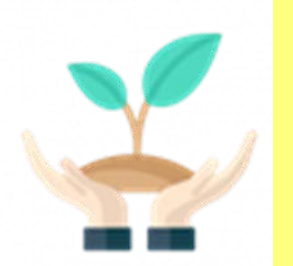


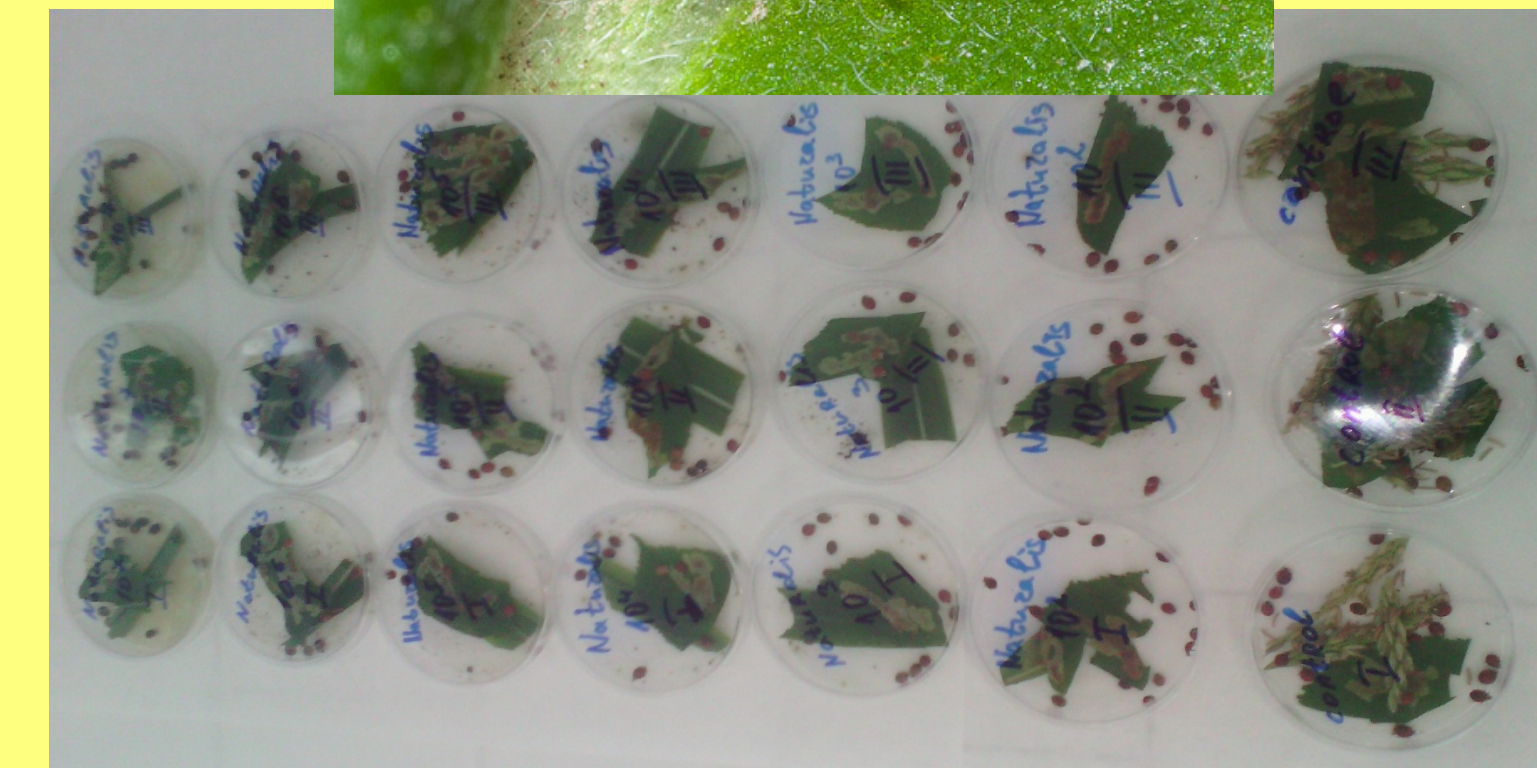
# A survey of a bioinsecticide for controlling insect pests under laboratory conditions

