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Effect of *Beauveria bassiana* (strain ATCC 74040) on two leaf beetle pests of maize under laboratory conditions

Ефект на *Beauveria bassiana* (щам ATCC 74040) върху два вида листояди вредители по царевицата в лабораторни условия

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ABSTRACT

In some European countries, the Western corn rootworm, *Diabrotica virgifera virgifera*, and the cereal leaf beetle, *Oulema melanopus*, are present in maize stands in high population density, occasionally causing damage of the crops. Susceptibility of adults of these species and larvae of the cereal leaf beetle to the commercially available product Naturalis® based on *Beauveria bassiana* strain ATCC 74040 was explored in laboratory conditions. The results of the bioassays showed that the fungal strain caused the highest corrected mortality on *O. melanopus* larvae with average values above 95% for all conidia concentrations (from 2.3 × 10^2 to 2.3 × 10^7 conidia/ml) tested. For the adults of the two pests, the average mortality increased positively with concentration of conidia and the values ranges from 9.5% to 94.2% for *O. melanopus* (concentrations from 2.3 × 10^2 to 2.3 × 10^7 conidia/ml) and from 19.3% to 92.4% for *D. v. virgifera* (concentrations from 2.3 × 10^3 to 2.3 × 10^7 conidia/ml). Accordingly, the LC_{50} values for *O. melanopus* larvae and adults and *D. v. virgifera* adults were determined as 4.6, 8.3 × 10^4 and 4.3 × 10^5 conidia/ml, respectively. Further studies to confirm the susceptibility of the larvae of the cereal leaf beetle to Naturalis® under field conditions should be conducted.

Keywords: *Diabrotica virgifera virgifera*, *Oulema melanopus*, mycoinsecticide, mortality, median lethal time, insect pest control

АБСТРАКТ

В някои европейски страни западният царевичен коренов червей *Diabrotica virgifera virgifera* и обикновената житна пиявица *Oulema melanopus* присъстват в царевичните площи във висока плътност, нанасяйки щети на културите. В лабораторни условия беше изследвана чувствителността на възрастните индивиди от тези видове и ларвите на обикновената житна пиявица към търговския продукт Naturalis®, който съдържа *Beauveria bassiana* (щам ATCC 74040). Резултатите от опитите показват, че гъбният щам причини най-високата коригирана смъртност на ларвите на *O. melanopus* със средни стойности над 95% за всички изпитани концентрации на конидиите (от 2.3 × 10^2 до 2.3 × 10^7 конидии/ml). За възрастните на двата вида вредители средната смъртност нараства с увеличаване на концентрацията на конидиите и стойностите варират от 9.5% до 94.2% за *O. melanopus* (концентрации от 2.3 x 10^2 до 2.3 × 10^7 конидии/ml) и от 19.3% до 92.4% за *D. v. virgifera* (концентрации от 2.3 × 10^3 до 2.3 × 10^7 конидии/ml). Стойностите на LC_{50} за ларвите и възрастните на *O. melanopus* и възрастните индивиди на *D. v. virgifera* бяха съответно 4.6, 8.3 × 10^4 и 4.3 × 10^5 конидии/ml. Необходимо е да се проведат допълнителни изследвания за потвърждаване на високата чувствителност на ларвите на обикновената житна пиявица към Naturalis® в полеви условия.

Ключови думи: *Diabrotica virgifera virgifera*, *Oulema melanopus*, микоинсектицид, смъртност, средно летално време, борба с насекоми вредители

INTRODUCTION

Maize, *Zea mays* L. (Poaceae) with global maize production amounts over 1.1 billion metric tons per year is one of the most widely grown cereal throughout the world (Grote et al., 2021). Maize in Europe is attacked by many insect pests, including two chrysomelid beetle species, the Western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, 1858 and the cereal leaf beetle *Oulema melanopus* (Linnaeus, 1758) (CLB), which are present in maize stands in high numbers, occasionally causing damage of the crops (Grozea et al., 2006; Meissle et al., 2010, 2012; Lysenko, 2018; Velchev et al., 2019; Ismailov et al., 2021). In addition to the direct damage, these two species are known to transmit the Maize Chlorotic mottle virus (Awata et al., 2019), which recently has been reported in Europe (Spain) (Achon et al., 2017).

WCR is native to North America and it was first discovered in Europe in 1992 near Belgrade airport, Serbia (former Yugoslavia) (Bača, 1994). The pest is currently distributed across 21 European countries (Bažok et al., 2021). *Diabrotica v. virgifera* is univoltine species, overwintering as an egg in the soil. Maize is the primary host of WCR larvae, which are the harmful stage damaging maize roots (Chiang, 1973) but alternative host plants may contribute to the invasion potential of the pest (Moeser and Vidal, 2004; Gloyna et al., 2011).

Oulema melanopus is spread across Europe, Asia and North Africa and North America (Bezděk and Baselga, 2015). It has one generation per year. Beetles overwinter in the soil, shelterbelts and wooded areas in the leaf litter and debris. The CLB has a wide host range, including wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), maize (*Z. mays*), rye (*Secale cereale* L.), sorghum (*Sorghum bicolor* (L.)) Moench, Sudan grass (*Sorghum × drummondii* (Steud.) Millsp. & Chas), triticale *Triticosecale* Witt. and many grass species (e.g., wheatgrass *Agropyron* sp., wild oat *Avena fatua* L., winter wild oat *A. sterilis* L., quackgrass *Elymus repens* (L.) Gould, tall fescue *Festuca arundinacea*, Schreb., *Hordeum bulbosum* L., *H. murinum* L., annual and perennial ryegrass *Lolium* L., timothy *Phleum pratense* L., Johnsongrass *Sorghum halepense* (L.) Pers.) (Maneva, 2013; Kamçili and Can, 2019; Wielkopolan et al., 2021). Both adults and larvae cause damages on cereal plants by feeding on the leaves, but the larvae (especially the larvae of the last instar of development) are considered to be more harmful stage (Zarubova et al., 2015; Wielkopolan et al., 2021).

