REDIA, XCV, 2012: 43-48

MONICA ORESTE (*) - GIOVANNI BUBICI (*) - MICHELE POLISENO (*)
ORESTE TRIGGIANI (*) - EUSTACHIO TARASCO (*)

PATHOGENICITY OF *BEAUVERIA BASSIANA* (BALS.-CRIV.) VUILL. AND *METARHIZIUM ANISOPLIAE* (METSCHN.) SOROKIN AGAINST *GALLERIA MELLONELLA* L. AND *TENEBRIO MOLITOR* L. IN LABORATORY ASSAYS

(*) *Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti - DI.S.S.P.A. Università degli Studi di Bari "Aldo Moro", Via Amendola 165/a, 70126 Bari, Italy;* e-mail: monica.oreste@agr.uniba.it

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

The pathogenicity of 23 isolates of *Beauveria bassiana* (Ascomycota, Hypocreales: Cordycipitaceae) and four of *Metarhizium anisopliae* (Ascomycota, Hypocreales: Clavicipitaceae) was tested against *Galleria mellonella* (Lepidoptera: Galleriidae) and *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvae in laboratory assays, using $2 \cdot 10^6$ conidia mL^{-1} fungal suspensions. The commercial myco-insecticide Naturalis (Intrachem Bio Italia, Italy), containing the ATCC 74040 *B. bassiana* strain, was included in the assays for comparison. Mycosed larvae were counted 1, 2, 3, 7, 10, 14 and 17 days after inoculation and the cumulative mortality data were used to calculate mean survival time (MST) and lethal times (LT_{50} and LT_{95}). No difference between *B. bassiana* and *M. anisopliae* were detected in the pathogenicity against the two insect species. However, a wide variability occurred among fungal isolates within species. The two *B. bassiana* isolates AL1 and ALB55 killed *G. mellonella* larvae within the shortest time (MST of 2.2 and 2.3 days, respectively), as well as the ALB55 did against *T. molitor* larvae (MST of 2.8 days). Naturalis was superior to these two *B. bassiana* isolates, causing a MST of 1.1 day or shorter on the insect larvae. Overall, *G. mellonella* resulted more sensitive than *T. molitor*, as showed also by the non-inoculated controls, for which MSTs were 7.7 and 8.4 days, respectively. Due to the rapid and effective insecticide action, the ALB55 *B. bassiana* isolate can be considered as a new promising candidate for the microbial pest control.

KEY WORDS: entomopathogenic fungi, virulence, myco-insecticide, microbial pest control.

INTRODUCTION

The entomopathogenic fungi are common natural enemies of arthropods, in particular insects, and they can be used as potential microbial control agents in biological or integrated pest management strategies, both in agro-forestry and urban ecosystems (LACEY and KAYA, 2007). The most important species, *Beauveria bassiana* (Vuill.) Balsam and *Metarhizium anisopliae* (Metch.) Sorok. belong to the Phylum Ascomycota, order Hypocreales, families Clavicipitaceae and Cordycipitaceae, respectively (HIBBET *et al.*, 2007). Their natural habitat is the soil which may influence their survival and distribution. The entomopathogenic fungi may be found both in primary and secondary ecosystems (forest and cultivated soils, respectively), although the frequency of isolation in forest habitats is higher, due to the larger host biodiversity, the undisturbed soil condition and the permanence of habitat. Several studies have shown the prevalence of *B. bassiana* in uncultivated soils, where *M. anisopliae* is less frequently recovered (TARASCO *et al.,* 1997; CHANDLER *et al.,* 1997; KLEESPIES *et al.,* 1989). On the other hand, *M. anisopliae* is quite common in agricultural soils, particularly in association with herbaceous crops (BIDOCHKA *et al.*, 1998; KELLER *et al.*, 2003).

