Scientific Bulletin. Series F. Biotechnologies, Vol. XXVI, No. 2, 2022
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

PATHOGENICITY OF *Beauveria bassiana*, *B. pseudobassiana*, AND *Metarhizium anisopliae* INDIGENOUS ISOLATES AGAINST *Plodia interpunctella* AND *Galleria mellonella* IN LABORATORY ASSAYS

Daniel COJANU[1, 2], Maria Cristina LUMÎNARE[1, 2], Monica Mihaela DINU[1, 2], Ana-Cristina FĂTU[1]

[1]Research - Development Institute for Plant Protection, 8 Ion Ionescu de la Brad Blvd., District 1, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: cristina.fatu@icdpp.ro

Abstract

Using two different bioassay methods, the pathogenicity of three isolates of Beauveria bassiana, one of B. pseudobassiana and one of Metarhizium anisopliae, was evaluated against two model insects, Plodia interpunctella and Galleria mellonella in larval stage. In laboratory conditions, the insects were treated by immersion in a conidial suspension of 1x10^8 UFC/ml and by dusting. Larval mortalities were recorded daily, 14 days post-exposure. All the fungal isolates have been shown to be pathogenic to test insects. Thus, two isolates of B. bassiana determined the highest mycosis percentages for test insects both by immersion and dusting. One B. bassiana isolate (BbTd1) killed P. interpunctella larvae in the shortest time (6 days), and the other isolate (BbTd2) determined the highest mycoses percentage, followed by B. pseudobassiana isolate (BpPa) by both treatment methods. The highest mycosis percentage was determined by BbTd2 and BPa isolates in the dusting treatment. G. mellonella larvae proved to be the least sensitive to fungal treatments applied by immersion (MST>50%). In the dusting treatment, the BbTd1 and MaF isolates induced the highest percentage of mycosis of G. mellonella larvae. All isolates have pathogenicity against test insects, indicating their possible use for biocontrol.

Key words*: Beauveria bassiana, B. pseudobassiana, Metarhizium anisopliae, immersion, dusting.*

INTRODUCTION

By 2050, the number of people to be fed will be as significant as nine billion (Hawkins et al., 2018). Sustainable food production for all populations should be considered, and crop protection has an essential role in maintaining soil and crop yields healthy (Godfray et al., 2010). Pests, pathogens, and unfavorable growing conditions are responsible for up to 26% of crop losses, estimated at over $470 billion worldwide (Bamisile et al., 2021). The evolution of pesticide resistance of pests and pathogens in agriculture and concerns for sustainable agriculture has led to a reassessment of pest control options. In recent years, awareness of the impacts of chemical plant protection products on the environment, soil, biodiversity, and human health has resulted in struggles to reduce dependence on chemical pesticides (Committee on the Environment, Public Health and Food Safety of European Parliament, 2019).

Entomopathogenic fungi are currently used as biological control agents (BCA) in biological control programs and integrated crop protection strategies (IPM) against pests, being an environmentally friendly alternative to chemical pesticides. The idea of using entomopathogenic microorganisms to control harmful insects appeared about two hundred years ago (Tanada and Kaya, 1993). After discovering the entomopathogenic character of some fungi, numerous studies on the interaction with arthropods have been conducted (Steinhaus, 1975). To date, more than 700 species have been described and have been characterized as insect-pathogenic fungi (Khachatourians & Qazi, 2008). The most studied entomopathogenic fungal species are *Beauveria bassiana* (Balsamo-Crivelli), Vuillemin, *Isaria fumosorosea* Wize,

Metarhizium anisopliae (Metchnikoff) Sorokin, and *Lecanicillium lecanii* (Zimmerman) Viegas (Li et al., 2011; Chen et al., 2015). The most common mycopesticides are products formulated from *B. bassiana*, *M. anisopliae*, *B. brongniartii*, and *I. fumosorosea* (Bamisile et al., 2021).

In recent years, an increasing body of molecular evidence has shown that the *Beauveria* genus is divided into cryptic lineages, and new species were described, *B. asiatica*, *B. australis*, *B. kipukae*, *B. pseudobassiana*, *B. sungii*, and *B. varroae* (Rehner et al. 2011).

B. pseudobassiana has many morphological similarities to *B. bassiana* (Wang et al., 2020) and probably it has been confused with this species before. Maybe this is the reason for only a few studies available on host diversity and virulence of *B. pseudobassiana*. Recently *B. pseudobassiana* has been shown to have great potential in the biocontrol of numerous insect pests (Wang et al., 2020).

The path from discovering a BCA in a natural outbreak to its commercialization as a bioinsecticide goes through many laboratory, field, and greenhouses experiments (Dent, 1998).

