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BACKGROUND

Increased emergence and spread of resistance against current insecticides amongst a wide range of both disease vector and agricultural pest insects has rendered many traditional forms of chemical control ineffective. This trend poses serious threats to public health, food security and economies relying on agriculture. Furthermore, the negative impact of many insecticides on human and animal health, and the environment, has resulted in new regulations favoring the development of 'green' biopesticides within the European Union.

OBJECTIVE

The overarching aim of this project is to identify suitable microorganisms for pest control and develop them into prototype biopesticides. As a first step towards this goal, we prospected the Greek island of Crete for environmental samples from which we isolated fungal strains that were screened against larvae of the mosquito *Culex pipiens* biotype *molestus*.

METHODOLOGY

Seventeen locations in Crete were selected based on geographic and environmental characteristics, and sample collections were mostly focused on rhizosphere soil, plant roots and stems, and dead insects. In three locations, batches of bulk soil (ca. 4 kg) were also collected.

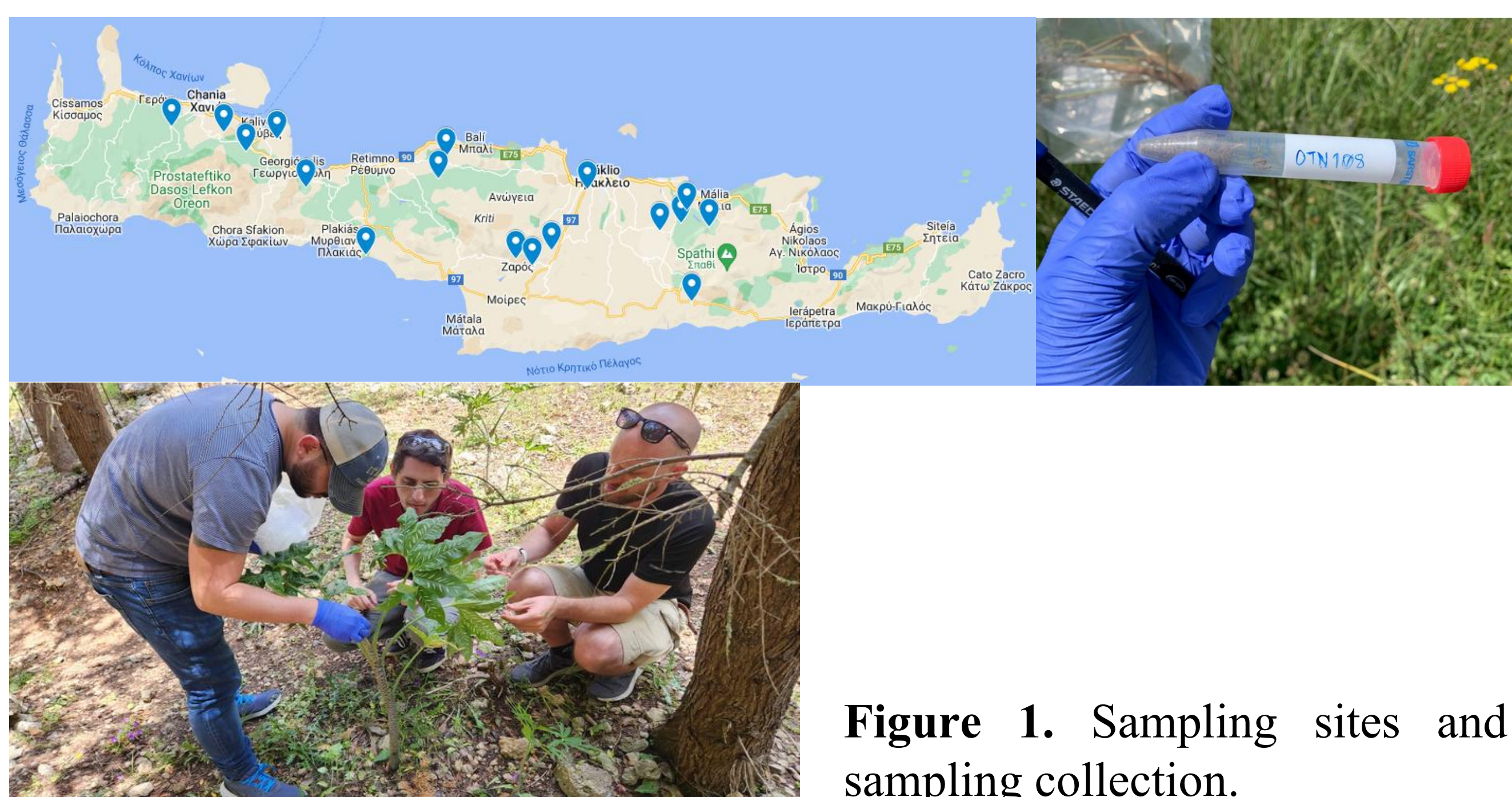
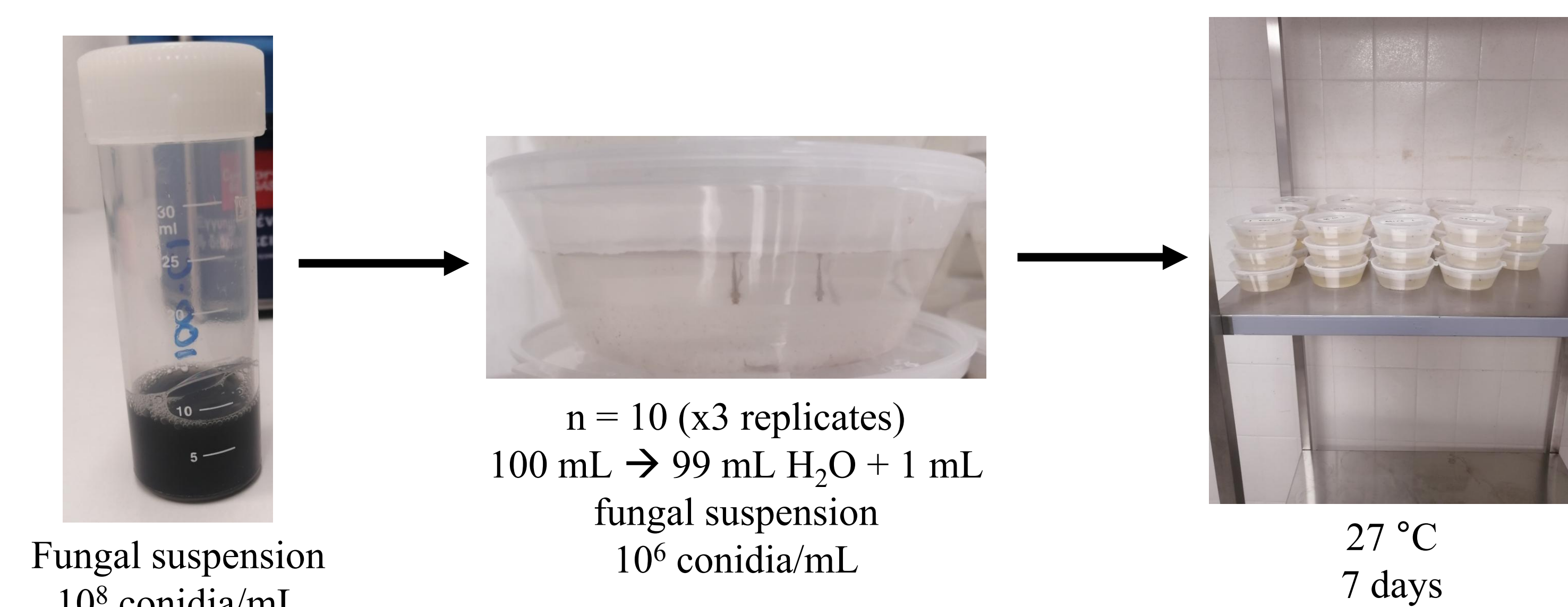


Figure 1. Sampling sites and sampling collection.

