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ENTOMOPATHOGENICITY OF *BEAUVERIA BASSIANA* (BALS.) VUILL. AND *METARHIZIUM ANISOPLIAE* (METSCH.) SOROKIN ISOLATED FROM DIFFERENT SOURCES IN THE CONTROL OF *RHYNCHOPHORUS FERRUGINEUS* (OLIVIER) (COLEOPTERA CURCULIONIDAE) ([1])

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Francardi V., Benvenuti C., Roversi P.F., Rumine P., Barzanti G. – Entomopathogenicity of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin isolated from different sources in the control of *Rhynchophorus ferrugineus* (Olivier) (Coleoptera Curculionidae).

The entomopathogenicity of an indigenous *Beauveria bassiana* strain isolated from soil and of *B. bassiana* and *Metarhizium anisopliae* strains isolated in Italy from naturally infected *Rhynchophorus ferrugineus* (RPW) adults, was tested against larvae and adults of RPW in laboratory bioassays. The individuals were infected via direct contact on sporulated mycelia grown on Sabouraud Dextrose Agar or on wheat substrata. *M. anisopliae* obtained from *R. ferrugineus* showed the highest efficacy against RPW larvae and adults particularly against individuals contaminated on sporulated wheat, which showed values of cumulative larval mortality of 100% and adult mortality of 90%; LT_{50} was obtained in 13.1 days in both larvae and adults. *B. bassiana* strain isolated from soil recorded a lower cumulative mortality on larvae (13%) and adults (13%) treated on inoculated Sabouraud Dextrose Agar. *B. bassiana* strain isolated from RPW showed cumulative mortality values higher than 50% against larvae treated on inoculated wheat (55%) and Sabouraud Dextrose Agar (53%); LT_{50} was obtained in 15 days and 21.8.days respectively.

Results are discussed with regard to the potential employment of the virulent indigenous strain of *M. anisopliae* for microbiological control of *R. ferrugineus* as part of an integrated pest management program.

KEY WORDS: RPW, entomopathogenic fungi, infecting substratum, biological control, virulence.

INTRODUCTION

Rhynchophorus ferrugineus (Olivier) (RPW) is a invasive pest of great phytosanitary importance on palms of ornamental and economic importance (FERRY & GOMEZ, 2002; EPPO, 2008). The curculionid is recorded in various Asian and Mediterranean countries, Oceanic islands (LI *et al*., 2009) and recently also in California (USA) (EPPO, 2010). Preventive and curative treatments against RPW populations generally consist in the periodic use of chemical insecticides which may involve problems of environmental pollution, reduction of entomophagous fauna and human health especially when carried out in urban areas. Microbiological insecticides based on the entomopathogenic fungi *Metarhizium anisopliae* (Metsch) Sorokin and *Beauveria bassiana* (Bals.) Vuill., although lacking a rapid "pulling down" efficacy against the curculionid population, may represent an important integrated or alternative control measure against RPW.

Increasing interest in *M. anisopliae* and *B. bassiana* as biocontrol agents of the curculionid has stemmed from their frequent isolation from *R. ferrugineus* larvae, pupae and adult cadavers collected on palms in several countries in new infested areas and by the consequent possibility to find wild indigenous strains highly virulent against the RPW. In this regards bioassay were recently carried out to test the virulence of *M. anisopliae* and *B. bassiana* isolates from RPW against different life stage of the curculionid, also in comparison with *M. anisopliae* and *B. bassiana* strains obtained from different sources (soil, various insect species). The investigations led to the detection of more active isolates against RPW individuals in laboratory and field trials (GAZAVI & AVAND-FAGHIH, 2002; SHAJU *et al*., 2003;EL-SUFTY *et al*., 2007; GINDIN *et al.,* 2006; TARASCO *et al*., 2007; EL-SUFTY *et al*., 2009; SEWIFY *et al*., 2009; DEMBILIO *et al*., 2010; SHAWIR & AL-JABR 2010); in Egypt a local virulent *B. bassiana* isolate was genetically characterized and patented (SEWIFY, 2007).

The aim of the present study was to evaluate the entomopathogenicity of indigenous *B. bassiana* and *M. anisopliae* obtained in Italy against larvae and adults of *R. ferrugineus* in order to identify indigenous strains potentially suitable for RPW biological control and to study the influence of infecting substrata in supporting fungal virulence.