The entomopathogenic fungi act by contact and are able to infect insects with piercing-sucking mouthparts, at every stages. They have horizontal and vertical transmissions, viz. inside the population (among individuals) and over time (across the progenies), respectively. Despite these advantages, a number of factors, such as the difficulty in environmental adaptability, resistance to chemicals, and lower ready-effect compared to the chemicals may limit the use of these fungi for the pest management in greenhouse and, especially, in field (SHAH and PELL, 2003; QUESADA MORAGA *et al.,* 2006a; WRAIGHT *et al.,* 2007). Nevertheless, a wide variability within and among species has been detected in pathogenicity, virulence and ecological features of a lot of entomopathogenic fungi. This variability has been considered and analyzed to select candidates for myco-insecticides production (QUESADA MORAGA *et al.,* 2006a; WRAIGHT *et al.*, 2007).

This research was aimed at evaluating the pathogenicity of some *B. bassiana* and *M. anisopliae* isolates, recovered from soil samples in Italy, Albania and Algeria, against the two model host insects *Galleria mellonella* L. and *Tenebrio molitor* L..

MATERIALS AND METHODS

FUNGAL ISOLATES

Twenty-three isolates of *B. bassiana* and four of *M. anisopliae* were used in the experiments (table 1). The fungal isolates were maintained in collection, stored at 4°C, in glass tubes provided with a cotton cap and containing potato-sucrose-agar. They were isolated from soil samples of Italy, Albania and Algeria within previous

– Received 16 July 2012 Accepted 24 September 2012 4 December 2012

Table 1 – Fungal isolates.

Fungal Species	Isolates	Geographical origin	Site of isolation	Habitat
Beauveria bassiana	M44	Italy	Canale di Pirro, Martina Franca (Taranto)	Pinewood
	RA1	Italy	Rapolla (Potenza)	Vineyard
	AL1	Italy	Altamura (Bari)	Wasteland
	GM6	Italy	Massafra (Taranto)	Oakwood
	ZA17	Italy	Zapponeta (Foggia)	Asparagus
	SP15	Italy	Spinazzola (Bari)	Wheat
	CG2	Italy	Ceglie Messapica (Brindisi)	Olive
	SA3	Italy	Sammichele (Bari)	Cherry
	OF13	Italy	Casalonga, S.Ferdinando (Foggia)	Wheat
	OF64	Italy	Canale Posticchio, Canosa (Foggia)	Cane
	OF26	Italy	S.Ferdinando (Foggia)	Riverbed
	OF68	Italy	Loconia (Foggia)	Vineyard
	OF50	Italy	Loconia (Foggia)	Olive
	OF49	Italy	Postaplana, Canosa (Foggia)	Wasteland
	OF22	Italy	La Palata, Ofanto (Foggia)	Oliveto
	41ALG	Algeria	Chrèa, Blinda	Oakwood
	28ALG	Algeria	Hassi El Gara Menaa, Djerif	Palms
	21ALG	Algeria	Bainem	Oakwood
	40ALG	Algeria	Djelfa	Pineta
	42ALG	Algeria	Tikdja, Bouira	Cedar
	ALB55	Albania	Koplik, Shkoder	Vineyard
	ALB59	Albania	Fikas, Elbasan	Olive
Metarhizium anisopliae	OF55	Italy	La Palata, Ofanto (Foggia)	Olive
	CIST8	Italy	Cisternino (Brindisi)	Wasteland
	OF14	Italy	La Palata, Ofanto (Foggia)	Cabbage
	OF31	Italy	S.Ferdinando (Foggia)	Olive

research works, and their thermal regimes were already determined (ORESTE *et. al*., 2011; TARASCO *et al.,* 1997; TARASCO and TRIGGIANI, 2007; SCATIGNA *et al.,* 2007; ZAMOUM *et al.,* 2008; TARASCO and POLISENO, 2005). The commercial biological insecticide Naturalis (Intrachem Bio Italia, Italy), containing at least $2.3 \cdot 10^7$ conidia mL^{-1} of the ATCC 74040 *B. bassiana* strain, was included in the experiments as control.

In order to prepare the inoculum, each fungal isolate was grown in Petri dishes (90 mm diameter) containing 2% malt extract-agar, at 25°C in the dark for 15 days. Then, conidia were harvested by pouring in the plate 25 mL sterile distilled water added with 0.002% Tween 80 (Sigma Aldrich). Conidia concentration was estimated by means of the Malassez chamber, and adjusted by diluting conidia to a final concentration of $2 \cdot 10^6$ conidia mL^{-1}. The same conidial concentration was used for Naturalis.