To be used as a mycoinsecticide, it is necessary to establish the pathogenicity, virulence, temperature ranges, and humidity requirements for germination, infection, sporulation, etc., for each fungal isolate.

The Indian meal moth *P. interpunctella* (Lepidoptera: Pyralidae) is a common cosmopolitan household principally on stored food products and processed food commodities. It is one of the most used lepidopterans as a test insect (Takov et al., 2020) for different research topics such as sexual selection (Gage, 1998; Lewis et al., 2011) and host-parasite dynamics (Sait et al., 1994; Knell et al., 1996).

G. mellonella is widely used as a model insect to evaluate bacterial pathogenesis and virulence (Jønsson et al., 2017; Morales et al., 2019). The size of the larvae makes their manipulation easy. Monitoring survival is also very timely because larvae acquire a dark color due to strong melanization when they die (Contador & Zaragoza, 2014).

G. mellonella and *P. interpunctella* are known to be susceptible to entomopathogenous fungi like *B. bassiana* and *M. anisopliae* so they are used as model host insects in pathogenic investigations (Būda & Pečiulytė, 2008; Hussein, 2011; Baydar et al., 2016; Vertyporokh et al., 2020) and also relatively easy to grow in the laboratory.

This paper aimed to evaluate the pathogenicity of some *Beauveria bassiana*, *B. pseudobassiana,* and *Metarhizium anisopliae* indigenous isolates recovered from insects against the two model test insects, *Plodia interpunctella* and *Galleria mellonella.*

MATERIALS AND METHODS

Test insects: *Galleria mellonella* and *Plodia interpunctella* larvae were obtained from cultures held at Research-Development Institute for Plant Protection (RDIPP) using insect colonies maintained in the laboratory at 23±2°C, in sterile glass gears, 14:10 h, L:D. The larvae were reared on Hydak medium amended with bee wax (350 ml/kg medium).

Fungal material: Three isolates of *Beauveria bassiana*, one isolate of *B. pseudobassiana*, and one isolate of *M. anisopliae* were used in the experiments (Table 1).

Table 1. Origin of fungal isolates

Code	Species	Host insect	Region of isolation
BbTd1	*Beauveria bassiana*	*Tanymecus dilaticollis* (adult)	Tulcea
BbTd2	*Beauveria bassiana*	*Tanymecus dilaticollis* (adult)	Ilfov
BbIt	*Beauveria bassiana*	*Ips typographus* (adult)	Botosani
BpPa	*Beauveria pseudobassiana*	*Pyrrhocoris apterus* (adult)	Giurgiu
MaF	*Metarhizium anisopliae*	*Anoxia villosa* (larva)	Ialomița

They were isolated from natural infected insects and maintained as pure cultures in the Culture Collection of Entomopathogenic Fungi, Department of Useful Organisms (RDIPP Bucharest).

All fungal isolates were grown on sterile barley grains in polypropylene bags (30 x 50 cm). The aerial conidia of *Beauveria* and *Metarhizium* isolates were produced by two-stage and one-stage techniques, respectively (Mascarin and

Jaronski, 2016). After 30 days of incubation at 25˚C, 50 grams of barley kernels colonized by fungus (0.8-9 x10^9 UFC/g) was washed with 40 ml sterile distilled water containing Tween 80 (0.01%). After homogenization, in order to remove coarse impurities, the suspension was filtered through sterile cotton wool. Conidial concentrations were determined using a Burker hemocytometer, at a 400x magnification. The adjustment of the suspension titer was made by dilution with sterile distilled water containing Tween 80 (0.01%).

Bioassay: Batches of 15 or 20 larvae were pre-sorted into separate 80 ml pp tubes (4cm Ø) with snap-top lids just before the testing. The larvae were exposed to the fungus by immersion or by dusting.

In immersion treatment, each batch of insects was transferred on the bottom of a Sartorius funnel covered with filter paper connected with vacuum pump over which 30 ml of each fungal suspension was applied. Sterile-distilled water with Tween 80 was used for control experiments. After 10 seconds, the liquid was absorbed and after one minute, the larvae were transferred individually in sterile, compartmentalized plastic boxes to avoid cross-contamination. Equal amounts of diet were distributed for each insect. There were three replicates (boxes) per treatment and insect species and 15 to 20 individuals per box. Insects were incubated at 25°C. Larval mortalities were recorded daily, for 14 days, after-exposure.