MATERIALS AND METHODS

R. FERRUGINEUS REARING

Larvae and adults of *R. ferrugineus* employed in the laboratory tests were sent to our CRA-ABP Agrobiology and Pedology Research Centre of Florence (Italy) in 2009-2010 from the Department of Science and Phytosanitary

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Technologies, Catania University, Sicily (Italy). The insects, once arrived at our Centre, were put separately into plastic boxes closed with a hermetic cover in which a circular hole was plugged with a fire-glued metallic fine-knit mesh; they were maintained on pieces of Golden apples for two weeks to verify their good health before individuals were chosen for the bioassays. Insect breeding was carried out in a climatic room at 24±2°C, 70±5% RH, larvae in the dark, adults with a photoperiod of 12:12 (N:D).

B. BASSIANA AND *M. ANISOPLIAE* ISOLATION AND CULTURE

Isolation from soil - The *B. bassiana* (B.01/T02) strain was isolated from soil in Tuscany (I) according to the method of ZIMMERMANN (1986). About 5 kg of soil were placed in a plastic box with air-holes in the cover. Five larvae (L_3-L_4) of the wax moth *Galleria mellonella* were added to the soil substrate to be infected by the entomopathogenic fungi. The box was kept for 2-3 weeks at room temperature (20-25°C) and a ca. 80% humidity level was ensured inside the box by periodic water sprays. Dead larvae were collected, placed on wet filter paper in Petri dishes and put in an incubator at 25°C. The mycelium and spores that emerged on larvae were transferred directly onto growth medium (PDA - Potato Dextrose Agar) in Petri dishes and incubated at 25°C for 10 days; the isolates were then purified by repeated transplanting.

Isolation from RPW cadavers - The *B. bassiana* (B.09/I01) and *M. anisopliae* (M.08/I05) strains were obtained from naturally infected RPW adults collected in Lazio and in Sicily, respectively. To promote conidial growth, mycosed RPW adult cadavers were placed singly on filter paper saturated daily with water to achieve ca. 100% RH inside Petri dishes. The dishes were maintained at room temperature (20-25C°). Parts of fungal propagules grown on the cadavers were then transferred, with sterile needles, into Petri dishes with SDAY1/4 (Sabouraud Dextrose Agar Fluka supplemented with yeast extract ¼ of concentration) and kept in an incubator at 25°C. Pure fungal colonies were then stored on PDA (Potato Dextrose Agar) and MEA (Malt Extract Agar) slants in bacteriological glass tubes at 4°C.

All the strains are stored in the CRA-ABP entomopathogenic fungi collection. The *B. bassiana* isolates were determined by sequencing analyses of the 18SrRNA gene and the internal transcribed spacer (ITS1) (Professor A. Alma, University of Turin), while *M. anisopliae* was sequenced for the 5' region of the nuclear gene elongation factor-1 alpha (EF-1alpha) (Dr. Stephen Rehner, Systematic Mycology and Microbiology Laboratory, Beltsville, Maryland - USA).

INFECTING SUBSTRATA

Preparation of inoculated Sabouraud Dextrose Agar (I.SDA) - B. bassiana and *M. anisopliae* were grown in Petri dishes on SDAY1/4 lined with a sterile cellophane disc in the dark for two months..

Preparation of inoculated wheat substratum (I.W) - 400 g of wheat were prepared according to the procedure of GINDIN *et al.* (2006) for a solid rice-based medium; 200 g of the whole wheat were transferred into each of two sterilized conical glass Erlenmeyer flasks (500 ml). Inoculation was performed by transferring about 1 cm^2 of sporulated mycelium of *B. bassiana* and *M. anisopliae,* grown on SDA cultures in Petri dishes, into the wheat. The flasks, inoculated with *M. anisopliae* and *B. bassiana* respectively, were then plugged with sterilized cotton and placed in a climatic cell at 24±2°C. After about one month, the wheat in each flask was transferred separately into 2 plastic boxes (500 ml) and RPW larvae and adults were put inside the boxes for the treatments, as reported below. Concentrations of $7x10^6$ conidia of *B. bassiana* and *M. anisopliae* were estimated on 0.3 g of sporulated wheat (I.W) by means of a Toma-Zeiss-counting chamber.

LABORATORY BIOASSAYS

Five laboratory tests (3 tests with RPW larvae and 2 tests with adults) were separately carried out. The experimental design is summarized in tables 1-5.