INSECTS

Before experiments, *G. mellonella* and *T. molitor* mature larvae, obtained from commercial stocks, were maintained in plastic cages at 23±2°C and 12 h photoperiod. *G. mellonella* was fed with an artificial diet (250 g honey, 220 ml glycerol, 340 g wheat meal,100 g yeast powder, 50 g pure beeswax, 1.75 g nipagin) and *T. molitor* with bread and flavour, periodically added with fresh vegetables.

PATHOGENICITY ASSAYS

The experiments were carried out in plastic boxes (10×15×10 cm in size) lined on the bottom with a filter paper, and closed on the top with a plastic net (1 mm mesh). Twenty larvae of *G. mellonella* or *T. molitor* were put in each box and treated with a single fungal isolate. A 10 mL conidial suspension of each isolate was sprayed in the boxes containing larvae. This conidial suspension volume ensured homogeneous wetting of larvae and box inner surface. Sterile distilled water added with 0.002% Tween 80 was used as control. Boxes were incubated in a climatic chamber at 25°C and 75% relative humidity. A complete randomized block design with five replicates (boxes) was used. Dead larvae were counted (and removed) 1, 2, 3, 7, 10, 14 and 17 days after inoculation. Reisolation of the fungi was attempted from random samples at each counting to confirm the infections.

STATISTICAL ANALYSIS

For calculations and statistical analyses, cumulative mortality was calculated. Mean survival time (MST) and lethal time (LT) were determined by the Kaplan-Meier procedure (KAPLAN and MEIER, 1958) and the probit analysis, respectively (FINNEY, 1971). The lethal times LT_{50} and LT_{95}, meaning time to kill 50 and 95% individuals, respectively, were calculated.

Since MST and LT data violated the assumptions for parametric statistics, they were analyzed with the nonparametric method of SHAH & MADDEN (2004). The method consists in the analysis of variance-type statistics performed on ranked data in a heteroscedastic framework. Independent variables were: fungal species (FS), fungal isolate (FI) nested within FS, and insect species (IS). Pairwise comparisons among fungal isolates were done with Conover-Iman's test, which uses a Student distribution and corresponds to a *t* test performed on the ranks (CONOVER and IMAN, 1981). Analysis of variance-type statistics were performed with SAS 9.0 (SAS Institute, Cary, NC), while the other analyses with XLSTAT 2011 (Addinsoft).

RESULTS

The analysis performed by Kaplan-Meier procedure was used to calculate the MST's, the main values (together with LT50 and LT95, calculated by probit analysis) to compare the isolates effectiveness. By analyzing data separated by insect species, the analysis of variance-type statistics demonstrated that the pathogenicity of *B. bassiana* was not different from that of *M. anisopliae* on both *G. mellonella* and *T. molitor* in terms of MST and LTs (table 2). However, a significant variability was detected among fungal isolates within species ($P<0.0001$). Moreover, the significant interaction of Fungal isolate×Insect species (see pooled data in table 2) indicated that the several fungal isolates affected diversely the mortality of the two insect species. Thus, the pairwise comparisons were conducted separately by insect species (tables 3 and 4).

In the control, the mortality of *G. mellonella* reached 44% 17 DAI; 50% larvae dead in 14 days, and 95% larvae were estimated to die in 27.5 days. The control mortality of *T. molitor* 17 DAI (34%) was lower than that of *G. mellonella* ($P=0.0489$): different MST resulted for the two insect, viz. 7.7 days for *G. mellonella* and 8.4 days for *T. molitor*. 50% *T. molitor* larvae were estimated to die in 22.5 days, and 95% larvae in 45 days.