In dusting treatment, groups of 15 larvae of *P. interpunctella* or 20 larvae of *G. mellonella* were sorted into sterile plastic tubes with a capacity of 80 ml (4 cm diameter) provided with a lid over which barley kernels colonized by fungus were inserted to cover the larvae completely and left in contact an hour, after which they were individually transferred to sterile compartmentalized boxes with lids. Each insect was distributed equal portions of food. Insects were incubated at 23± 1ºC, 16:8 L: D, and 50-60% humidity. Mortality of larvae was recorded daily, during 14 days after inoculation. The dead larvae were removed from the box and placed in Petri dishes provided with moistened filter paper and incubated at 25°C for 3-5 days to stimulate the appearance of fungal mycelium. Death due to fungus infections were confirmed by conidia formation on cadavers.

Statistical analysis: the effectiveness of fungal isolates was expressed as a cumulative percentage of mycosis. The virulence of each isolate on each insect species, was estimated by the values of median survival time (MST), calculated by Kaplan Meyer survival curves which were modelled using GraphPadPrism V7 and a log-rank (Mantel-Cox) test was applied to the significance threshold $p < 0.05$ (GraphPad Software, San Diego California USA). The individuals who survived until the end of the observation period were considered censored.

The percentages of larval mortality were transformed (arcsin) and analyzed using variant analysis. The averages were compared using the Tukey test and were considered statistically different at a signification level of 5%.

RESULTS AND DISCUSSIONS

Both insect species have manifested diseases caused by treatment by immersion in conidial suspensions or by direct contact with barley kernels colonized by fungus (dusting). The mycelium that covered the treated larvae confirmed that they died from fungal infection. There were no deaths due to fungal infection in control.

The effect of treatments on the larvae of *Plodia interpunctella*

Immersion treatment

The lowest percentage of survival (the highest mortality) was recorded in the case of larvae treated with *B. bassiana* isolate (BbTd2) after ten days of incubation, followed by the isolate of *B. pseudobassiana* (BpPa), after eight days of incubation (Figure 1).

The comparison of survival curves showed a significant difference in susceptibility of *P. interpunctella* larvae to immersion treatment (X^2=14.56, p=0.0057) with different fungal isolates. All fungal isolates resulted in a significantly lower percentage survival of *P. interpunctella* larvae compared to the control demonstrated by log-rank analyses (Table 2). The laboratory results of the virulence bioassay show the median survival time (MST) for the larvae of test insect, *P interpunctella* assessed at 14 d is presented in Table 3.

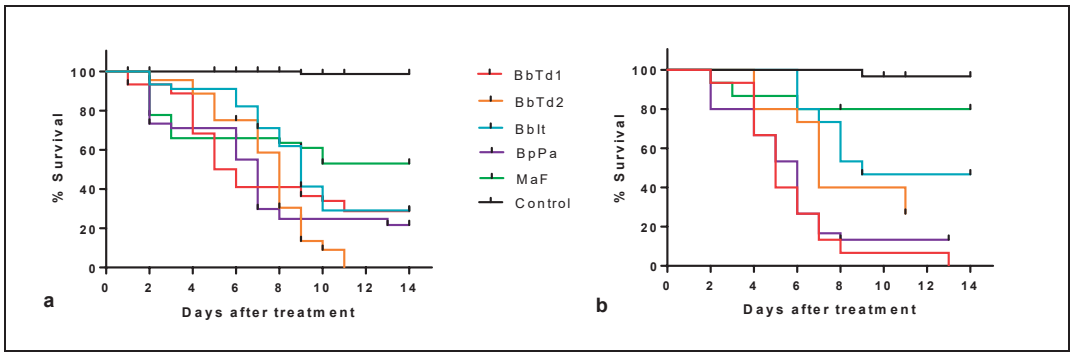

Figure 1. Percentage survival of *Plodia interpunctella* larvae after immersion (a) and dusting (b) treatments with three fungal isolates of *Beauveria bassiana* (BbTd1, BbTd2, and BbIt), one isolate of *B. pseudobassiana* (BpPa), and one isolate of *Metarhizium anisopliae* (MaF), recorded after 14 days

Table 2. Pairwise treatment comparisons using Log-Rank (Mantel-Cox) test based on Kaplan-Meir survival analysis of *Plodia interpunctella* larvae after immersion and dusting treatment

Immersion										
Isolate	**BbTd1**		**BbTd2**		**BbIt**		**BpPa**		**MaF**	
	X^2	P	X^2	P	X^2	P	X^2	P	X^2	P
BbTd1	-	-								
BbTd2	0.50	0.47	-	-						
BbIt	1.64	0.199	9.19	0.002	-	-				
BpPa	0.08	0.769	0.23	0.631	5.81	0.015	-	-		
MaF	2.75	0.096	10.25	0.001	1.91	0.166	7.24	0.007	-	-
Control	15.70	<0.0001	27.58	<0.0001	15.56	<0.0001	20.58	<0.0001	9.04	0.002