Collected dead insects and plant roots and stems were surface sterilized and plated on Sabouraud Dextrose Agar (SDA). Soil samples were suspended in saline and plated on SDA. After incubation at 27 ± 1 °C for 7–14 days, colonies were isolated on SDA. Isolation of fungi from bulk soil batches was performed through insect baiting, using larvae of *Tenebrio molitor*. Every two days, dead larvae were collected, surface sterilized and placed in moist chambers at 27 ± 1 °C for 7 days. Fungi that emerged from the cadavers were isolated on SDA.



After a course, semi-high-throughput larvicidal screening against *Culex pipiens* biotype *molestus*, nine fungal isolates were selected for a virulence bioassay. Fungal suspensions were prepared washing sporulated plates with sterile distilled water + 0.05% Tween 80; the adjusted concentration was 10⁸ conidia/mL. Then, 1 mL was transferred to plastic cups containing 99 mL of distilled water, resulting in a final concentration of 10⁶ conidia/mL. In the control, it was added 1 mL of a 0.05% Tween 80 solution. Each cup had 10 3rd-instar larvae, and there were 3 cups per isolate. Larvae were fed with pulverized fish food. Mortality was evaluated daily up to 7 days. The bioassay was repeated three times.

RESULTS

A total of 34 environmental samples were collected from various sites in Crete, from which 46 fungal isolates were obtained.

Table 1. List of isolates selected for a virulence assay, based on a preliminary screening. Identification at the genus level was performed through Sanger sequencing of the ITS region of the rRNA.

Isolate code	Genus	Origin
OTN100-C1	<i>Cladosporium</i>	Soil
OTN103-C1	<i>Cladosporium</i>	Insect
OTN108-CD3	<i>Talaromyces</i>	Soil
OTN145-C3	<i>Mortierella</i>	Insect
OTN159-C1	<i>Cladosporium</i>	Insect
OTN181-CD1	<i>Purpureocillium</i>	Soil
BS1-C4	<i>Penicillium</i>	Soil
BS1-C6	<i>Cladosporium</i>	Soil
BS2-C3	<i>Metarhizium</i>	Soil

- Survival of larvae was affected by the treatments. All isolates resulted in higher mortality than the control (Figure 3).
- Isolate BS2-C3 was the most virulent, resulting in larval mortality over 90% and LT₅₀ of 1.8 days.
- Isolate BS1-C4 achieved over 50% of mortality and had a LT₅₀ of 5.4 days.
- The other isolates showed a less potent entomopathogenic activity.

