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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 insect control and develop them into prototype bioinsecticides. 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 and adults of the mosquito *Culex pipiens 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.

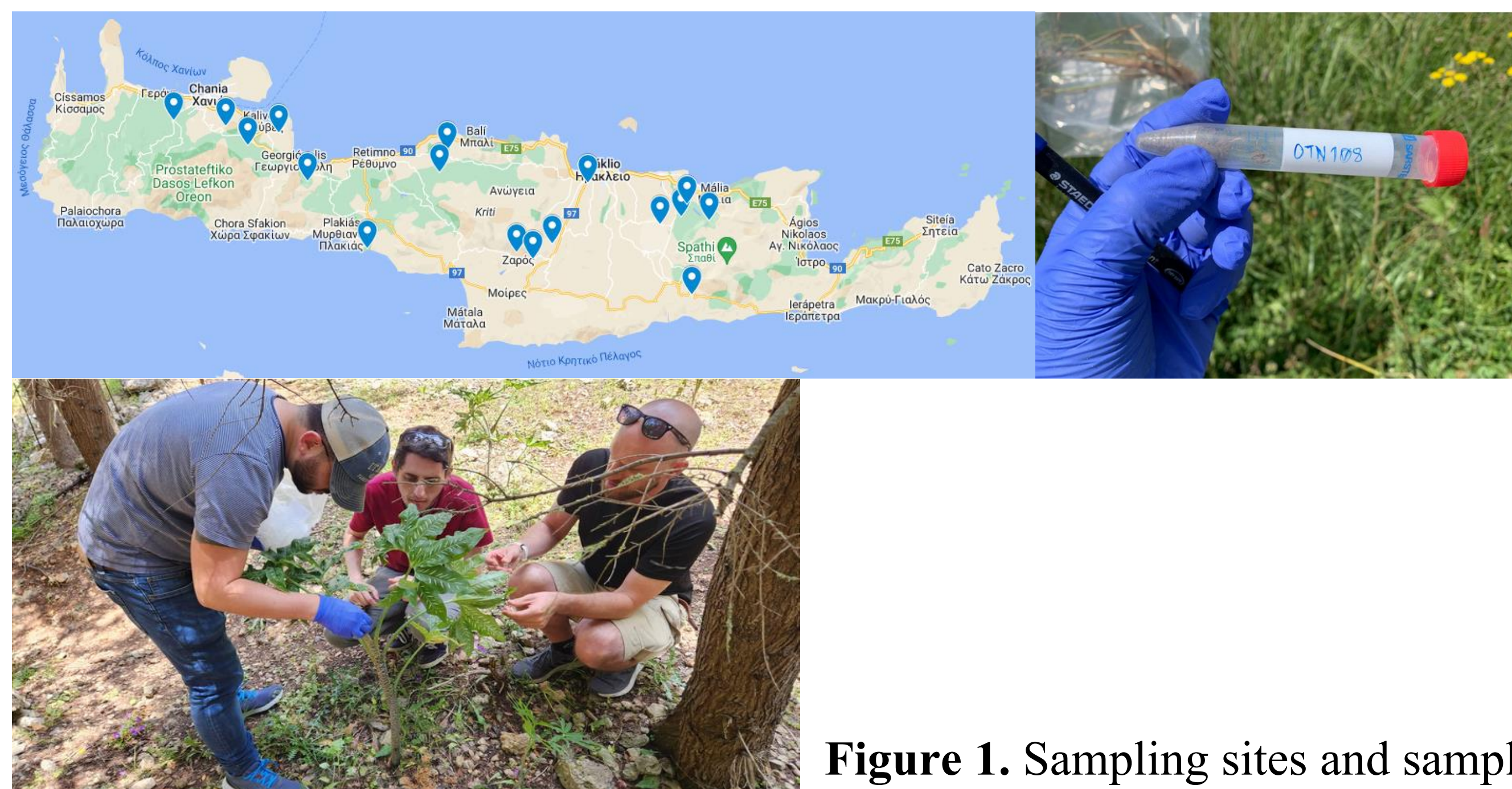


Figure 1. Sampling sites and sampling collection.

Among the 36 samples, we identified 46 fungal isolates. Following preliminary bioassays against larvae of *C. pipiens molestus*, eight isolates were selected for further characterization of larvicidal and adulticidal activities.

**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
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

After a course, semi-high-throughput larvicidal screening against *C. pipiens molestus*, eight fungal isolates were selected for virulence bioassays. Fungal suspensions were prepared by washing sporulated plates with a solution of 0.05% Tween 80; the adjusted concentration was  $10^8$  conidia/mL.

For larvicidal bioassays, 1 mL was transferred to plastic cups containing 99 mL of distilled water, resulting in a final concentration of  $10^6$  conidia/mL. Controls were treated with 1 mL of a 0.05% Tween 80 solution. Mortality was evaluated daily for 7 days.

For adulticidal bioassays, 2 mL of the suspensions were sprayed to plastic cups (300 mL). Controls were sprayed with 2 mL of a 0.05% Tween 80 solution. After drying the cups, ten adult mosquitoes (5–7 days old) were transferred to each cup. A 10% sugar solution was offered as a food source. Mortality was evaluated daily for 10 days.