G. mellonella larvae inoculated with *B. bassiana* or *M. anisopliae* isolates had on average a MST of 5 days, a LT_{50} of 3.6 days and a LT_{95} of 5.5 days (table 3). All fungal isolates were significantly different from the non-inoculated control, in terms of mortality, MST and LTs, except 40ALG, which however caused 88% mortality of *G. mellonella* larvae 17 DAI. The commercial bio-insecticide Naturalis was the most effective against both insect species, since MST of *G. mellonella* larvae was 1.1 days, and that of *T. molitor* larvae was shorter than 1 day. Besides Naturalis, the two isolates AL1 and ALB55 resulted the most virulent against *G. mellonella*, since killed 100% larvae within 3 days, thus determining MSTs of 2.2 and 2.3 days, respectively. ALB55 was the most virulent isolate against *T. molitor* as well, with a MST of 2.8 days (table 4).

DISCUSSION AND CONCLUSIONS

In the present research work, a screening for the pathogenicity of *B. bassiana* and *M. anisopliae* isolates against *G. mellonella* and *T. molitor* was carried out in laboratory assays. The fungal isolates were obtained within previous researches, and their thermal regimes were already determined (ORESTE et. al., 2011; TARASCO *et al.,* 1997; TARASCO and TRIGGIANI, 2007; SCATIGNA *et al.,* 2007; ZAMOUM *et al*., 2008; TARASCO and POLISENO, 2005). *G. mellonella* and *T. molitor* are known to be susceptible to these two entomopathogenic fungal species, so that they are even used as baits for recovering entomopathogenic fungi from the soil, as model host insects (ZIMMERMAN, 1986; BIDOCHKA *et al.,* 2002; MONTESINOS-MATÍAS *et al.,* 2011; TARASCO *et al.,* 2011) or, recently, as probe for pathogenicity to other insect species (BHARADWAJ *et al.,* 2011). Overall, *G. mellonella* larvae were more sensitive to the fungal infections than *T. molitor* and had a higher natural mortality in the untreated control. Although a high variability in virulence was detected among isolates within each fungal species, the two *B. bassiana* isolates AL1 and ALB55 were the most virulent against *G. mellonella*. These isolates caused the highest mortality of *G. mellonella* larvae within the first 3 days after inoculation, thus combining virulence and speed of action, which are the basic requirements for the effectiveness evaluations. The ALB55 isolate was the most virulent also against *T. molitor.* Larvae of *G. mellonella* and *T. molitor* treated with ALB55 had MSTs of 2.3 and 2.8 days, respectively, and LT_{50} of 1.7 and 2.4 days,

Table 2 – Analysis of variance-type statistics for the effects of isolates of *Beauveria bassiana* and *Metarhizium anisopliae* on the mortality of larvae of *Galleria mellonella* and *Tenebrio molitor* in a 17-day laboratory assay.

Sources of variability [a]	Mortality 17 DAI [b] (%)	Mean survival time (days)	LT_{50} [c] (days)	LT_{95} [c] (days)
Pooled data				
FS [d]	0.0128	0.4797	0.0920	0.1856
FI(FS)	<0.0001	<0.0001	<0.0001	<0.0001
IS	<0.0001	<0.0001	<0.0001	<0.0001
FS×IS [d]	<0.0001	0.9542	0.4211	0.2286
FI(FS)×IS	<0.0001	<0.0001	<0.0001	<0.0001
Data separated by insect species				
G. mellonella				
FS [d]	0.7646	0.4054	0.2253	0.2393
FI(FS)	0.0214	<0.0001	<0.0001	<0.0001
T. molitor				
FS [d]	0.0678	0.6077	0.1566	0.0660
FI(FS)	<0.0001	<0.0001	<0.0001	<0.0001

[a] FS = Fungal species; FI = Fungal isolate; IS = Insect species.
[b] DAI = Days After Inoculation.
[c] LT = Lethal time for 50% or 95% individuals.
[d] Variance of FI(FS) was used as error term (denominator variance) in the tests of null hypothesis, because FI effect was nested within FS effect.

Table 3 – Effects of applications of *Beauveria bassiana* or *Metarhizium anisopliae* conidia on larvae of *Galleria mellonella* in a 17-day laboratory assay[a].