Several approaches can be used to control *D. v. virgifera* and *O. melanopus*: chemical insecticides, agricultural practices, development and exploitation of resistant host plant lines, behavioural control (by behavior-modifying chemicals) or biological pest control (Bažok et al., 2021; Wielkopolan et al., 2021). The use of conventional insecticides against important agricultural pests in past decades has led to serious problems, including detrimental effects on non-target arthropods, contamination of food, problems in human health, and development of insecticide resistance. Currently, conventional insecticides have a reduced role in *D. v. virgifera* management programs but are still used as complementary tactics with other management approaches (Meinke et al., 2021). Nowadays adopting cropping system approaches and fostering the adoption of nonchemical tactics is a challenge toward a reduced reliance on chemical insecticides (Lamichhane et al., 2016). The application of biopesticides based on entomopathogens (bacteria, fungi, baculoviruses and nematodes), essential oils, plant extracts, naturally-occurring biochemicals and inert dusts appears to be a complementary or alternative methodology to the conventional insecticides (Ruiu, 2018; Stankovic et al., 2020).

Fungal diseases of insects are common and widespread and contribute to the natural regulation of insect populations. Most entomopathogenic fungi (EPF) in the group of Ascomycetes are usually found in the soil and can cause natural outbreaks on their own when environmental conditions are favorable. Insect-pathogenic fungal species have been in use for more than 150 years since *Metarhizium anisopliae* (Metschn.) Sorokīn was tested against the wheat chafer *Anisoplia austriaca* (Herbst, 1783) and the sugar beet weevil

Asproparthenis punctiventris (Germar, 1824) (Clarksson and Charnley, 1996). *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin is among the highly effective biological agents that can infect insect pests from different insect orders, especially from Hemiptera, Coleoptera, Lepidoptera, Thysanoptera, Orthoptera and Diptera and causing white muscadine disease (Dannon et al., 2020). Different strains of this fungus are available in more than 58 commercial formulations for pest management in agriculture, greenhouses, nurseries and home gardens (de Faria and Wraight, 2007). It is well known that the secondary metabolites (beauvericin and bassianolide, a variety of beauverolides, oosporein, bassianin, and tenellin) produced by *B. bassiana* have diverse functions including insecticide properties (Zimmermann, 2007).

Mycoinsecticide Naturalis® (CBC (Europe), S.r.l., Italy), based on *B. bassiana* strain ATCC 74040 (isolated from the cotton boll weevil *Anthonomus grandis* (Boheman, 1843) in Texas, USA), is used against the following target pests: whiteflies, thrips, mites, aphids, lace bugs, weevils, wireworms, fruit flies, olive flies (Wright, 1992; Ladurner et al., 2008). The strain acts primarily by contact, infesting the host through the cuticle by cuticle-degrading enzymes and causing death due to depletion of nutrients or dehydration (Ladurner et al., 2009; Ortu et al., 2009). Recently, demonstrated endophytic properties of this strain (Rondot and Reineke, 2018; Homayoonzadeh et al., 2022) increase the interest to application of the commercial product in insect pest management. In addition to insecticide and endophytic properties, Rondot and Reineke (2017) reported also avoidance behaviour of *Otiorhynchus sulcatus* (Fabricius, 1775) adults toward plants treated with Naturalis®.

The control of multiple insect pests by one pathogenic fungal strain is helpful in IPM strategies and also in organic growing systems. Laboratory assessment of entomopathogenic fungi is an important step in determining pathogenic strain prior to use in the field for insect pest control. The insecticide activity of *B. bassiana* strain ATCC 74040 on other important pest of maize in Eastern Europe, the grey maize weevil, *Tanymecus dilaticollis* Gyllenhal, 1834 (Curculionidae), have been demonstrated under laboratory and field conditions (Toshova et al., 2021). The present study aimed to investigate the effectiveness of Naturalis® against WCR adults and larvae and adults of CLB under laboratory conditions.

MATERIAL AND METHODS

Insects and fungal strain

Adults of CLB and WCR were collected from a maize field (1 ha) belonging to the Maize Research Institute, Knezha (43°28'48.85"N; 24°3'22.03"E) (north-western Bulgaria) on 17 June 2019 and 20 July, 2020 for CLB and WCR, respectively. Larvae of CLB were collected on 7 June 2021 from an oat crop in the same area and were with a size 4-6 mm and a width of head capsule corresponding to last instar stages (3rd - 4th) (Hoxie and Wellso, 1974). Before the beginning of the bioassays adults of *D. v. virgifera* and *O. melanopus* were placed at 5-6 °C in a refrigerator for at least 15 min to render beetles less mobile. Chilling has been commonly used as an insect anesthetic for many years in behavioral and ecological investigations (Rayl and Wratten, 2016).

The commercial bioinsecticide Naturalis® containing *B. bassiana* strain ATCC 74040 (CBC (Europe), S.r.l., Italy) with a concentration of 2.3×10^7 viable conidia/ml was purchased by Amititsa Ltd. (Kresna, Bulgaria).

Contact bioassays on WCR adults and CLB larvae and adults

For each bioassay the following serial dilutions of Naturalis® were prepared in distilled water: 2.3×10^6, 2.3×10^5, 2.3×10^4, 2.3×10^3 and 2.3×10^2 conidia/ml. One milliliter of a conidial concentration including the undilluted product with a concentration of 2.3×10^7 was spread evenly across the surface of the white filter paper disc on the bottom of a plastic Petri dish (ISOLAB, Laborgeräte GmbH; 85 mm diameter) following a method previously described (Draganova et al., 2012). Insects in control variants were exposed to 1 ml of distilled water (i.e., no fungus). Different groups of field-collected insects were transferred into each Petri dish in direct contact with conidial concentrations and controls. Bioassays were performed with three replications for each concentration/control variant with the following exception - the lowest concentration, 2.3×10^2 conidia/ml, was not tested on *D. v. virgifera* adults due to insufficient number of specimens. In the bioassay with WCR, groups of 24 ± 1 (mean number ± SE) adults were transferred into the Petri dishes. In the bioassays with *O. melanopus*, 20 and 21 specimens were added to each Petri dish for the larvae and adults, respectively.