Dusting										
Isolate	**BbTd1**		**BbTd2**		**BbIt**		**BpPa**		**MaF**	
	X^2	P	X^2	P	X^2	P	X^2	P	X^2	P
BbTd1	-	-								
BbTd2	5.66	0.0170	-	-						
BbIt	16.10	<0.0001	1.38	0.238	-	-				
BpPa	0.51	0.4735	4.54	0.033	11.04	0.0009	-	-		
MaF	17.89	<0.0001	5.98	0.0145	2.412	0.12	14.87	0.0001	-	-
Control	32.39	<0.0001	17.2	<0.0001	9.308	0.002	22.77	<0.0001	2.86	0.09

Table 3. Median survival time (MST) of the *Plodia interpunctella* larvae treated with isolates of *B. bassiana*, *B. pseudobassiana,* and *Metarhizium anisopliae* for 14 days

Isolate	MST (days)	
	Immersion	Dusting
BbTd1	6	5
BbTd2	8	7
BbIt	9	9
BpPa	7	6
MaF	-	-
Control	-	-

MST= Median Survival Time

The shortest median survival time was registered for larvae treated with suspensions of *B. bassiana* isolate BbTd1 (6 d) followed by *B. pseudobassiana* isolate, BpPa (7 d). The log-rank analyses indicate that the two treatments are statistically similar in terms of survival percentage (X^2=0.08, p=0.769). Because survival of larvae over 14 days was more than 50% (Figure 1), the MST for *P. interpunctella* untreated control and MaF treatment could not be determined. This is according to another experiment, where more than 50% of *P. interpunctella* larvae survived during 14 days after being sprayed with *Paecilomyces farinosus* conidial suspension (2.6×10^6 conidia ml^{-1}) (Būda & Pečiulytė, 2008).

Regarding the percentage of mycosis, statistical analysis indicated that there are no significant differences between treatments. However, the highest rate of mycosis was recorded in the case of treatment with *B. bassiana* BbTd2 isolate (82.2%), and the lowest in the case of treatment with *M. anisopliae* MaF isolate (44.4%) (Figure 2, a). Similar results were

registered by Mantzoukas et al. (2021), who noticed an 86.5% and 50% mortality of *P. interpunctella* larvae when were sprayed with $1x10^8$ conidia ml^{-1} of *Isaria fumosorosea* and *Gnomoniopsis castaneae*, respectively.

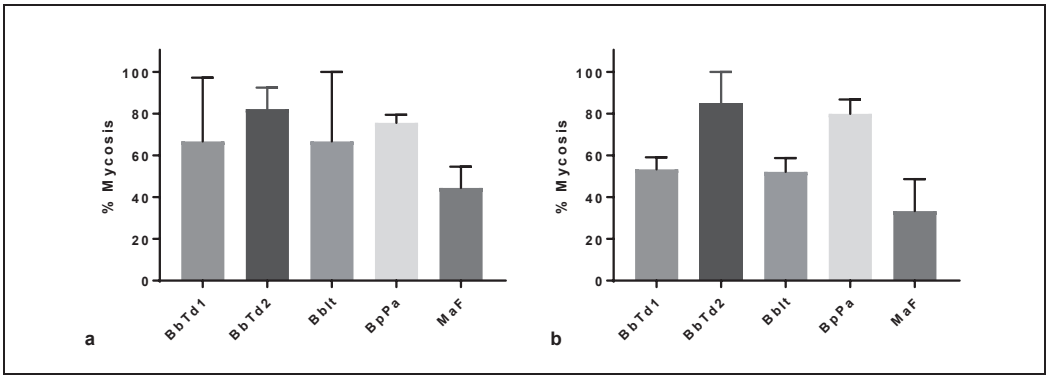

Figure 2. Cumulative percentage of mycosis (±SD) (before arcsin transformation) of *Plodia interpunctella* larvae treated by immersion (a) and dusting (b), with three different isolates of *Beauveria bassiana* (BbTd1, BbTd2 and BbIt), one isolate of *B. pseudobassian* (BpPa) and one isolate of *Metarhizium anisopliae* (MaF)

Dusting treatment

The lowest survival percentage was recorded in the case of larvae treated with BbTd1 followed by BpPa, after eight days of incubation (Figure 1). The log-rank analyses indicate that the two treatments are statistically similar in terms of survival percentage (X^2=0.51, p=0.473). As in the immersion treatment, those two isolates also determined the shortest MST of 5 and 6 days, respectively.

The comparison of survival curves indicated a significant difference in the susceptibility of *P. interpunctella* larvae to treatment with different fungal isolates (X^2=33.34, p=0.0001) by dusting. All fungal isolates resulted in a significantly lower survival percentage of *P. interpunctella* larvae compared to the control demonstrated by log-rank analyses (Table 2).