Species	Isolate	Mortality 17 DAI[b] (%)	Mean survival time (days)	LT_{50}[c] (days)	LT_{95}[c] (days)
B. bassiana	Naturalis	100.0 c	1.1 a	0.1 a	2.2 a
	ATCC 74040	100.0 c	2.0 b	1.7 b	2.5 a
	AL1	100.0 c	2.2 b	1.7 b	2.7 a
	ALB55	100.0 c	2.3 b	1.7 b	2.6 a
	28ALG	100.0 c	3.0 c	1.7 b	3.3 b
	41ALG	100.0 c	3.0 c	1.7 b	3.3 b
	21ALG	100.0 c	4.4 de	1.9 cd	5.5 e
	RA1	100.0 c	3.1 c	2.0 c	3.6 c
	31ALG	100.0 c	3.5 cd	2.1 c	3.6 c
	ZA17	100.0 c	3.8 d	2.1 bc	3.6 c
	OF50	100.0 c	4.1 de	2.3 cd	3.7 cd
	SP15	100.0 c	3.9 d	2.4 cd	3.8 cd
	CG2	100.0 c	5.0 ef	2.8 de	4.1 de
	OF49	100.0 c	5.0 f	3.0 e	4.2 e
	OF68	100.0 c	5.7 fg	3.3 e	4.4 ef
	OF13	100.0 c	6.6 h	3.7 f	4.7 g
	M44	100.0 c	6.3 gh	3.8 f	4.8 fg
	ALB59	98.0 bc	6.3 gh	4.2 fg	7.7 i
	OF26	99.0 c	6.5 h	4.2 f	6.0 gh
	GM6	100.0 c	6.8 hi	4.2 fg	5.1 g
	SA3	100.0 c	6.9 i	4.7 g	5.4 h
	OF22	100.0 c	7.0 i	4.9 g	6.8 hi
	OF64	100.0 c	7.2 ij	5.5 g	8.7 i
	42ALG	93.0 bc	6.9 i	5.6 g	10.4 i
	40ALG	88.0 b	8.0 j	7.6 h	13.4 j
	Average	**99.1**	**5.0**	**3.6**	**5.5**
M. anisopliae	CIST8	100.0 c	3.6 d	1.9 bc	3.4 bc
	OF14	100.0 c	3.6 d	2.1 c	3.6 c
	OF31	100.0 c	4.4 de	2.6 d	4.0 d
	OF55	100.0 c	4.8 e	2.9 d	4.1 d
	Average	**100.0**	**4.2**	**2.4**	**3.8**

[a] Means with different letters are significantly different according to Conover-Iman's test ($P<0.05$).
[b] DAI = Days After Inoculation.
[c] LT = Lethal time for 50% or 95% individuals

respectively. These results are interesting if compared with those obtained in similar experiments. Among 61 *B. bassiana* isolates ($1{\cdot}10^7$ conidia mL^{-1}), the shortest LT_{50} were found to be 3.2 and 4.7 days for *G. mellonella* and *T. molitor*, respectively (BIDOCHKA *et al.,* 2002). In another experiment, MSTs ranged from 5.9 to 7.4 days for *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* Westwood nymphs (Hemiptera: Aleurodidae) treated with *B. bassiana* isolates (QUESADA MORAGA *et al.,* 2006a). MSTs ranging from 5.4 to 14.4 days, and LT50 ranging from 4.6 to 6.1 days, were found in a screening of 10 *B. bassiana* and 5 *M. anisopliae* isolates ($1{\cdot}10^7$ or $1{\cdot}10^8$ conidia mL^{-1}) for the pathogenicity against *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (QUESADA MORAGA et al., 2006b). A LT_{50} of 4.22 days was due to a *M. anisopliae* isolate ($1{\cdot}10^8$ conidia mL^{-1}) applied to neonate larvae of *Capnodis tenebrionis* (L.) (Coleoptera: Buprestidae) (MARANNINO *et al.,* 2008). Also, MSTs ranging from 4.2 to 4.7 days were found on adults of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) treated with three isolates of each *B. bassiana* and *M. anisopliae* ($1{\cdot}10^7$ conidia mL^{-1}) (LIU and BAUER, 2006).

In our assays, the commercial bio-insecticide Naturalis killed all the larvae of both insect species within one day after inoculation, while the ATCC 74040 *B. bassiana* strain, contained into the same product, resulted not significantly different from our more effective isolates AL1 and ALB55 (with MST against *G. mellonella* and *T. molitor* 2.0 and 2.6 days respectively). The rapid action of Naturalis is probably related to the improving effect of co-formulants, in term of adhesion and persistence on targets.