Field collected maize parts (young leaves, silks, immature seeds of maize) were presented at two-day-intervals for feeding WCR adults. The food for CLB adults and larvae were fresh Johnsongrass (*S. halepense*) and oat *(A. sativa*) plants, respectively. The food plants were collected from the field and were added every two days into the Petri dishes. Mortality was recorded at 24-h intervals for ten days after treatment. Insect was categorized as dead when no leg or antennal movements were observed. Dead specimens were removed daily. They were placed in new plastic Petri dishes with filter paper moistened with distilled water (1 ml) and incubated at 24 ± 1 °C for up to 14 days to observe for fungal pathogen exhibition.

Insects were categorized as infected with *B. bassiana* when fungus mycelia were visible on insects' integument.

Data and statistical analyses

For all the treatments, mortality of adults assessed was corrected with Schneider-Orelli's formula taking into account natural insect mortality. The LC_{50} (median lethal concentration), LC_{90} (lethal concentration that kills 90% of tested individuals) and LT_{50} (median lethal time) values with associated 95% confidence intervals (CIs) were calculated by probit analysis (Finney, 1971). Two-way ANOVA was used to determine the influence of conidia concentration and time post treatment on insect mortality. One-way ANOVA was then used to evaluate the effect of concentrations on cumulative mortality. Means were compared by a LSD post hoc test. The log(x+1) transformation was used on all percentage values subjected to ANOVA. Statistical analyses were performed using Statistica, version 7.0 (StatSoft, Inc). The significant difference was set at $P<0.05$.

RESULTS

Bioassay on Diabrotica v. virgifera

Ten days after the beginning of the bioassay, insects in the control group had an average cumulated mortality rates of 22% (± 1%).

The mean corrected mortality of WCR adults in response to the conidia concentrations of Naturalis® after 10 days post treatment is illustrated in Figure 1, and it was in the range from 19% to 92% for the lowest to the highest conidia concentration, respectively. We found significant effects of the concentration ($F_{(4,150)}=49.78$; $P<0.001$) and time ($F_{(9,150)}=9.05$; $P<0.001$) on *D. v. virgifera* mortality. There was no interaction between concentration and the date post treatment ($F_{(36,150)}=0.97$; $P=0.223$). At the highest concentration tested (2.3×10^7 conidia/ml), Naturalis® caused significantly higher mortality than the other conidial concentrations (ANOVA followed by LSD test, $P<0.05$).

The mortality rates caused by the concentrations from 2.3×10^3 to 2.3×10^6 conidia/ml were low (19-37%) and they did not differ significantly from each other on 10th day after treatment (LSD test; $P<0.05$). *Diabrotica v. virgifera* adults died faster at highest conidial concentrations (2.3×10^7 conidia/ml) than at lower conidial concentrations. The average mortality rate of 91% was reached with the highest concentration in a period of 24 hours after application of the commercial product.

The conidial concentration, which cause LC_{50} of WCR adults after ten days was 4.3×10^5 conidia/ml (95% CIs: 9.0×10^3 – 3.0×10^7 conidia/ml), whereas LC_{90} was 3.3×10^7 conidia/ml (95% CIs: 4.7×10^5 – 2.2×10^9 conidia/ml). Treatment with the highest conidia concentration (2.3×10^7 conidia/ml) reached LT_{50} for 2.13 min (95% CIs: 0.56 to 8.15 min). The LT_{50} values ranged between 18 days and 29 days ten days after treatment for the conidia concentrations of 2.3 × 10^6 conidia/ml and 2.3 × 10^3 conidia/ml, respectively (Figure 2). No mycelia were observed on cadavers of WCR adults in the control treatment while only the cadavers of specimens treated with the highest concentration of *B. bassiana* exhibited external mycelial growth (100%).

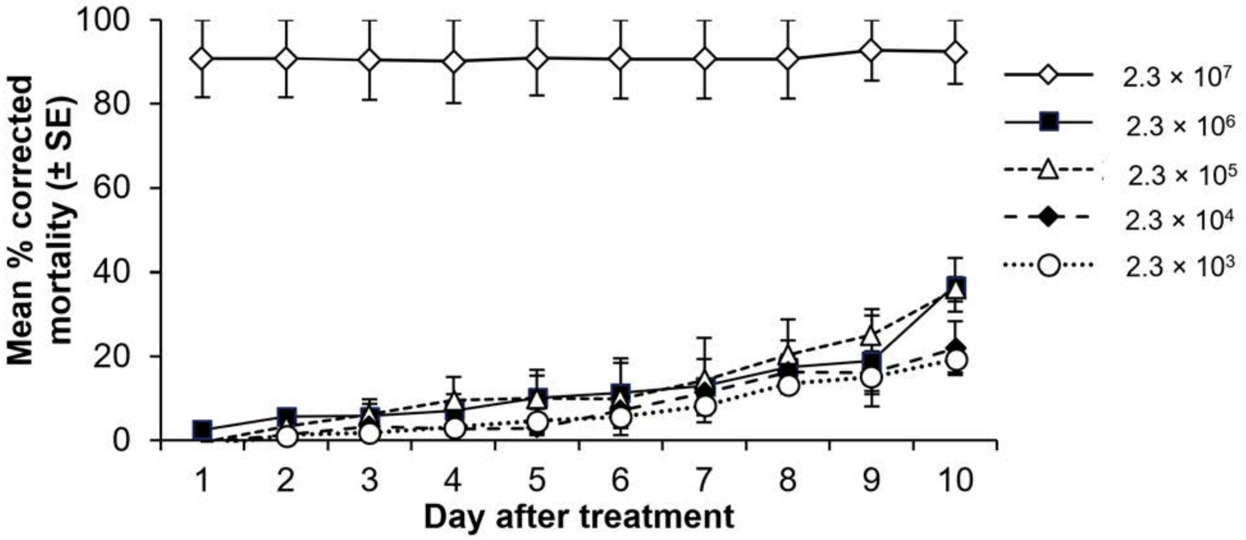

Figure 1. Cumulative mortality (%) of *D. v. virgifera* adults caused by different concentrations of *B. bassiana* (2.3 × 10^3 – 2.3 × 10^7 conidia/ml)