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**ABSTRACT:** The purpose of this study was to evaluate the efficiency of the mycoinsecticide Naturalis® (BioIntrachem, Italy) against field-collected adults of the grey maize weevil, *Tanymecus dilaticollis*, the cereal leaf beetle, *Oulema melanopus*, and the multicolored Asian lady beetle, *Harmonia axyridis*. Naturalis® was tested in six concentrations ( $2.3 \times 10^2$  -  $2.3 \times 10^7$  conidia/ml) in glass Petri dishes. The median lethal concentration (LC<sub>50</sub>) after ten days of fungal treatment ranged from  $2.0 \times 10^3$  conidia/ml to  $2.1 \times 10^6$  conidia/ml for the different species. Naturalis® was most efficient against *T. dilaticollis* adults causing significantly higher mortality at all concentrations tested compared to the control treatment (water). The possibility of using mycoinsecticide to control coleopteran pests in Bulgaria is discussed.



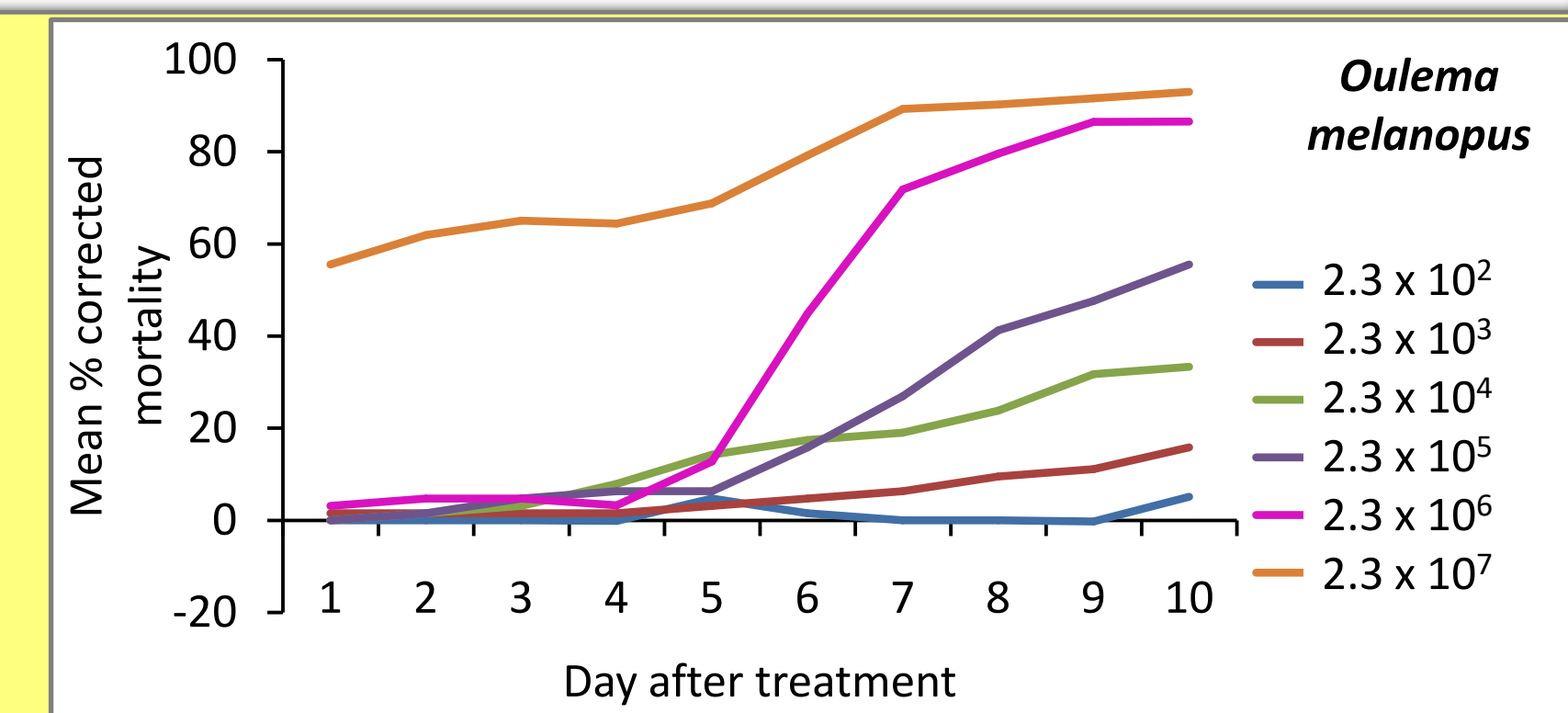
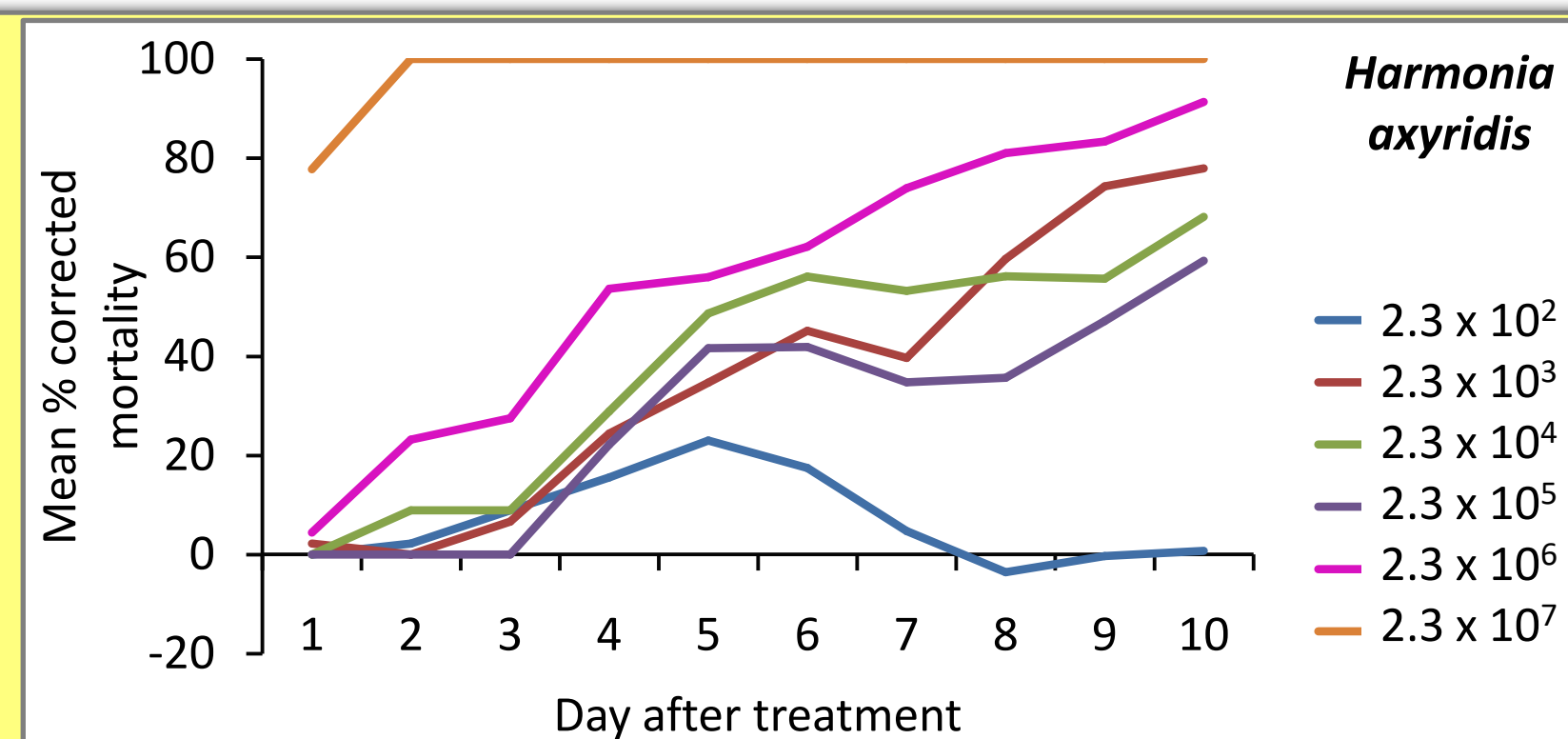
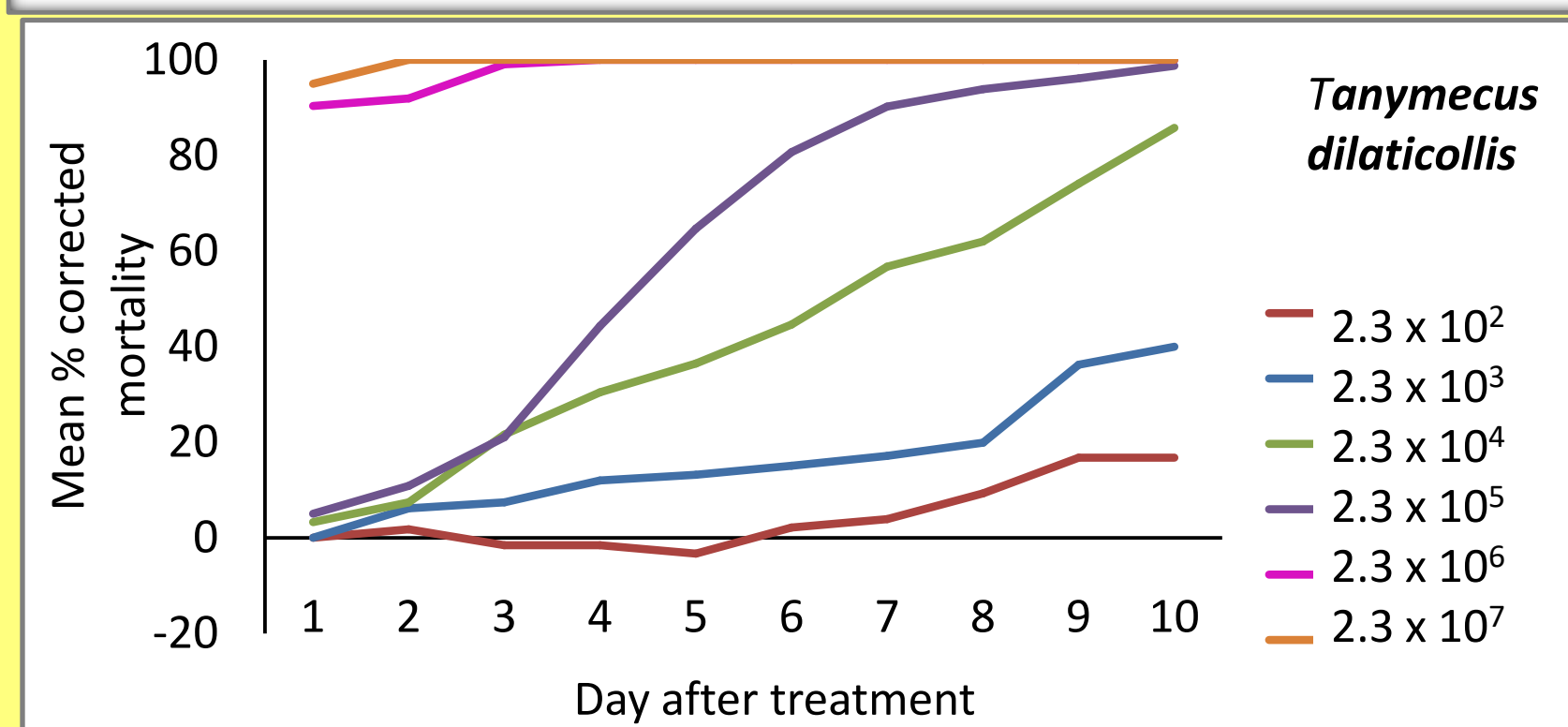
## A bioassay method for evaluating Naturalis® (*Beauveria bassiana* strain ATCC 74040) effectiveness