Due to the very high virulence, the *B. bassiana* ALB55 isolate can be reasonably considered as a new promising pest biocontrol candidate.

REFERENCES

BHARADWAJ A., STAFFORD III K.C., 2011 – *Potential of* Tenebrio molitor *(Coleoptera: Tenebrionidae) as a bioassay probe for* Metarhizium brunneum *(Hypocreales: Clavicipitaceae) activity against* Ixodes scapularis *(Acari: Ixodidae).* - Journal of Economic Entomology, 104(6): 2095-2098.

BIDOCHKA *M.J.,* KASPERSKI *J.E.,* WILD *G.A.M., 1998* – *Occurrence of the entomopathogenic fungi* M. anisopliae *and* B. bassiana *in soils from temperate and near-northern habitats*. - Canadian Journal of Botany, 76: 1198 - 1204.

BIDOCHKA M.J., MENZIES F.V., KAMP A.M., 2002 – *Genetic groups of the insect-pathogenic fungus* Beauveria bassiana

Table 4 – Effects of applications of *Beauveria bassiana* or *Metarhizium anisopliae* conidia on larvae of *Tenebrio molitor* in a 17-day laboratory assay[a].

Species	Isolate	Mortality 17 DAI[b] (%)		Mean survival time (days)		LT_{50}[c] (days)		LT_{95}[c] (days)	
B. bassiana	Naturalis	100.0	h	1.0[d]	a	–[e]		–[e]	
	ATCC 74040	100.0	gh	2.6	b	2.3	a	5.7	a
	ALB55	98.0	gh	2.8	b	2.4	a	5.7	a
	OF50	94.0	f-h	6.7	de	5.1	c	12.5	cd
	OF26	58.0	a-e	7.0	de	5.1	bc	7.2	ab
	OF49	84.0	d-h	6.3	d	5.6	c	15.3	d
	SA3	95.0	f-h	7.3	ef	5.7	cd	10.3	bc
	CG2	96.0	f-h	6.6	d	5.9	cd	10.9	bc
	OF64	93.0	f-h	7.1	de	5.9	cd	11.4	cd
	OF68	99.0	h	7.7	f	5.9	cd	9.7	bc
	41ALG	89.0	e-h	7.4	ef	6.5	d	13.2	cd
	31ALG	83.0	c-h	7.2	de	6.9	de	16.2	d
	RA1	86.0	d-h	7.4	ef	6.9	de	14.3	cd
	OF13	85.0	d-h	7.4	ef	6.9	de	15.5	d
	AL1	78.0	c-h	4.0	c	7.0	de	21.0	e
	M44	82.0	c-h	7.9	f	7.8	de	15.2	d
	SP15	72.0	a-f	8.0	f	8.9	e	19.5	de
	GM6	73.0	b-g	8.2	f	9.1	ef	18.6	de
	OF22	63.0	a-e	8.9	g	11.9	fg	23.0	e
	ZA17	60.0	a-e	10.2	g	12.1	g	21.0	e
	21ALG	53.0	a-d	10.0	g	13.1	g	23.2	e
	ALB59	41.0	a-c	8.9	fg	17.5	gh	32.2	f
	28ALG	43.0	a-c	8.8	fg	19.3	gh	41.2	g
	42ALG	22.0	a	10.1	g	19.7	h	32.0	f
	40ALG	30.0	ab	7.9	f	24.6	h	62.8	h
	Average	**74.0**		**7.4**		**9.9**		**20.3**	
M. anisopliae	OF14	100.0	h	6.4	d	4.1	b	5.0	a
	OF55	89.0	e-h	7.4	ef	6.6	de	12.9	cd
	CIST8	82.0	c-h	7.8	f	7.4	de	15.7	d
	OF31	99.0	h	9.8	g	11.5	fg	20.6	e
	Average	**92.5**		**7.2**		**5.8**		**10.2**	
Control		**34.0**	ab	**8.4**	fg	**22.5**	h	**45.0**	g

[a] Means with different letters are significantly different according to Conover-Iman's test ($P<0.05$).
[b] DAI = Days After Inoculation.
[c] LT = Lethal time for 50% or 95% individuals.
[d] Mean survival time, here, may be 1 day or fewer, because mortality 1 DAI was already 100%.
[e] Lethal time could not be calculated because mortality 1 DAI was already 100%. Therefore it should be shorter than 1 day.

are associated with habitat and thermal growth preferences. - Archives of Microbiology, 178: 531-537.