The method of treating the larvae of *P. interpunctella* by dusting highlighted again the effectiveness of the BbTd2 isolate, which determined the highest percentage of mycosis (85%) (Figure 2, b).

The effect of treatments on the larvae of *Galleria mellonella*

Immersion treatment

The larvae of *G. mellonella* proved to be less sensitive to fungal treatments applied by immersion than the larvae of *P. interpunctella*, the percentage of survival being more than 50% (Figure 3, a), which is why the average survival time could not be calculated. All fungal isolates resulted in a significantly lower percentage survival of *G. mellonella* larvae than the control demonstrated by log-rank analyses (Table 4). Mycosis percentages were also reduced, with the highest being induced by the strains BbTd1 (43.3%) and MaF (37.7%) (Figure 4, a).

This could be due to poor conidial attachment of conidia from aqueous suspension to the insect cuticle. This is in contrast with results from other similar experiments in which *G. mellonella* proves to be more sensible to *Beauveria* action. In a study of genes involved in *B. bassiana* infection to *G. mellonella*, Chen et al. (2018) obtained 90% G. *mellonella* larvae mortality after 99 h post treatment. In another experiment evaluating the virulence of various isolates, a mortality of 100% of *G. mellonella* larvae was due to *B. bassiana* and *M. anisopliae* treatment at concentrations of 10^5 or 10^6 conidia ml^{-1} (Hussein et al., 2011).

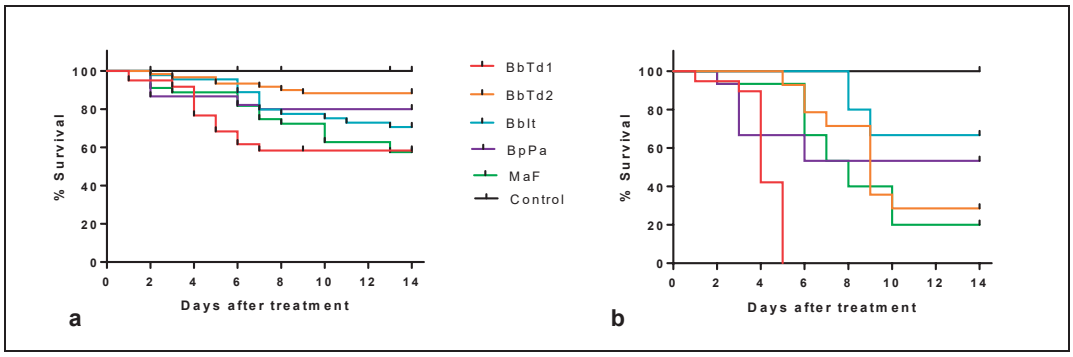

Figure 3. Percentage survival of *Galleria mellonella* larvae after immersion (a) and dusting (b) treatments with three fungal isolates of *Beauveria bassiana* (BbTd1, BbTd2 and BbIt), one isolate of *B. pseudobassiana* (BpPa) and one isolate of *Metarhizium anisopliae* (MaF) recorded after 14 days

Table 4. Pairwise treatment comparisons using Log-Rank (Mantel-Cox) test based on Kaplan-Meir survival analysis of *Galleria mellonella* larvae after immersion and dusting treatment

Immersion										
Isolate	BbTd1		BbTd2		BbIt		BpPa		MaF	
	X^2	P	X^2	P	X^2	P	X^2	P	X^2	P
BbTd1	-	-								
BbTd2	14.29	0.0002	-	-						
BbIt	2.86	0.090	4.78	0.028	-	-				
BpPa	4.85	0.0270	1.51	0.218	0.65	0.418	-	-		
MaF	0.14	0.699	12.20	0.0005	1.62	0.202	3.97	0.046	-	-
Control	35.73	<0.0001	8.26	0.004	22.63	<0.0001	15.06	0.0001	35.49	<0.0001

Dusting										
Isolate	BbTd1		BbTd2		BbIt		BpPa		MaF	
	X^2	P	X^2	P	X^2	P	X^2	P	X^2	P
BbTd1	-	-								
BbTd2	27.3	<0.0001	-	-						
BbIt	31.45	<0.0001	3.84	0.049	-	-				
BpPa	8.21	0.0042	0.23	0.626	1.38	0.239	-	-		
MaF	24.95	<0.0001	0.50	0.475	7.71	0.005	1.08	0.296	-	-
Control	31.45	<0.0001	16.2	<0.0001	5.81	0.015	8.86	0.002	20.14	<0.0001