Tests with R. ferrugineus larvae - In total, 180 larvae (2 to 4 cm long) were employed in three tests. T1: 60 larvae were divided into 3 groups, each of 20 individuals; one group was treated on I.SDA contaminated with *B. bassiana* (B.01/T02), another on I.SDA contaminated with sporulated mycelia of *M. anisopliae* (M.08/I05) and the last group was an untreated control (SDA) (tab. 1). T1 was a preliminary test carried out to compare virulence parameters of of *B. bassiana* and *M. anisopliae* isolated from different sources. T2: 60 larvae were divided into 4 groups, each of 15 individuals; one group was treated on I.SDA contaminated with *B. bassiana* (B.01/T02), another on I.SDA with *B. bassiana* (B.09/I01), a third on I.SDA with *M. anisopliae* (M.08/I05) and the last group was an untreated control (SDA) (tab. 2). T3: 60 larvae were divided into 3 groups, each of 20 individuals; one group was treated on I.W contaminated with *B. bassiana* (B.09/I01), another on I.W with *M. anisopliae* (M.08/I05) and the last group was an untreated control (W) (tab. 3). *B. bassiana* (B.01/T02) was not employed in T3 in consideration of the low virulence parameters recorded in the T2 assay.

Larvae were infected *via* direct contact by rolling individuals on I.SDA with sporulated mycelium in Petri

Table 1 – Treatments with *B. bassiana* and *M. anisopliae* strains against *R. ferrugineus* larvae. Larvae were contaminated on sporulated mycelium grown on Sabouraud Dextrose Agar (I.SDA). In the Gehan-Wilcoxon test column, the same letters indicate that the treatment effects are not significantly different (P=0.05).

Trial	Substratum	Treatment	No. larvae	Cumulative mortality 28 days (%)	Abbott (%)	Lethal time (LT_{50}) (days)	Gehan-Wilcoxon test
T1	I.SDA	*B. bassiana* (Bba01/T02)	20	50	33	27	AB
T1	I.SDA	*M. anisopliae* (Man08/I05)	20	75	67	13	B
T1	SDA	Control	20	25	/	ND	A

Table 2 – Treatments with *B. bassiana* and *M. anisopliae* strains against *R. ferrugineus* larvae. Larvae were contaminated on sporulated mycelium grown on Sabouraud Dextrose Agar (I.SDA). In the Gehan-Wilcoxon test column, the same letters indicate that the treatment effects are not significantly different (P=0.05).

Trial	Substratum	Treatment	No. larvae	Cumulative mortality 28 days (%)	Abbott (%)	Lethal time (LT_{50}) (days)	Gehan-Wilcoxon test
T2	I.SDA	*B. bassiana* (Bba01/T02)	15	13	/	ND	A
T2	I.SDA	*B. bassiana* (Bba09/I01)	15	53	46	21.8	B
T2	I.SDA	*M.* anisopliae (Man08/I05)	15	60	53	19.5	B
T2	SDA	Control	15	13	/	ND	A

Table 3 – Treatments with *B. bassiana* and *M. anisopliae* strains against *R. ferrugineus* larvae. Larvae were contaminated on sporulated mycelium grown on wheat (I.W). In the Gehan-Wilcoxon test column, the same letters indicate that the treatment effects are not significantly different (P=0.05).

Trial	Substratum	Treatment	No. larvae	Cumulative mortality 28 days (%)	Abbott (%)	Lethal time (LT_{50}) (days)	Gehan-Wilcoxon test
T3	I.W	*B. bassiana* (Bba09/I01)	20	55	25	15	A
T3	I.W	*M. anisopliae* (Man08/I05)	20	100	100	12.2	B
T3	W	Control	20	40	/	ND	A

Table 4 – Treatments with *B. bassiana* and *M. anisopliae* strains against *R. ferrugineus* adults. Adults were contaminated on sporulated mycelium grown on Sabouraud Dextrose Agar (I.SDA). In the Gehan-Wilcoxon test column, the same letters indicate that the treatment effects are not significantly different (P=0.05).