CHANDLER D., HAY D., REID A.P., 1997 – *Sampling and occurrence of entomopathogenic fungi and nematodes in UK soils*. - Applied Soil Ecology, 5: 133 -143.

CONOVER W.J., IMAN R.L., 1981 – Rank Transformations as a Bridge between Parametric and Nonparametric Statistics. - The American Statistician, 35: 124 -129.

FINNEY D.J., 1971 – *Probit Analysis, Third Edition*. - London, Cambridge University Press.

HIBBETT D.S., BINDER M., BISCHOFF J.F., BLACKWELL M., CANNON P.F., ERIKSSON O.E., HUHNDORF S., JAMES T., KIRK P.M., LÜCKING R., THORSTEN LUMBSCH H., LUTZONI F., MATHENY P.B., MCLAUGHLIN D.J., POWELL M.J., REDHEAD S., SCHOCH C.L., SPATAFORA J.W., STALPERS J.A., VILGALYS R., AIME M.C., APTROOT A., BAUER R., BEGEROW D., BENNY G.L., CASTLEBURY L.A., CROUS P.W., DAI Y.C., GAMS W., GEISER D.M., GRIFFITH G.W., GUEIDAN C., HAWKSWORTH D.L., HESTMARK G., HOSAKA K., HUMBER R.A., HYDE K.D., IRONSIDE J.E., KÕLJALG U., KURTZMAN C.P., LARSSON K.H., LICHTWARDT R., LONGCORE J., MIADLIKOWSKA J., MILLER A., MONCALVO J.M., MOZLEY-STANDRIDGE S., OBERWINKLER F., PARMASTO E., REEB V., ROGERS J.D., ROUX C., RYVARDEN L., SAMPAIO J.P., SCHÜSSLER A., SUGIYAMA J., THORN R.G., TIBELL L., UNTEREINER W.A., WALKER C., WANG Z., WEIR A., WEISS M., WHITE M.M., WINKA K., YAO Y.J., ZHANG N., 2007 – *A higher-level phylogenetic classification of the Fungi*. - Mycological Research, 111 (5): 509-547.

KAPLAN EL., MEIER P., 1958 – *Non parametric estimation from incomplete data*. - Journal of American Statisticians Association, 53: 457-481.

KELLER S., KESSLER P., SCHWEIZER C., 2003 – *Distribution of insect pathogenic soil fungi in Switzerland with special reference to* Beauveria brongniartii *and* Metharhizium anisopliae. – BioControl, 48 (3): 307 - 319.

KLEESPIES R., BATHON H., ZIMMERMAN G., 1989 – *Investigations into the natural occurrence of entomopathogenic fungi and nematodes in various soils from the Darmstadt area (in German).* - Gesund Pflanzen, 41: 350 - 355.

LACEY L.A., KKAYA H.K., 2007 – *Field manual of techniques in invertebrate pathology*. Springer, Dordrecht, The Netherlands, 868 pp.

LIU H., S. BAUER, L., 2006 – *Susceptibility of* Agrilus planipennis (Coleoptera: Buprestidae) *to* Beauveria bassiana *and* Metarhizium anisopliae. - Journal of Economic Entomology, 99 (4): 1096 - 1103.

MARANNINO P., SANTIAGO ALVAREZ C., DE LILLO E., QUESADA MORAGA E., 2008 – *Evaluation of* Metarhizium anisopliae (Metsch) Sorok. *to target larvae and adults of* Capnodis tenebrionis (L.) (Coleoptera: Buprestidae) *in soil and fiber band applications*. - Journal of Invertebrate Pathology, 97: 237-244.