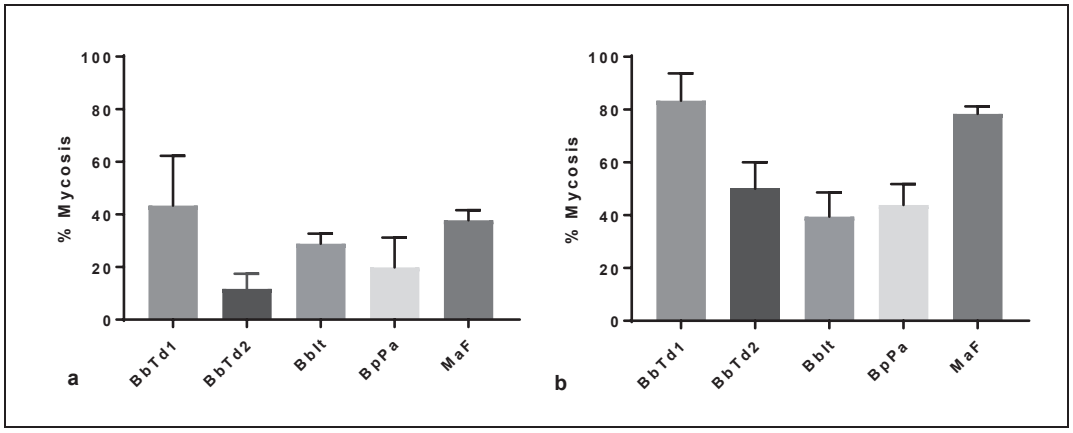

Figure 4. Cumulative percentage of mycosis (±SD) (before arcsin transformation) of *Galleria mellonella* larvae treated by immersion (a) and dusting (b), with three different isolates of *Beauveria bassiana* (BbTd1, BbTd2 and BbIt), one isolate of *B. pseudobassian* (BpPa) and one isolate of *Metarhizium anisopliae* (MaF)

When Oreste et al. (2012) evaluated the pathogenicity of 23 isolates of *B. bassiana* and four of *M. anisopliae* against *G. mellonella* and *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvae in laboratory assays, a mortality of 80-100% after 17 days was reported, using 2×10^6 conidia ml^{-1} fungal suspensions, and that two *B. bassiana* isolates killed *G. mellonella* larvae within 2.2 and 2.3 days, respectively. But Kryukov et al. (2018) reported 63% mortality after 12 days due to mycosis of *Galleria* larvae treated with 10^8 conidia ml^{-1} of *B. bassiana* occurred. The authors state that the increase of the mycosis percent was due to the transmission of conidia between wax moth larvae in clusters. Other entomopathogenic species like *Purpureocillium lilacinus* prove to be highly infectious for *G. mellonella,* causing 100% larval mortality within seven days post-immersion with 1×10^8 conidia ml^{-1} and a median lethal time (LT50) value of 1.83 days (Demirci & Altuntaş, 2019). Using the agar surface technique, *P. lilacinus* demonstrated very low infectivity of only 30% larval mortality on *G. mellonella* in 10 days and an LT50 value of 16.16 days (Baydar et al., 2016).

Dusting treatment

The comparison of survival curves (Figure 3) indicated a significant difference in the susceptibility of *G. mellonella* larvae to treatment with various fungal isolates (X^2=55.01, p<0.0001) by dusting.

The comparison of survival curves indicated that *B. bassiana* isolate BbTd1 is significantly more effective than all the other fungal isolates (Table 4) killing the larvae treated by dusting within the shortest time (4 d) (Table 5).

Table 5. Median survival time (MST) of the *Galleria mellonella* larvae treated with isolates of *Beauveria. bassiana*, *B. pseudobassiana* and *Metarhizium anisopliae* for 14 days

Isolate	MST (days)	
	Immersion	Dusting
BbTd1	-	4
BbTd2	-	9
BbIt	-	-
BpPa	-	-
MaF	-	8
Control	-	-

MST= Median Survival Time

For the BbIt and BpPa isolates, the average survival times could not be calculated. Dusting treatments with BbTd1 and MaF isolates induced the highest percentage of mycosis of *G. mellonella* larvae of 83% and 78%, respectively (Figure 4).

About the efficacy of treatment related to the kind of bioassay (with "dry" conidia by direct contact or dusting and "wet" conidia by dipping or spray), our study shows that it depends on the target pest, *G. mellonella* being more susceptible to fungus infection applied by dusting than by immersion. The treatments of *Rhynchophorus ferrugineus* with dry conidia of *B. bassiana* induced significant adult mortality compared to the dipping method (Ricaño et al., 2013; Güerri-Agulló et al., 2010). The laboratory and greenhouse trials results found dry conidia of *M. anisopliae* to be more effective than wet conidia in infecting mosquitoes (*Culicoides nubeculosus*), causing 100% mortality after five days (Ansari et al., 2011). In contrast, the mortality of 1st larval instar of both *H. variegata* and *C. undecimpunctata* and pupal stage of *C. undecimpunctata* were significantly increased with the spray method only (Sayed et al., 2021).