- Adults of *T. dilaticollis*, *Oulema melanopus* and *Harmonia axyridis* were collected from a maize field belonging to the Maize Research Institute, Knezha, Bulgaria (43°28'48.85"N; 24°3'22.03"E)
- Naturalis® was tested at six concentrations:  $2.3 \times 10^2$  -  $2.3 \times 10^7$  conidia/ml.
- 1 ml from each concentration was applied separately on the white filter paper disc covering the bottom of a Petri dish.
- Adults in control variants were treated with 1 ml of distilled water.
- The experiments were replicated → 3 times for each species.
- Insect mortality was recorded at 24-h intervals during 10 days after treatment.
- The mortality data were corrected using Schneider-Orelli's formula, where corrected mortality (%) =  $(a - b/100 - b) \times 100$  (where a = percentage mortality data from the treated group and b = percentage mortality from the control group)
- Probit analysis was used to estimate the median lethal concentration (LC<sub>50</sub>) (time necessary to kill 50% of the tested individuals) and lethal concentration 90 (LC<sub>90</sub>), the lethal time LT<sub>50</sub> and the 95% confidence intervals of these features.

LC<sub>50</sub> and LC<sub>90</sub> of different concentrations of Naturalis® against the adults of the three coleopteran species under laboratory conditions.

Species	LC <sub>50</sub> conidia/ ml	Confidence intervals		LC <sub>90</sub> conidia/ ml	Confidence intervals	
		Lower bound	Upper bound		Lower bound	Upper bound
<i>T. dilaticollis</i>	$2.0 \times 10^3$	$8.7 \times 10^2$	$5.8 \times 10^3$	$5.2 \times 10^4$	$1.6 \times 10^4$	$5.8 \times 10^5$
<i>H. axyridis</i>	$1.4 \times 10^4$	$1.6 \times 10^3$	$6.5 \times 10^5$	$5.0 \times 10^5$	$2.7 \times 10^4$	$1.0 \times 10^8$
<i>O. melanopus</i>	$2.2 \times 10^5$	$2.8 \times 10^4$	$5.2 \times 10^6$	$1.7 \times 10^6$	$8.9 \times 10^3$	$4.9 \times 10^5$

Cumulative mortality (%) of adults of *T. dilaticollis*, *H. axyridis* and *O. melanopus* caused by Naturalis® at different conidial concentrations (conidia/ml) ten days after treatment



Mycosis of *O. melanopus* adults caused by the fungus *Beauveria bassiana*,  $2.3 \times 10^5$  -  $2.3 \times 10^7$  conidia/ml.



Median lethal time (LT<sub>50</sub>) in days at the highest concentrations of Naturalis® for the adults of *T. dilaticollis*, *H. axyridis* and *O. melanopus* under laboratory conditions.

Concentration conidia/ml	LT <sub>50</sub> (lower and upper confidence intervals), days		
	<i>Tanymecus dilaticollis</i>	<i>Harmonia axyridis</i>	<i>Oulema melanopus</i>
$2.3 \times 10^4$	5.75 (5.07 - 6.70)	9.51 (5.16 - 36.06)	74.13 (35.40 - 229.09)
$2.3 \times 10^5$	3.53 (3.16 - 4.04)	6.62 (5.32 - 8.79)	15.18 (10.23 - 29.51)
$2.3 \times 10^6$	0.25 (0.19 - 0.30)	3.85 (3.27 - 4.75)	6.17 (4.47 - 10.07)
$2.3 \times 10^7$	0.01 (0.001 - 0.02)	0.09 (0.03 - 0.16)	1.30 (1.24 - 1.39)

- To our knowledge these are the first results on the susceptibility of the adults of *T. dilaticollis*, *H. axyridis* and *O. melanopus* to Naturalis® (BioIntrachem, Italy).
- It was established that the adults of the grey maize weevil were most sensitive test-insects to the mycoinsecticide under laboratory conditions.
- For *T. dilaticollis*, the mycoinsecticide caused 100 % corrected mortality of exposed weevils when tested at concentrations over  $2.3 \times 10^5$  conidia/ml. There was no significant difference in mortality between treatments with concentrations above  $2.3 \times 10^4$  conidia/ml.
- For *H. axyridis*, the highest concentrations tested evoked 91-100 % mortality.
- For *O. melanopus*, the effectiveness of Naturalis® was relatively low – the highest mortality was in the range of 87-93 % for the concentrations of  $2.3 \times 10^6$  -  $2.3 \times 10^7$  conidia/ml . received at the end of the bioassay.
- Results showed potential of this mycoinsecticide for its use for microbial control of *T. dilaticollis* adults.