Trial	Substratum	Treatment	No. adults	Cumulative mortality 28 days (%)	Abbott (%)	Lethal time (LT_{50}) (days)	Gehan-Wilcoxon test
T4	I.SDA	*B. bassiana* (Bba01/T02)	15	13	7	ND	A
T4	I.SDA	*B. bassiana* (Bba09/I01)	15	20	14	N.D.	AB
T4	I.SDA	*M. anisopliae* (Man08/I05)	15	53	50	26.8	B
T4	SDA	Control	15	7	/	ND	A

Table 5 – Treatments with *B. bassiana* and *M. anisopliae* strains against *R. ferrugineus* adults. Adults contaminated on sporulated mycelium grown on wheat (I.W). In the Gehan-Wilcoxon test column, the same letters indicate that the treatment effects are not significantly different (P=0.05).

Trial	Substratum	Treatment	No. adults	Cumulative mortality 28 days (%)	Abbott (%)	Lethal time (LT_{50}) (days)	Gehan-Wilcoxon test
T5	I.W	*B. bassiana* (Bba09/I01)	20	20	11	N.D.	A
T5	I.W	*M. anisopliae* (Man08/I05)	20	90	89	13.1	B
T5	W	Control	20	10	/	ND	A

dishes or on fungi-sporulated wheat (I.W) inside plastic boxes for about 5 minutes. In the controls, larvae were rolled on uncontaminated agar (SDA) or sterilized wheat (W) for the same time.

Treated and control larvae were then placed individually in screw cups (150 ml) with a central hole closed with a fire-glued metallic fine-knit mesh and left to feed on Golden apple pieces. Larvae were kept in the dark in a controlled room at 27°C and 45-60% RH. In test T1,T2, and T3 each larva is a replication.

Tests with R. ferrugineus adults - In total, 120 adults were employed in two experiments. T4: 60 adults were divided into 4 groups, each of 15 individuals; one group was treated on I.SDA contaminated with *B. bassiana* (B.01/T02), another on I.SDA with *B. bassiana* (B.09/I01), a third on I.SDA with *M. anisopliae* (M.08/I05) and the last group was an untreated control (tab. 4). T5: 60 adults were divided into 3 groups, each of 20 individuals; one group was treated on I.W contaminated with *B. bassiana* (B.09/I01), another on I.W with *M. anisopliae* (M.08/I05) and the last group was an untreated control (W) (tab. 5). In accordance to the overcited bioassay with larvae, *B. bassiana* (B.01/T02) was not used in T5 test on wheat substratum in consideration of the low virulence parameters recorded in the T4 assay.

RPW adults were infected by letting them walk on I.SDA with sporulated mycelium in Petri dishes or on fungi-sporulated I.W in plastic boxes for about 15 minutes. In the controls, adults were allowed to walk on uncontaminated agar (SDA) or sterilized wheat (W) for the same time.

Treated and control adults were then placed individually in plastic containers (500 ml) with holes in each cap and left to feed on Golden apple pieces in a climatic room at 27°C, 45-60% RH and a photoperiod of 12:12 (N:D). In test T4 and T5 each adult is a replication.

In all bioassays, the Golden apple pieces were changed once a week. Larval and adult mortality was controlled daily. The tests ended 28 days after the treatments. Dead specimens were placed individually on moistened filter paper inside Petri dishes at room temperature; only cadavers showing external growth of *B. bassiana* and *M. anisopliae* strains were considered in the analysis.

Data analysis

For both RPW stages, fungal virulence was expressed as cumulative mortality (%) and treatment efficacy (Abbott %) within 28 days after treatment. Lethal time (LT_{50}) was also considered inside this period to put more in evidence fungal impact in the RPW control. Survival analyses were performed separately for each trial with the Gehan-Wilcoxon post-hoc test (Peto & Peto, 1972) using SPSS 15.0.