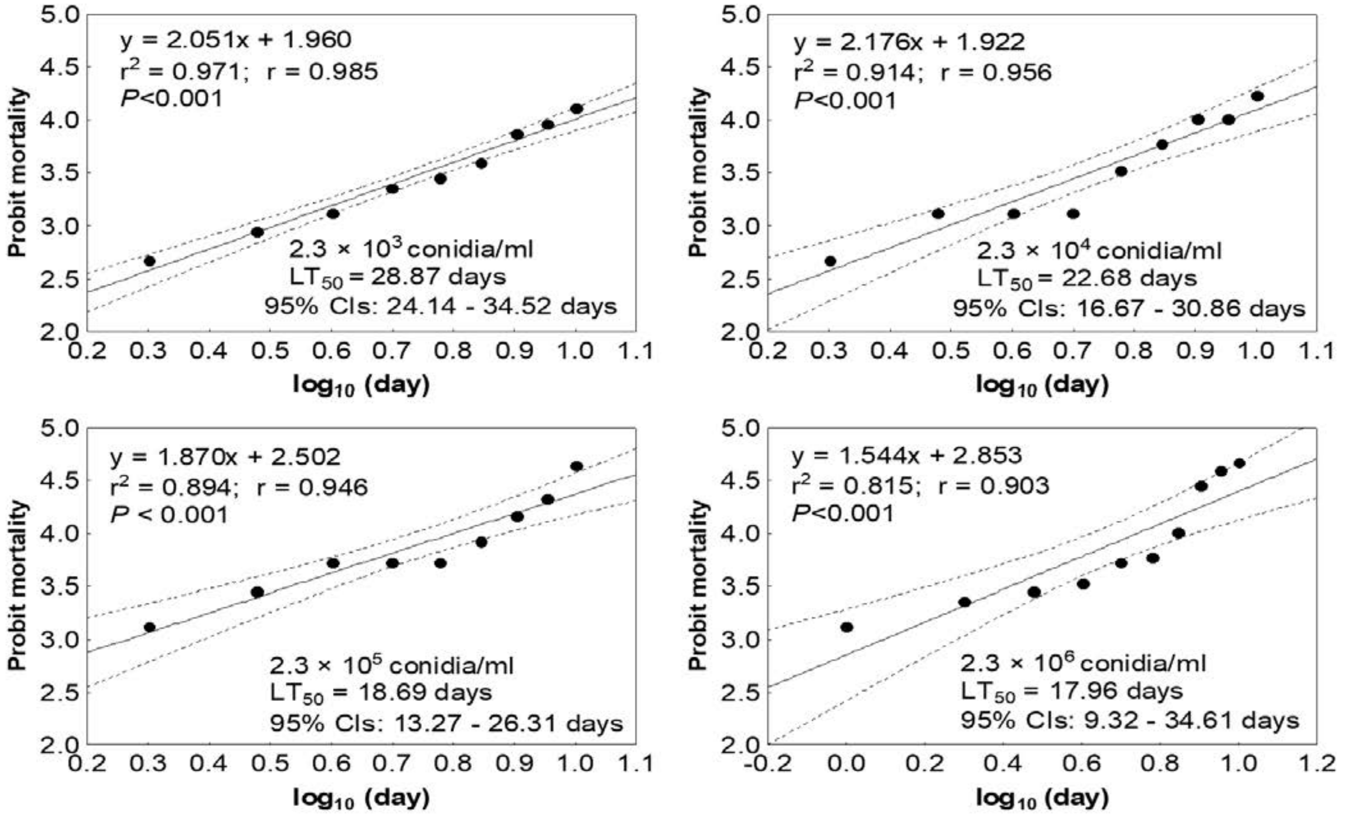

Figure 2. Probit analysis on LT_{50} of *D. v. virgifera* adults treated with *B. bassiana* at different concentrations: (2.3 × 10^3 – 2.3 × 10^6 conidia/ml)

Bioassays on O. melanopus larvae

Control mortality averaged 26.7±1.7% for CLB larvae. The mean percent mortality of larvae treated with *B. bassiana* strain was concentration dependent and increased with the increase in conidial concentration and time (Figure 3). High corrected mortality (> 95 %) of larvae at all concentrations of Naturalis® was observed at the end of the bioassay. *Oulema melanopus* larvae treated with the highest concentration (2.3 × 10^7 conidia/ml) died within 24 h after exposure to conidia. The analysis of variance regarding *B. bassiana* concentration ($F_{(5,180)}$=55.66; *P*<0.001), time ($F_{(9,180)}$=218.74; *P*<0.001), and their interaction ($F_{(45,180)}$=10.65; *P*<0.001) showed highly significant effects on the mortality rates of CLB larvae.

The conidial concentration, which cause LC_{50} of *O. melanopus* larvae after 10 days was 4.6 conidia/ml (95% CIs: 0.09 – 1.1 × 10^4 conidia/ml), whereas LC_{90} was 6.2 × 10^2 conidia/ml (95% CIs: 0.26 – 1.5 × 10^6 conidia/ml). LT_{50} values ranged from two to four days for the concentrations at 2.3 × 10^3 - 2.3 × 10^6 conidia/ml (Figure 4). With respect to mycelium development, infected larvae showed 100% confirmed mortality at all conidial concentrations of *B. bassiana* while no cadavers in the control showed symptoms of mycelium growth.