MONTESINOS-MATÍAS R., VINIEGRA - GONZÁLEZ G., ALATORRE-ROSAS R., LOERA O., 2011 – *Relationship between virulence and enzymatic profiles in the cuticle of* Tenebrio molitor *by 2-deoxy-d-glucose-resistant mutants of* Beauveria bassiana (Bals.) Vuill. - World Journal of Microbiology and Biotechnology, 27: 2095-2102.

ORESTE M., TARASCO E., POLISENO M., BUBICI G., TRIGGIANI O., 2011 – *In Vitro thermal regimes of the entomopathogenic fungi* Beauveria Bassiana *(Vuill.)* Balsam and Metarhizium anisopliae (Metch.) Sorok. - IOBC/WPRS Bulletin, 66: 159-162.

QUESADA MORAGA E., MARANHAO E.A.A., VALVERDE GARCIA P., SANTIAGO ALVAREZ C., 2006a – *Selection of* Beauveria bassiana *isolates for control of the whiteflies* Bemisia tabaci *and* Trialeurodes vaporariorum *on the basis of their virulence, thermal requirements, and toxicogenic activity.* - Biological Control, 36: 274-287.

QUESADA MORAGA E., Ruiz GARCIA A., SANTIAGO ALVAREZ C., 2006b – *Laboratory Evaluation of Entomopathogenic Fungi* Beauveria bassiana *and* Metarhizium anisopliae *Against Puparia and Adults of* Ceratitis capitata (Diptera: Tephritidae). - Journal of Economic Entomology, 99(6): 1955-1966.

SCATIGNA M.A.E., POLISENO M., VLORA A., ZIMMERMAN G., TARASCO E., 2007 – *Entomopathogenic fungi in riparian soils of Ofanto River (Italy, Apulia Region).* IOBC/WPRS Bulletin, 30 (1): 143-146.

SHAH D.A., MADDEN L.V., 2004 – *Nonparametric Analysis of Ordinal Data in Designed Factorial Experiments*. - Phytopathology, 94: 33 - 43.

SHAH P.A., PELL J.K., 2003 – *Entomopathogenic fungi as biological control agents*. - Applied Microbiology and Biotechnology, 61: 413-423.

TARASCO E., POLISENO M., 2005 – *Preliminary survey on the occurrence of entomopathogenic nematodes and fungi in soils from Albania*. - IOBC/wprs Bulletin, 28 (3): 165 -168.

TARASCO E., TRIGGIANI O., 2007 – *Occurrence of entomopathogenic nematodes and fungi in oak wood soils from southern Italy.* - 5th Meeting IOBC, WG "Integrated Protection in oak forests", Tlemcen (Algeria), 22 - 25 October 2007: 14.

TARASCO E., de BIEVRE C., PAPIEROK B., POLISENO M., TRIGGIANI O., 1997 – *Occurrence of entomopathogenic fungi in soils in Southern Italy.* - Entomologica, 31: 157 - 166.

TARASCO E., SANTIAGO ALVAREZ C., TRIGGIANI O., QUESADA MORAGA E., 2011 – *Laboratory studies on the competition for insect haemocoel between* Beauveria bassiana *and* Steinernema ichnusae *recovered in the same ecological niche*. - Biocontrol Science and Technology, 21 (6): 693-704.

WRAIGHT S.P., INGLIS G.D., GOETTEL M.S., 2007 – *Fungi.* In: Field Manual of Techniques in Invertebrate Pathology, Lacey L.A., Kaya H.K. Ed. Springer, Dordrecht, The Netherlands, pp. 223-248.

ZAMOUM M., BERCHICHE S., SAI K., TRIGGIANI O., TarAsco E., 2008 – *Premiéres observations sur la présence des organismes entomopathogènes nématodes et des champignons en Algérie.* - International Meeting "Entomological Research in Mediterranean Forest Ecosystem", Med Insect 2008, Estoril, Portugal, 5-8 May 2008: 39.

ZIMMERMANN G., 1986 – *The "*Galleria *bait method" for detection of entomopathogenic fungi in soil.* - Journal of Applied Entomology, 102: 213-215.