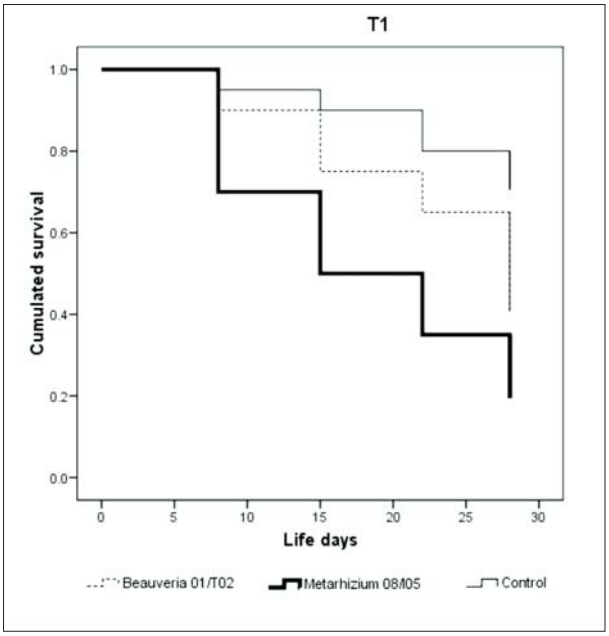

Fig. I – Survival Analysis. Cumulative survival of larvae treated on I.SDA substratum inoculated with *B. bassiana* and *M. anisopliae* strains.

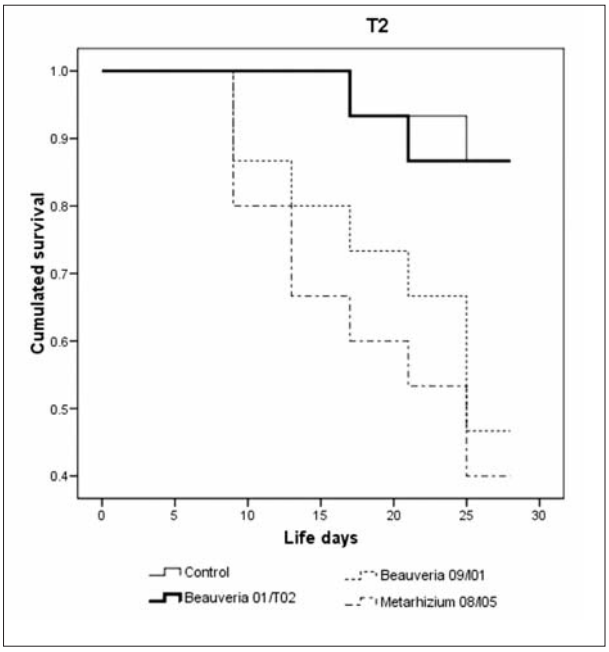

Fig. II – Survival Analysis. Cumulative survival of larvae treated on I.SDA substratum inoculated with *B. bassiana* and *M. anisopliae* strains.

RESULTS

Data are reported in tables 1-5 and the cumulative survival curves of RPW larvae and adults for each trial are reported in Figures I-V.

The virulence parameters recorded in the bioassays showed that, among tested fungal strains, *M. anisopliae* isolated from *R. ferrugineus* (M.08/I05) had the highest efficacy of control against RPW larvae and adults treated on both infected substrata (I.SDA and I.W). On I.W in particular, the larval cumulative mortality reached 100% and adult mortality 90%, the treatment efficacy was 100% and 89% respectively and LT_{50} was obtained in 13.1 days (tab. 3, 5). The cumulative survivals of larvae and adults treated with *M. anisopliae* (M.08/I05) on both substrata were always significantly different from the control (tab. 1, 2, 3, 4 and 5) (Fig. I, II, III, IV and V).

RPW larvae treated on I.SDA inoculated with *M. anisopliae* (M.08/I05) had cumulative mortality and treatment efficacy over 50% (tab. 1, 2); the highest mortality value was 75% and treatment efficacy 67%; LT_{50} was reached in 13 days after treatment (tab. 1). The

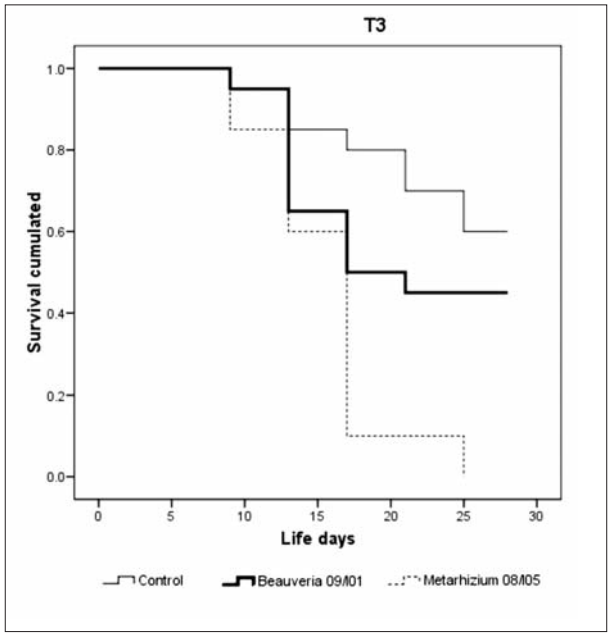

Fig. III – Survival Analysis. Cumulative survival of larvae treated on I.W substratum inoculated with *B. bassiana* and *M. anisopliae* strains.