Bioassays on O. melanopus adults

Control treatments showed 17.6 ±1.6% mortality of CLB larvae ten days after treatment. Higher conidial concentrations caused higher and faster larval mortality than the lower ones (Figure 5). The average mortality rates due to *B. bassiana* concentrations ranged between 9.48% and 94.23 % at 10 days post-treatment. The effects of the concentration ($F_{(5,180)}$=63.88; *P*<0.001) and exposure time ($F_{(9,180)}$=23.23; *P*<0.001) on *O. melanopus* adult mortality were significant. There was an interaction between concentration and time ($F_{(45,180)}$=1.57; *P*=0.029).

The difference in mortality rate between CLB adults treated with different conidia concentrations was significant ($F_{(5,18)}$=7.42; *P*=0.003). High (> 88%) level of mortality was observed only for the two highest conidia concentrations, 2.3 × 10^6 and 2.3 × 10^7 conidia/ml and there was significant difference with the mean mortality percent caused by the concentrations of 2.3 × 10^2 - 2.3 × 10^4 conidia/ml (ANOVA followed by LSD test; *P*<0.05). The conidial concentrations, which cause LC_{50} and LC_{90} of *O. melanopus* adults were 8.3 × 10^4 conidia/ml (95% CIs: 1.1 × 10^4 – 9.1 × 10^5 conidia/ml) and 8.2 × 10^6 conidia/ml (95% CIs: 7.5 × 10^5 – 8.9 × 10^7 conidia/ml), respectively. LT_{50} values varied between two days at the highest conidia concentration to 26 days at the lowest concentration (Figure 6). Fungal mycelium was observed only on the

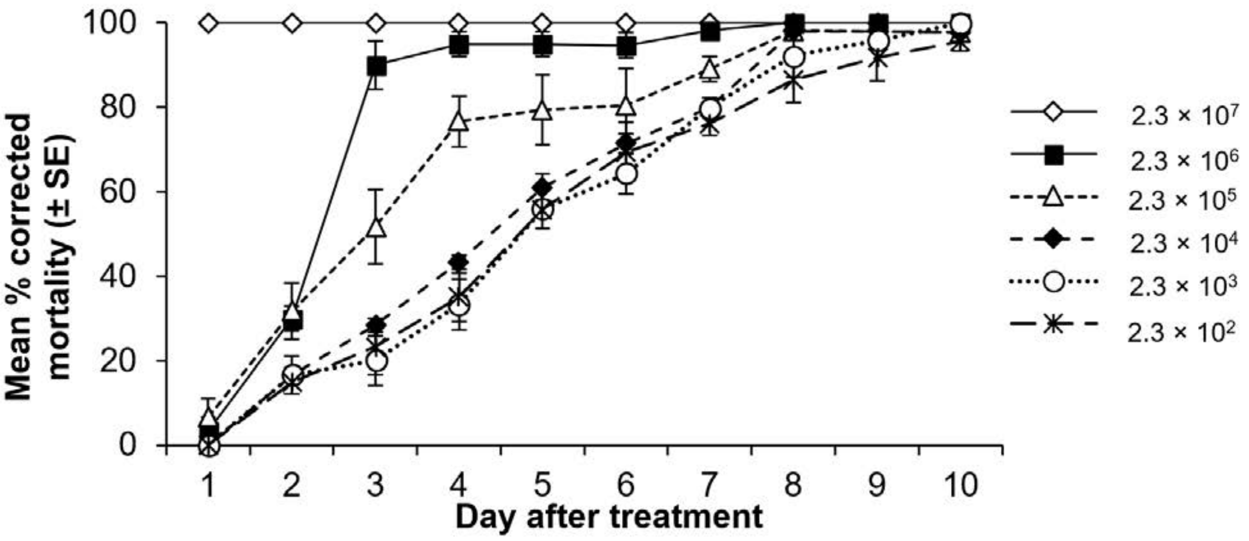

Figure 3. Cumulative mortality (%) of *O. melanopus* larvae treated by different concentrations of *B. bassiana* (2.3 × 10^2 – 2.3 × 10^7 conidia/ml)

