, 1758): Essai de synthese bibliographique. CIRAD-PRIFAS.

De Gregorio, R. (1989). Listecommentee des travauxconsacres a la morphologie, la biologie, láethologie, lálimentation et le polymorphisi mesaisonnier du croquet puant, *Zonocerus*. 11: Alimentation et polymorphis mesaisonnier (Orthoptera: Pyrogomorphidae). *Bulletin of Society of Entomology France*, 94:5-6.

De Visscher, M.N. (1990). Resultat de lénquete SAS 1989 sur *Zonocerus variegatus* (Linne, 1758), AcridienravageurcommunementappeleCriquetpuantouCriquetbariole. RIFAS, Montpellier;.

Dias, B.A., Neves, P.M., Furlaneto-Maia, L., Furlaneto, M.C. (2008). Cuticle-degrading proteases produced by the entomopathogenic fungus *Beauveria bassiana* in the presence of coffee berry borer cuticle. *Brazilian Journal of Microbiology*, 39: 301-306.

Farenhorst, M. and Knols, B.G. (2007). Fungal entomopathogens for the control of adult mosquitoes: a look at the issues. *Biological Control*, 18: 51-59.

Feng, M., Poprawski, T.J. and Khachatourians, G. (1994). Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: Current status. *Biocontrol Science and Technology*, 4(1): 3-34.

Gyawali, K. (2018). Pesticide Uses and its Effects on Public Health and Environment. *Journal of Health Promotion*, 6: 28–36.

Holder, D.J. and Keyhani, N.O. (2005). Adhesion of the entomopathogenic

fungus *Beauveria*(coryceps) *bassiana* to substrata. *Applied environmental Microbiology*, 71: 5260-5266.

Jaber, L.R. and Ownley, B.H. (2018). Can we use entomopathogenic fungi as endophytes for dual biological control of insect pests and plant pathogens? *Biological Control*, 116: 36-45.

Kekeunou, S., Weise, S., Messi, J. and Tamo, M. (2006). Farmers' perception on the importance of variegated grasshopper (*Zonocerus variegatus* (L.)) in the agricultural production systems of the humid forest zone of Southern Cameroon. *Journal of Ethnobiology and Ethnomedicine*, 2(1): 17.

Kekeunou, S., Weise, S. and Messi, J. (2007). Abundance of *Zonocerus variegatus* (L.) (Orthoptera: Pyrgomorphidae) in the natural herbaceous follow and planted forest: Effect of *Chromolaena odorata* (Asteraceae). *Journal of Entomology*, 4: 457-462.

Kovac, M., Gorczak, M., Wrzosek, M., Tkaczuk, C. and Pernek, M. (2020). Identification of entomopathogenic fungi as naturally occurring enemies of the invasive oak lace bug, *Corythucha arcuata* (Say) (Hemiptera: Tingidae). *Insects*, 11(10): 679.

Kuzhuppillymyal-Prabhakarankutty, L., Ferrara-Rivero, F.H., Tamez-Guerra, P., Gomez-Flores, R., Rodríguez-Padilla, M.C. and Ek-Ramos, M.J. (2021). Effect of *Beauveria bassiana*-Seed Treatment on Zea mays L. Response against *Spodoptera frugiperda*. *Applied Science*, 11: 2887. https://doi.org/10.3390/app11072887

Lacey, L.A. and Brooks, W.M. (1997). Initial handling and diagnosis of diseased insects. In: Lacey, L. A. (ed.) Manual *of Techniques in Insect Pathology. Academic Press*, San Diego. California, pp, 1-15.

Li, Z.Z., Li, C.R. and Huang, B. (2001). Discovery and demonstration of the teleomorph of *Beauveria bassiana* (Bals.) Vuill., an important entomogenous fungus. *China Scientific Bulletin*, 46(9): 751-753.

Lockwood, A.J., Anderson-Sprecher, R. and Schell, S.P. (2002). When less is more: Optimization of reduced agent-area treatments (RAATS) for management of rangeland grasshoppers. *Crop Protection*, 21: 551-562.

Lockwood, J.A., Anderson-Sprecher, R. and Schell, S.P. (2000). Grasshoppers and Grassland Health: Managing grasshopper outbreaks within risking environmental disaster. Dordrecht, Netherland: Kluwer Acad. Publication.

Long, Z., Michel, L., Alexandre, L. and David, H. (2019). Locust and grasshopper management. *Annual Review of Entomology*, 64: 15-34.

Mannino, M.C., Huarte-Bonnet, C. and Davyl-Colo, B. (2019). Is the insect cuticle the only entry gate for fungal infection? Insight into alternative modes of action of entomopathogenic fungi. *Journal of Fungi*, 5: 33.

McNary, T.J., Shambaugh, B.A., Elliston, R.J. and Brown, C.L. (2011). Cooperative rangeland grasshopper suppression in Wyoming (USA) in 2010. *Metaleptea,* 31: 10-12.

Nwilene, F., Traore, A., Asidi, A., Sere, Y., Onasanya, A. and Abo, M. (2009). New records of insect vectors of *Rice yellow mottle virus* (RYMV) in Cote d'Ivoire, *West Africa. Crop Protection*, 21(4): 265-275.

Oku, E., Arong, G. and Bassey, D. (2011). Species composition of grasshoppers (Orthoptera) in open plots and farmlands in Calabar Metropolis, Southern Nigeria. *Pakistian Journal of Biological Sciences*, 14(8): 507-510.

Oliveira, D.G.P., Pauli, G., Mascarin, G.M. and Delalibera, I. (2015). A protocol for determination of conidial viability of the fungal entomopathogens *Beauveria bassiana* and *Metarhizium anisopliae* from commercial products. *Journal of Microbiological Methods*, 119: 44–52. https://doi.org/10.1016/j.mimet.2015.09.021

Ray, D., Peace, A., Moore, R., Petr, M., Grieve, Y., Convery, C. and Ziesche, T. (2016). Improved prediction of the climate-driven outbreaks of *Dendrolimus pini* in *Pinus sylvestris* forests. *Forestry*, 89: 230-244.

Schabel, S. (1976). Oral infection of Hylobius pales by *Metarhzium anisopliae*. *Journal of Invertebrate Pathology,* 383: 377-383.

Shahid, A.A., Rao, A.Q., Bakhsh, A. and Husnain, T. (2012). Entomopathogenic fungi as biological controllers: new insights into their virulence and pathogenicity. *Archive of Biological Sciences*, 64: 21-42.

Skrzecz, I., Slusarski, S. and Tkaczyk, M. (2020). Integration of science and practice for *Dendrolimus pini* (L.) management-A review with special reference to Central Europe. *Forest Ecology and Management*, 455(2020): 117697.

Sukovata, L. and Jaworski, Y. (2010). The abundance of the nun moth and lappet moth larvae on trees of different trunk thickness in Scots pine stands in theNotec forest complex. *Forest Research Papers*, 71: 231-237.

Sukovata, L. Dziuk, A., Parratt, M., Bystrowski, C., Dainton, K., Polaszek, A. and Moore, R. (2020). The importance of trap type, trap colour and capture liquid for catching *Dendrolimus pini* and their impact on by-catch of beneficial insects. *Agricultural and Forest Entomology*, 22(4): 319-327.

Tumialis, D., Skrzecz, I., Mazurkiewicz, A. and Pezowicz, E. (2018). Sensitivity of caterpillars of the pine tree lappet moth *Dendrolimus pini* to native strains of entomopathogenic nematodes. *International Journal of Pest Management*, https://doi.org/10.1080/09670874.2018.1519203