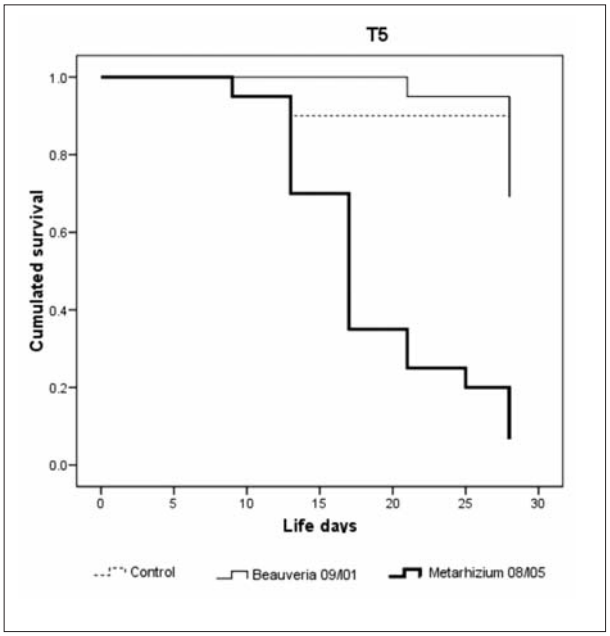

Fig. V – Survival Analysis. Cumulative survival of adults treated on I.W substratum inoculated with *B. bassiana* and *M. anisopliae* strains.

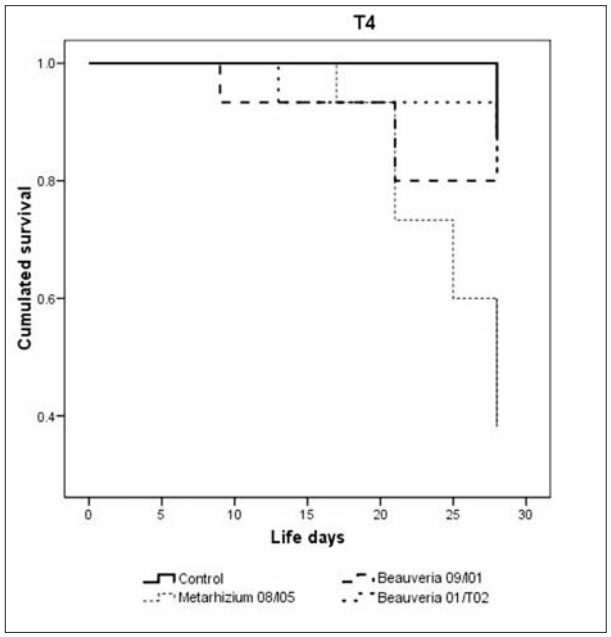

Fig. IV – Survival Analysis. Cumulative survival of adults treated on I.SDA substratum inoculated with *B. bassiana* and *M. anisopliae* strains

cumulative survival of larvae was significantly different from control survival (tab. 1, 2) (Fig. I, II) but not from survival of larvae treated on the same substratum with *B. bassiana* (B.01/T02) in one test (tab. 1) (Fig. I) and with *B. bassiana* (B.09/I01) in another test (tab. 2) (Fig. II).

RPW adults treated with *M. anisopliae* (M.08/I05) on I.SDA showed higher cumulative mortality (53%) and treatment efficacy (50%) than those recorded for both *B. bassiana* (B.09/I01) and *B. bassiana* (B.01/T02). LT_{50} of adults treated with *M. anisopliae* (M.08/I05) was reached in 26.8 days, whereas in the two *B. bassiana* strains LT_{50} was not recorded within 28 days after treatment (tab. 4). The cumulative survival of (M.08/I05)-treated adults was significantly different from that of controls but not from the survival of adults treated with *B. bassiana* (B.09/I01) (tab. 4) (Fig. IV).