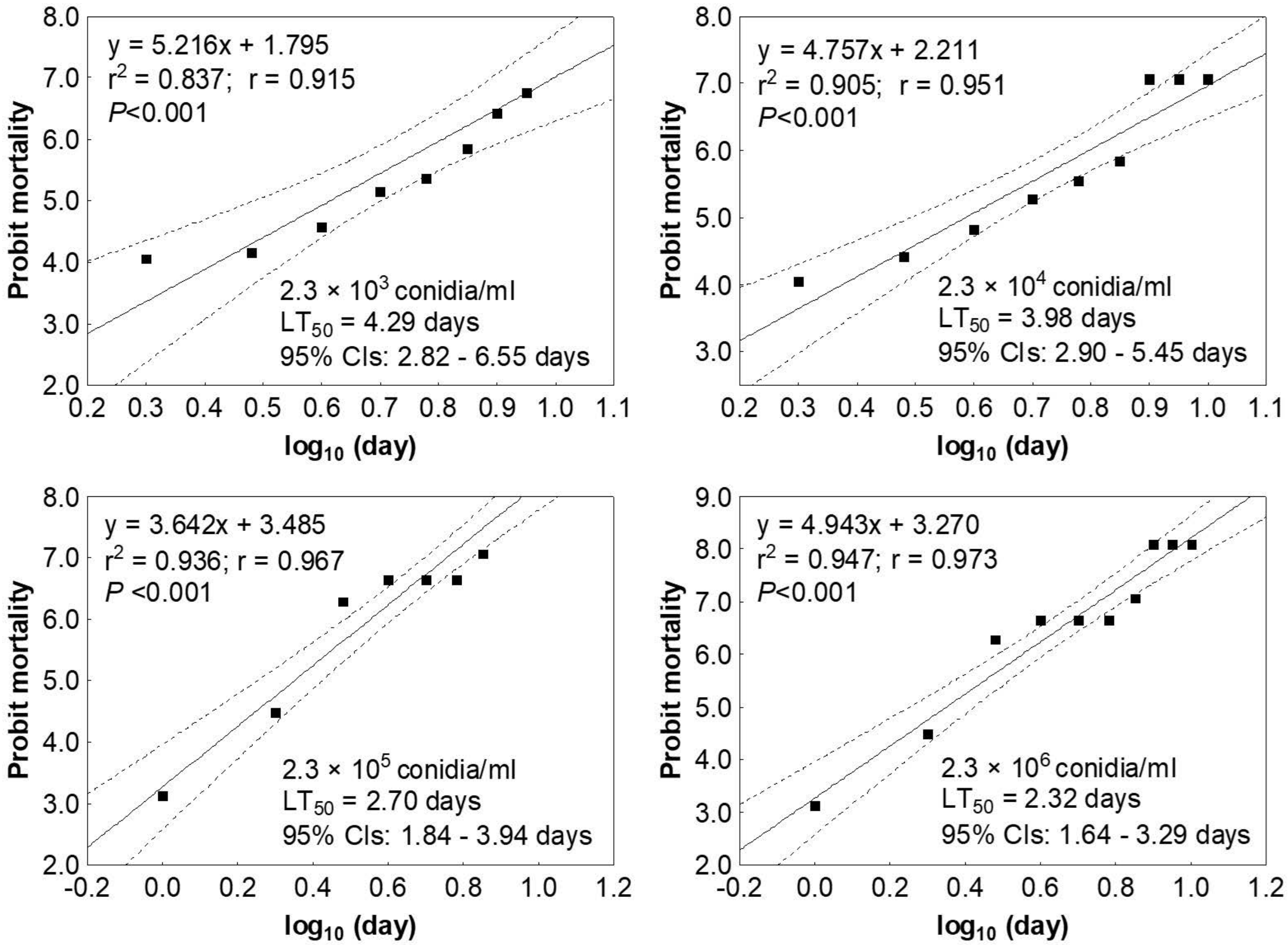

Figure 4. Probit analysis on LT_{50} of *O. melanopus* larvae treated with with *B. bassiana* at different concentrations (2.3 × 10^3 – 2.3 × 10^6 conidia/ml)

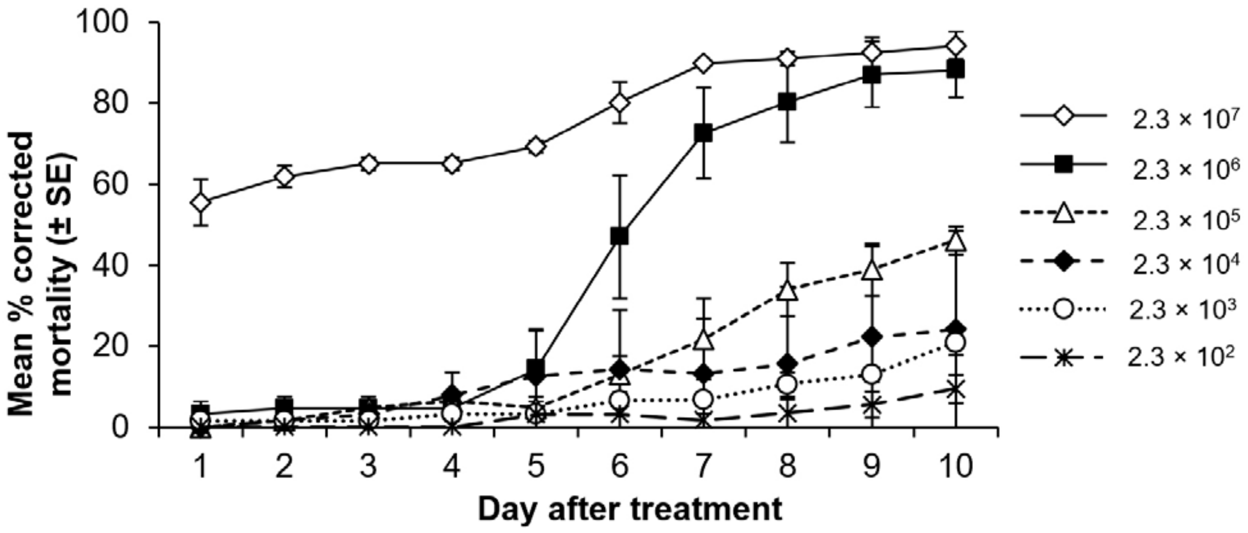

Figure 5. Cumulative mortality (%) of *O. melanopus* adults treated by different concentrations of *B. bassiana* (2.3 × 10^2 – 2.3 × 10^7 conidia/ml)

cadavers of the CLB adults treated with *B. bassiana*. The rate of mycelium presence increased with the conidial concentration tested. Maximum percent of mycelium growth was recorded at the highest concentration tested – only 2% of the dead adults were without mycelia. In the treatment with lowest fungal concentration 11% of the dead adults showed no signs of mycelium.