CONCLUSIONS

The obtained results demonstrated the efficacy of pathogenicity screening to isolate entomopathogenic fungi to control insect pests, and the availability of indigenous virulent entomopathogenic fungi, which can be exploited for the development of sustainable crop protection strategies.

ACKNOWLEDGEMENTS

This research was supported by the Romanian Ministry of Agriculture and Rural Development (MADR), ADER 1.5.6 project within the Sectorial program.

REFERENCES

Ansari, M. A., Pope, E. C., Carpenter, S., Scholte, E. J., & Butt, T. M. (2011). Entomopathogenic fungus as a biological control for an important vector of livestock disease: the Culicoides biting midge. *PloS one*, 6(1), e16108.

Bamisile, B. S., Akutse, K. S., Siddiqui, J. A., & Xu, Y. (2021). Model application of entomopathogenic fungi as alternatives to chemical pesticides: prospects, challenges, and insights for next-generation sustainable agriculture. *Front. Plant Sci.* 12:741-804.

Baydar, R., Güven, Ö., & Karaca, I. (2016). Occurrence of entomopathogenic fungi in agricultural soils from Isparta province in Turkey and their pathogenicity to *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) larvae. *Egyptian Journal of Biological Pest Control*, *26*(2), 323-327.

Būda, V., & Pečiulytė, D. (2008). Pathogenicity of four fungal species to Indian meal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). *Ekologija*, *54*(4).

Chen, A., Wang, Y., Shao, Y., Zhou, Q., Chen, S., Wu, Y., Chen, H., & Liu, E. (2018). Genes involved in *Beauveria bassiana* infection to *Galleria mellonella*. Archives of microbiology, 200(4), 541-552.

Chen, X., Li, L., Hu, Q., Zhang, B., Wu, W., Jin, F., et al. (2015). Expression of dsRNA in recombinant *Isaria fumosorosea* isolate targets the TLR7 gene in *Bemisia tabaci*. *BMC Biotechnol.* 15:64.

Committee on the Environment, Public Health and Food Safety of European Parliament. (2019) Report on implementing Directive 2009/128/EC on the sustainable use of pesticides. Available: https://www.europarl.europa.eu/doceo/document/A-8-2019-0045_EN.html (accessed 01.03.2022).

Contador, N. T. & Zaragoza, O. (2014). Expanding the use of alternative models to investigate novel aspects of immunity to microbial pathogens, *Virulence*, 5:4, 454-456.

Demirci, S. N. Ş., & Altuntaş, H. (2019). Entomopathogenic potential of *Purpureocillium lilacinum* against the model insect *Galleria mellonella* (Lepidoptera: Pyralidae). Environmental and Experimental Biology, 17(2), 71-74.

Dent, D.R. (1998). Removing the barriers to commercialization: summary of the process and key issues for a mycoinsecticide for locust and grasshopper control. LUBILOSA, CAB International, Silwood Park, Ascot, UK.

Gage, M. J. G (1998). Influences of sex, size, and symmetry on ejaculate expenditure in a moth. *Behavioral Ecology* 9: 592–597.

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S., Thomas, S. M. & Toulmin, C. (2010). Food security: the challenge of feeding 9 billion people. *Science* 327, 812–818.

Güerri-Agulló, B., Gómez-Vidal, S., Asensio, L., Barranco, P., & Lopez-Llorca, L. V. (2010). Infection of the red palm weevil (*Rhynchophorus ferrugineus*) by the entomopathogenic fungus *Beauveria bassiana*: a SEM study. Microscopy research and technique, 73(7), 714-725.

Hawkins, N. J., Bass, C., Dixon, A., & Neve, P. (2018). The evolutionary origins of pesticide resistance. *Biological reviews of the Cambridge Philosophical Society*, 94(1), 135–155.

Hussein, K.A., Abdel-Rahman, M.A., Abdel-Mallek, A.Y., El-Maraghy, S.S., & Joo, J.H. (2011). Pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* against *Galleria mellonella*. *Phytoparasitica*, 40, 117-126.

Jønsson, R., Struve, C., Jenssen, H., Krogfelt, K. A. (2017). The wax moth *Galleria mellonella* as a novel model system to study Enteroaggregative *Escherichia coli* pathogenesis. *Virulence*, *8*, 1894–1899.

Khachatourians, G. G., & Qazi, S. S. (2008). Entomopathogenic fungi: biochemistry and molecular biology, in *Human and Animal Relationships*. eds. A. A. Brakhage and P. F. Zipfel (Berlin, Heidelberg: Springer), 33–61.