As regards the two *B. bassiana* strains, RPW larvae treated with (B.09/I01) on I.SDA recorded higher virulence parameters (cumulative mortality higher than 50%, a maximum treatment efficacy of 46%, LT_{50} reached within 21.8 days) and a cumulative survival significantly different from control survival while *B. bassiana* (B.01/T02)- treated larvae had a mortality of only 13% and a survival not significantly different from the control (tab. 2) (Fig. II). In contrast, the cumulative survival of larvae and adults infected with *B. bassiana* (B.09/I01) on I.W was not significantly different from control survival (tab. 3, tab.5) (Fig. III; Fig. V).

In all the bioassays, the survival of RPW larvae and adults treated with *B. bassiana* (B.01/T02) on I. SDA substratum was not significantly different from control survival (tab. 1, 2, 4) (Fig. I, II, IV); in only a test the cumulative survival of *B. bassiana* (Bba01/T02)-treated larvae not resulted significatively different from both survival of control and of *M. anisopliae* (Man08/I05)-treated larvae (tab. 1) (Fig. I). The lower virulence parameters of *B. bassiana* (Bba01/T02) on treated RPW larvae and adults in comparison with *M. anisopliae* (Man08/I05) and *B. bassiana* (Bba09/I01) was confirmed in T2 and T4 tests (tab. 2, tab. 4) (Fig. II, Fig.IV).

DISCUSSION

B. bassiana and *M. anisopliae* are cosmopolite fungal entomopathogens which may be isolated from various sources (soil, insect, plant) and are characterized by difference in virulence toward different insect species. Fungal virulence is determined by different intrinsic characteristics in the strains and their manifestation is also

related with biotic and abiotic variations (Hall & Papierok, 1982). Thus the individuation of virulent strains of entomopathogenic fungi towards the *R. ferrugineus* in the Countries of introduction represent a precious opportunity to increase studies on the microbiological control efficacy in view of a possible field applications. This prospect was supported by the recent detection in Egypt of an indigenous strain of *B. bassiana* obtained from mycosed RPW collected in field which showed good results in the control of *R. ferrugineus* in laboratory and field tests (EL-SUFTY *et al.,* 2007; SEWIFY *et al*., 2009). In Italy *B. bassiana* and *M. anisopliae* strains tested on the RPW evidenced different entomopathogenicity against the curculionid.

Between the indigenous entomopathogenic fungi isolated from *R. ferrugineus, M. anisopliae* (M.08/I05) showed the highest virulence against both RPW larvae and adults. In our study, *M. anisopliae* virulence appeared also influenced by the type of infecting substratum as resulted higher on larvae (100% mortality) and adults (90% mortality) treated on I.W than on those treated on I.SDA (60-75% larval mortality and 53% adult mortality). Furthermore, L_{50} was reached faster in *M. anisopliae* larvae and adults treated on I.W (in 13.1 days) than in I.SDA-treated larvae and adults (19.5 days and 26.8 days, respectively). These results suggest that the more physical "smearing" of RPW adult bodies, due to tunnelling inside the I.W substratum, favoured greater adhesion and persistence of the fungal conidia on the curculionid bodies, thus increasing the infecting efficacy of *M. anisopliae.*

The results of the present paper are in accordance with GINDIN *et al*. (2006) who compared the entomopathogenicity of *B. bassiana* and *M. anisopliae* strains obtained from different sources and also reported higher virulence of *M. anisopliae*. After spraying RPW larvae with spore suspensions ($2x10^7$ ml^{-1}) of *M. anisopliae* isolates from scarabeid beetles, the Authors recorded similar mortality (from 40% to 80%), but in a faster time (5 days). They also observed differences in *M. anisopliae* time efficacy against RPW adults according to the type of infecting treatment: there was 84.6% mortality of adults treated with dry spores obtained *in situ* on SDA medium in 14 days, but 100% mortality of adults sprayed with spore suspensions ($1x\ 10^8$ spores ml^{-1}) in a longer period (35 days).

Similarly SHAWIR AND AL-JABR (2010), studying the infectivity of *M. anisopliae* and *B. bassiana* isolates against *R. ferrugineus,* observed different mortality values according to the infecting method, with higher mortality of both larvae and adults infected with fungal spore suspensions ($1x10^7$ spores/ml) by injection than by the dipping technique. In the treatment by injection, *B. bassiana* caused 80-85% larval and adult mortality, while *M. anisopliae* caused 70% larval and adult mortality. In the dipping method, the mortality values were lower: *B. bassiana* caused 60% larval mortality and 40-55% adult (male-female) mortality, while *M. anisopliae* caused 60% larval and 35%-50% adult (male-female) mortality within 10 days after treatment.