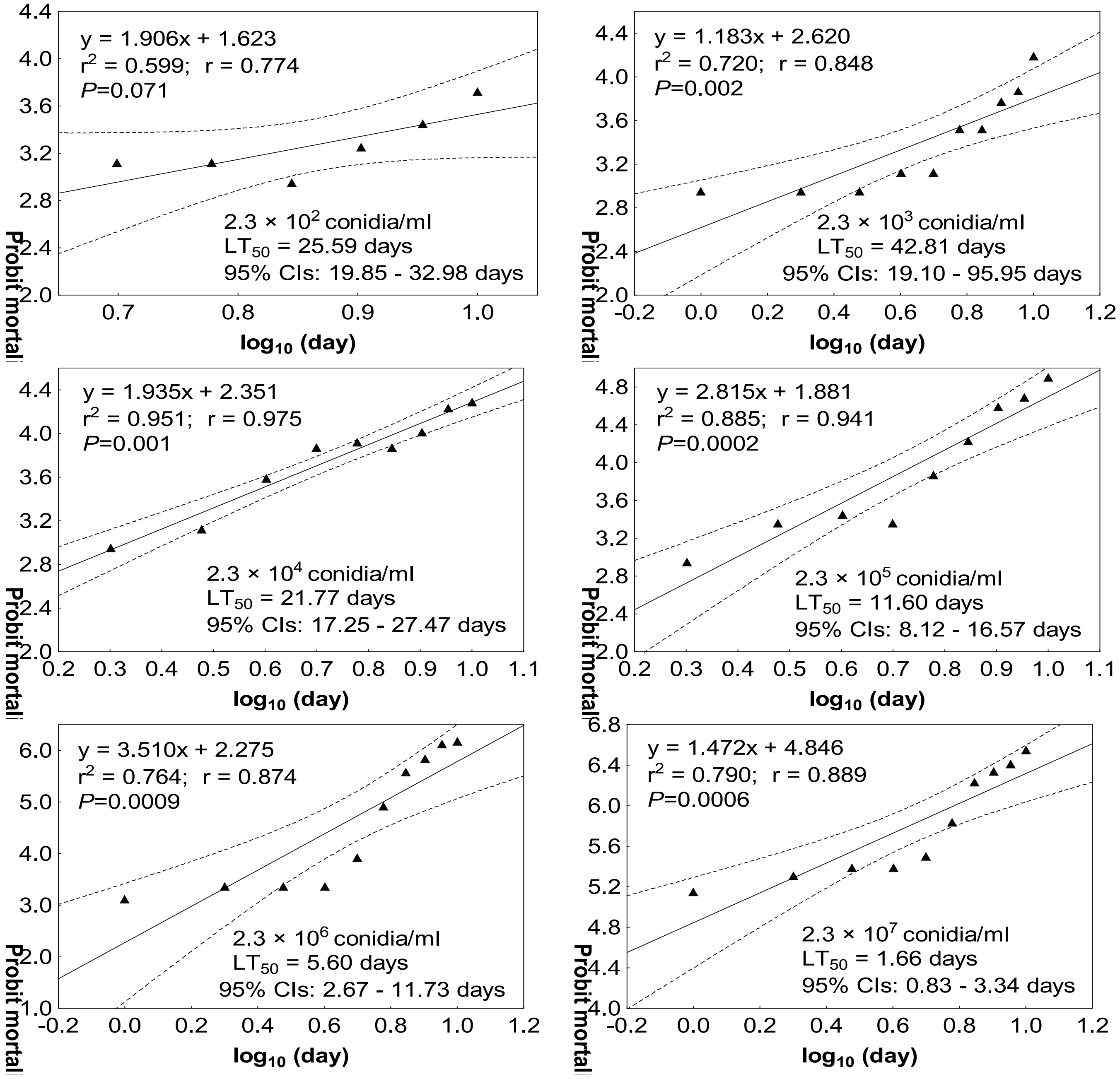

Figure 6. Probit analysis on LT_{50} of *O. melanopus* adults treated with *B. bassiana* at different concentrations (2.3 × 10^2 – 2.3 × 10^7 conidia/ml)

DISCUSSION

The entomopathogenic fungus *B. bassiana* is one of the most effective agents in biological control widely described in the literature (de Faria and Wraight, 2007). Natural infection of *O. melanopus* and *D. v. virgifera* with this fungal pathogen has been reported (Paschke, 1965; Toepfer et al., 2009).

Different factors may influence the results of application of EPF against insect pests including development stage of the insect, age of adults, fungal species, origin of the isolate (native or exotic), physiological properties of fungal strain, mechanisms of molecular interactions between fungi and hosts, formulations (aqueous, oil, foam), conidia concentration, application method (direct application, surface treatment of the substrate), exposure interval, frequency of application and environment factors (temperature, relative humidity, solar radiation, pesticides, competition with other microorganisms) (Wang and Wang, 2017; Islam et al., 2021; Umaru et al., 2022). Susceptibility of WCR to entomopathogenic fungi is well documented (reviewed bellow) while investigations on CLB are scarce (Kher et al., 2012). Testing twenty strains of *B. bassiana*, *B. brongniartii* (Saccardo) Petch and *M. anisopliae* at a concentration of 1 × 10^7 conidia/ml against larvae and adults of WCR in the laboratory Pilz et al. (2007) reported that isolates of *M. anisopliae* caused significantly higher mortalities than isolates of *B. brongniartii* and *B. bassiana*. Similarly, 21 days after inoculation, a strain of *M. anisopliae* (MA1) caused higher mortality of the WCR larvae than the strains of *B. bassiana* and *B. brongniartii*, all strains were tested at a concentration of 2 × 10^7 conidia/ml (Cagáň et al., 2019). The results of the same study showed that for a period of 14 days the mortality rate caused by *B. bassiana* strains varied between 12% and 52%. Rudeen et al. (2013) found that some *M. anisopliae* s.l. strains C20091 and C20092 collected from cornfields killed a higher proportion of *D. v. virgifera* larvae than a standard commercial F52 strain. The same authors showed that the mortality rates caused by *B. bassiana* strains (commercial GHA strain, and D2008 and D2009 isolated from *D. v. virgifera* cadavers) were lower than that of *M. anisopliae* strains C20091 and C20092. Recently, Toshova et al. (2022) evaluated the response of field-collected WCR adults to *Metarhizium pemphigi* (Driver and R. J. Milner) Kepler, S. A. Rehner and Humber, and reported low to moderate effectiveness against the tested insects depending of the conidial concentration.