Knell, R. J., Begon, M. & Thompson, D. J. (1996) Transmission dynamics of *Bacillus thuringiensis* infecting *Plodia interpunctella*: a test of the mass action assumption with an insect pathogen. *Proceedings of the Royal Society of London* B 263: 75–81.

Kryukov, V. Y., Kryukova, N. A., Tyurin, M. V., Yaroslavtseva, O. N., & Glupov, V. V. (2018). Passive vectoring of entomopathogenic fungus *Beauveria bassiana* among the wax moth *Galleria mellonella* larvae by the ectoparasitoid *Habrobracon hebetor* females. *Insect science*, 25(4), 643-654.

Lewis, Z., Wedell, N. & Hunt, J. (2011) Evidence for strong intralocus sexual conflict in the Indian meal moth, *Plodia interpunctella*. *Evolution* 65: 2085–2097.

Li, S.-J., Xue, X., Ahmed, M. Z., Ren, S.-X., Du, Y.-Z., Wu, J.-H. (2011). Host plants and natural enemies of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in China. *Insect Sci.* 18, 101–120.

Mantzoukas, S., Lagogiannis, I., Ntoukas, A., Tziros, G.T., Poulas, K., Eliopoulos, P.A., Avtzis, D.N. (2021). Could *Gnomoniopsis castaneae* Be Used as a Biological Control Agent against Insect Pests? Applied Sciences 11, 4066. https:// doi.org/10.3390/app11094066.

Mascarin, G. M., & Jaronski, S. T. (2016). The production and uses of *Beauveria bassiana* as a microbial insecticide. *World Journal of Microbiology and Biotechnology*, 32(11), 1-26.

Morales, S. R., Ocampo, M. F., Barboza,O. V., Martínez, V. K.,Pérez, J. A. & González, D. E. (2019). Assessing the pathogenicity of two bacteria isolated from the Entomopathogenic Nematode *Heterorhabditis indica* against *Galleria mellonella* and Some Pest Insects. *Insects*, 26;10(3):83.

Oreste, M., Bubici, G., Poliseno, M., Triggiani, O., & Tarasco, E. (2012). Pathogenicity of *Beauveria bassiana* (Bals.-Criv.) Vuill. and *Metarhizium anisopliae* (Metschn.) Sorokin against *Galleria mellonella* L. and *Tenebrio molitor* L. in laboratory assays. *Redia*, 95, 43-48.

Rehner, S. A., Minnis, A. M., Sung, G.-H., Luangsa-ard, J. J., Devotto, L., Humber, R. A. (2011). Phylogeny and systematics of the anamorphic, entomopathogenic genus *Beauveria*, 103(5), 1055–1073. doi:10.3852/10-302.

Ricaño, J., Güerri-Agulló, B., Serna-Sarriás, M. J., Rubio-Llorca, G., Asensio, L., Barranco, P., & Lopez-Llorca, L. V. (2013). Evaluation of the pathogenicity of multiple isolates of *Beauveria bassiana* (Hypocreales: Clavicipitaceae) on *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae) for the assessment of a solid formulation under simulated field conditions. Florida entomologist, 1311-1324.

Sait, S. M., Begon, M. & Thompson, D. J. (1994). The effects of a sublethal baculovirus infection in the Indian meal moth, *Plodia interpunctella. Journal of Animal Ecology* 63: 541–550.

Sayed, S., Elarrnaouty,S.-A., AlOtaibi, S., Salah, M. (2021). Pathogenicity and Side Effect of Indigenous *Beauveria bassiana* on *Coccinella undecimpunctata* and *Hippodamia v*ariegata (Coleoptera: Coccinellidae). Insects, 12, 42. https://doi.org/10.3390/insects 12010042.

Steinhaus, E. A. (1975). Disease in a Minor Chord. Ohio State University Press, Columbus. 488 pp.

Takov, D., Tchorbanov, A.m Pilarska, D. & Ostoich, P. (2020). Lepidopterans as model organisms in studies of insect immunity: a review. *Polish Journal of Entomology*. 89. 207-225.

Tanada, Y. & Kaya, H. K. eds. (1993). Insect Pathology. London: Academic Press, Inc.

Vertyporokh, L., Hułas-Stasiak, M., Wojda, I. (2020). Host-pathogen interaction after infection of *Galleria mellonella* with the filamentous fungus *Beauveria bassiana*. Insect Sci. Oct; 27(5):1079-1089. doi: 10.1111/1744-7917.12706.

Wang, Y., Tang, D. X., Duan, D. E., Wang, Y. B., & Yu, H. (2020). Morphology, molecular characterization, and virulence of *Beauveria pseudobassiana* isolated from different hosts. Journal of Invertebrate Pathology, 172, 107333.