In the present paper, *B. bassiana* (B.09/I01) showed higher efficacy against RPW larvae on infecting I.SDA (cumulative mortality 53%) than against adults contaminated on the same substratum (cumulative mortality 20%); L_{50} of larvae treated on I.SDA was reached in 21.8 days. *B. bassiana* (B.09/I01) also showed a higher virulence against larvae (cumulative mortality 55%) treated on infecting I.W than against adults (cumulative mortality 20%) on the same substratum; L_{50}.of larvae was reached in a shorter time,15 days.The higher mortality of RPW larvae treated with *B. bassiana* (B.09/I01) obtained from RPW is in agreement with data reported by EL-SUFTY *et al*. (2009) who, in the United Arab Emirates, observed higher susceptibility of larvae to conidial suspensions (10^8 con./ml) of local strains of *B. bassiana* isolated from *R. ferrugineus*. The Authors reported a mortality of 100%, 65-100% and 45-85% of young, middle-aged and mature larvae, respectively 5 days after treatment while RPW adults showed the highest mortality values (not reported) between 8 and 13 days.

The *B. bassiana* strain isolated from soil (B.01/T02) appeared to have scarce efficacy in RPW control; the larval cumulative survival analysis did not reveal a significant difference from the controls in the bioassays. This is in agreement with GINDIN *et al*. (2006) who, in tests to select the most pathogenic fungal isolates against *R. ferrugineus*, recorded no or low larval mortality (20%) in 5 days after spore suspension treatments with *B. bassiana* strains from soil.

In conclusion, *M. anisopliae* (M.08/I05) appeared to be a indigenous virulent strain which provided an effective control against RPW and its efficacy could be supported and/or enhanced by suitable insect host treatment. Anyway further studies have to be conducted on this topic in order to devise tools and strategies to infect successfully wild RPW with *M. anisopliae* (M.08/I05). The prospect is not only to infect and kill RPW but also to transmit the fungal inoculum inside curculionid population by contact among contamined and "healthy" individuals, following the advice of GINDIN *et al.* (2006), SEWIFY (2007) and DEMBILIO *et al*. (2010).The possibility to start epizoozies inside target insect pests represent a important aspect to get microbiological control methods more efficient and eco-sustainable

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RIASSUNTO

ENTOMOPATOGENICITÀ DI ISOLATI DI *BEAUVERIA BASSIANA* (BALS.) VUILL. E *METARHIZIUM ANISOPLIAE* (METSCH.) SOROKIN PER IL CONTROLLO DI *RHYNCHOPHORUS FERRUGINEUS* (OLIVIER)

L'entomopatogenicità di ceppi indigeni di *Beauveria bassiana* e *Metarhizium anisopliae* ottenuti da campioni di suolo e da adulti di *Rhynchophorus ferrugineus* (Olivier) raccolti in Italia, è stata studiata in laboratorio su larve e adulti di *R. ferrugineus* contaminati per contatto diretto con le spore dei

funghi entomopatogeni inoculati su due diversi substrati: Sabouraud Dextrose Agar e grano.

Il ceppo di *M. anisopliae* ha evidenziato i valori di mortalità più elevati, nei confronti di larve e adulti di *R. ferrugineus* in particolare su esemplari contaminati su grano: su questo substrato la mortalità di larve e adulti ha raggiunto rispettivamente il 100% e il 90%. LT_{50} è stata raggiunta in 13.1 giorni sia per le larve che per gli adulti. L'isolato di *B. bassiana* ottenuto da suolo ha registrato una mortalità cumulativa più bassa di larve (13%) e adulti (13%) del curculionide contaminati su Sabouraud Dextrose Agar. L'isolato di *B. bassiana* ottenuto da *R. ferrugineus* ha registrato percentuali di mortalità cumulativa superiori al 50% su larve contaminate su grano (55%) e Sabouraud Dextrose Agar (53%); LT_{50} è stata raggiunta, rispettivamente, dopo 15 giorni e 21,8 giorni dal trattamento.

I risultati sono discussi nella prospettiva di un potenziale impiego dell'isolato indigeno di *M. anisopliae* nel controllo microbiologico di *R. ferrugineus* nell'ambito di un programma di lotta integrata.

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