This is the first study that describes the susceptibility of WCR and CLB to different concentrations of the commercial *B. bassiana* strain ATCC 74040. Results of the current study showed that larvae of *O. melanopus* were very susceptible to Naturalis® with cumulative mortality above 96% for a wide range of concentrations (2.3 × 10^2 - 2.3 × 10^7 conidia/ml). High mortality rate of CLB and WCR adults was observed only with highest conidia concentrations tested - 2.3 × 10^6 - 2.3 × 10^7 conidia/ml and 2.3 × 10^7 conidia/ml, respectively. Differences in responses (mortality and mycelium development) to fungal treatments observed among the target species/stage of insect development could be linked to different factors as effect of application method used, conidia formulation, as well as differences in cuticle structure and the processes of conidial attachment on the cuticle, germination as well as strategies for activation of immune responses (Ortiz-Urquiza and Keyhani, 2013; Rohrlich et al., 2018). Ibrahim (2017) also demonstrated that mortality of the Arabian rhinoceros beetle *Oryctes agamemnon arabicus* (Burmeister, 1847) (Scarabaeidae) adults caused by Naturalis® was lower than that of the larval stage.

Previous laboratory studies have demonstrated insecticide activity of Naturalis® against different coleopteran pests. Atanasova and Vasilev (2020) reported that the efficacy of Naturalis® at a concentration of 0.2% reached 78 % and 68 % on the 5th day after treatment at laboratory conditions for larvae and adults of the Colorado potato beetle, *Leptinotarsa decemlineata* Say, 1824, respectively, and 100% on the 7th day after the treatment for both larvae and adults. Application of 3.75% (~8.6 × 10^5 conidia/ml) the product caused moderate mortality rate (in the range of 45-69%) on adults of four *Otiorhynchus species* – *O. sulcatus*, *O. dieckmanni* Mangano, 1979, *O. raucus* (Fabricius, 1777) and *O. crataegi* Germar 1824, but it was significantly higher than the mortality in the control variant (sterile water) after 28 days post treatment (Hirsch and Reineke, 2014). Toshova et al. (2021) reported high (> 86%) lethal effect of Naturalis® at concentrations above 2.3 × 10^4 conidia/ml on *T. dilaticollis* adults under laboratory conditions. Fătu et al. (2023) found that native *B. bassiana* strain (BbTd1) and native *B. pseudobassiana* Rehner et Humber strain (BbLy) were comparable in percentage of mycosis and virulence against the adults of this species to the pure ATCC 74040 strain. *Beauveria bassiana* ATCC 74040 caused 60% average mortality of the ambrosia beetle *X. germanus* at the highest dosage tested (approximately 600 conidia/mm^2) six days post-treatment; this strain was more effective than other commercial *B. bassiana* strain, GHA (Botanigard®, Laverlam International). High mortality rate (96%) have been shown for the granulate ambrosia beetle, *X. crassiusculus* treated with the same dose (Castrillo et al., 2013). Testing 23 isolates of *B. bassiana*, four isolates of *M. anisopliae* and Naturalis® against *Tenebrio molitor* L., 1758 (Tenebrionidae) larvae in laboratory assays, using 2 × 10^6 conidia/ml fungal suspensions, Oreste et al. (2012) reported that the commercial product was most effective - it caused 100% mortality for shortest time after application. Evaluation of the pathogenicity of the mycoinsecticide on *Aclees* sp. cf. *foveatus* Voss, 1932 (Curculionidae) adults by two methods, application of label concentation on the the soil in plastic cups and direct application of 1 µl suspension of the product on single adult, resulted in 100 % and 90% mortality of the test insects, respectively (Gargani et al., 2016). Sufyan et al. (2017) evaluated the effect of Naturalis® on wireworms (*Agriotes lineatus* (L., 1767) and *A. obscurus* (L., 1758)) (Elateridae) in the laboratory by application of the product on the wheat seeds (seeds put in the conidia solution for 10–15 min) and reported significantly higher mortality rate in the treatment with coated seeds and high number of wireworms (five individuals per box) than the rate in the control treatment (two individuals per box) and the treatment with coated seeds and low number of wireworms (three individuals per box). In contrast, Cuthbertson et al. (2012) and Clifton et al. (2020) demonstrated that *B. bassiana* strain ATCC 74040 failed to kill the Small hive beetle, *Aethina tumida* Murray, 1867 (Nitidulidae) and the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky, 1854) (Cerambycidae).

Naturalis® have been showed to be effective in field/semifiled conditions against several coleopteran pests (Paparatti and Speranza, 2005; Ladurner et al., 2009; Hirsch and Reineke, 2014; Sufyan et al., 2017; Toshova et al., 2021).

In the present study we concluded that Naturalis® was highly efficient against larval stage of *O. melanopus* development under laboratory conditions and further investigations to evaluate its potential to manage this pest in field conditions are needed. The results of the current study also indicate that application of the fungal product against *D. v. virgifera* adults is unsuitable.

The combined use of EPF and low doses of chemical insecticides has been found to be a promising alternative for reducing the amount of chemicals used for pest control (Ladurner et al., 2008; Pelizza et al., 2018). The combined application of *B. bassiana* with other bioinsecticides can improve insect pest control (Shrestha et al., 2020). Further study on the interactions (additive, synergistic, or antagonistic) between Naturalis® and synthetic insecticides or biological products to determine the possible advantages of combinations of these agents against insect pests of maize is required.

CONCLUSIONS

In the present study, we concluded that the commercial *B. bassiana* strain ATCC 74040 has a strong potential for the control of *O. melanopus* larvae. It showed high mortality rates and low LT_{50} of *O. melanopus* larvae at all conidial concentrations tested. However only increased *B. bassiana* concentration at 2.3 × 10^6 and 2.3 × 10^7 conidia/ml resulted in high mortality rates in adults of CLB and WCR adults, respectively.

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