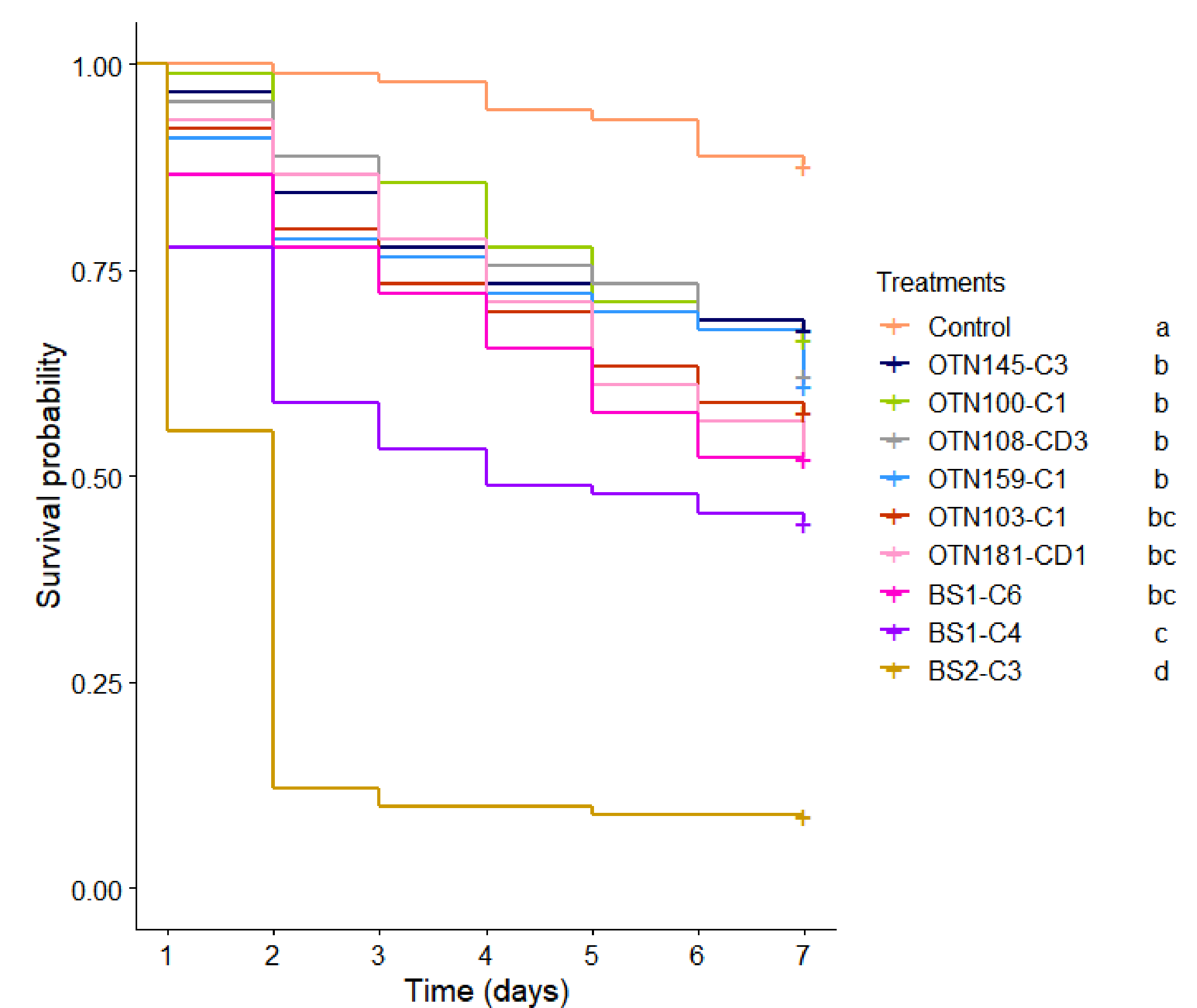


Figure 3. Daily cumulative survival probability (Kaplan-Meier) of *Culex pipiens* biotype *molestus* larvae exposed to conidial suspensions (10⁶ conidia/mL) of different genera of fungi. Treatments followed by different letters are significantly different (log-rank test, $\alpha = 0.05\%$).

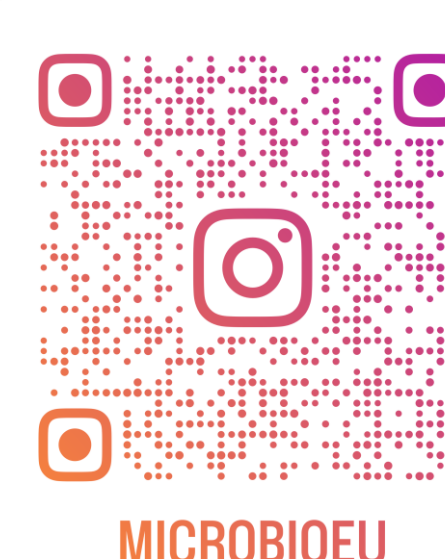
CONCLUSION

The nine selected isolates showed activity against mosquito larvae, although in different degrees, with BS2-C3 (*Metarhizium* sp.) showing the biggest potential against *C. pipiens* larvae.

ONGOING & FUTURE STUDIES

Ongoing studies are focusing on determining the activity spectrum of top hits by screening against larval and adult stages of additional vector mosquito species and agricultural pest insects, testing for non-target effects, and determining the nature of potential insecticidal metabolites and proteins.

LEARN MORE ABOUT THE PROJECT



MICROBIOEU



Website: microbiopest.eu/

ACKNOWLEDGEMENT

This project has received funding from the European Union's HORIZON-CSA (HORIZON-WIDERA-2022-TALENTS-01-ERA Chairs) programme under the grant agreement No 101087110.