In both assays (replicated three times), each isolate was tested in triplicate (n = 30 per isolate).

## RESULTS

Larvicidal assay:

- All isolates resulted in higher mortality than the control (Figure 2).
- Isolate BS2-C3 resulted in ~91% larval mortality, with an  $LT_{50}$  of 1.8 days.
- Isolate BS1-C4 achieved over 50% of mortality, with an  $LT_{50}$  of 5.4 days.

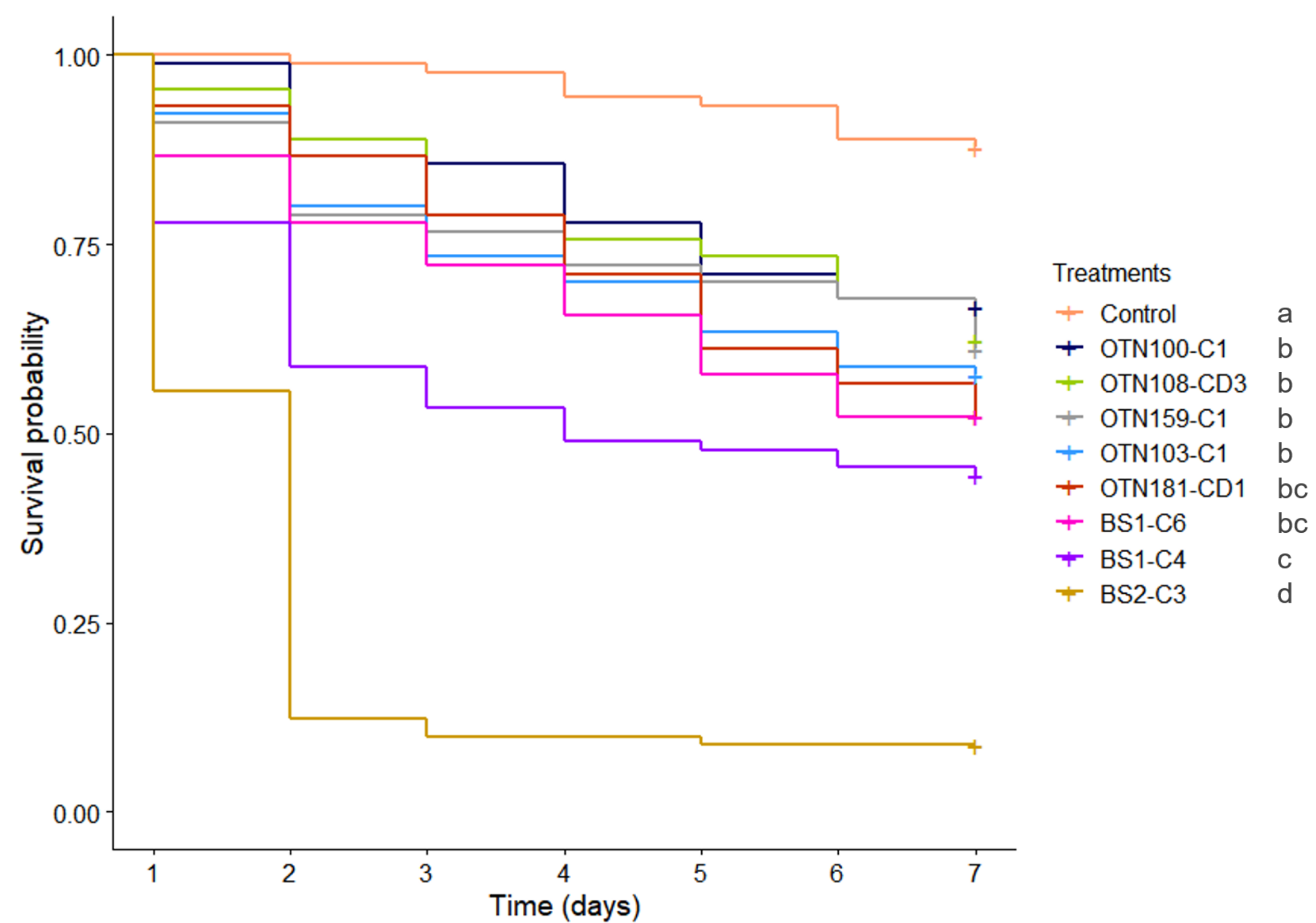


Figure 2. Daily cumulative survival probability (Kaplan-Meier) of *Culex pipiens molestus* larvae exposed to conidial suspensions ( $10^6$  conidia/mL) of different genera of fungi. Treatments followed by different letters are significantly different (log-rank test,  $\alpha = 5\%$ ).

Adulticidal assay:

- Seven isolates resulted in higher mortality than the control (Figure 3).
- Isolate BS2-C3 (*Metarhizium* sp.) resulted in ~97% adult mortality, with an  $LT_{50}$  of 4.1 days.
- Isolate OTN181-CD1 (*Purpureocillium* sp.) achieved 62% mortality, and OTN159-C1 (*Cladosporium* sp.) achieved 52% mortality.

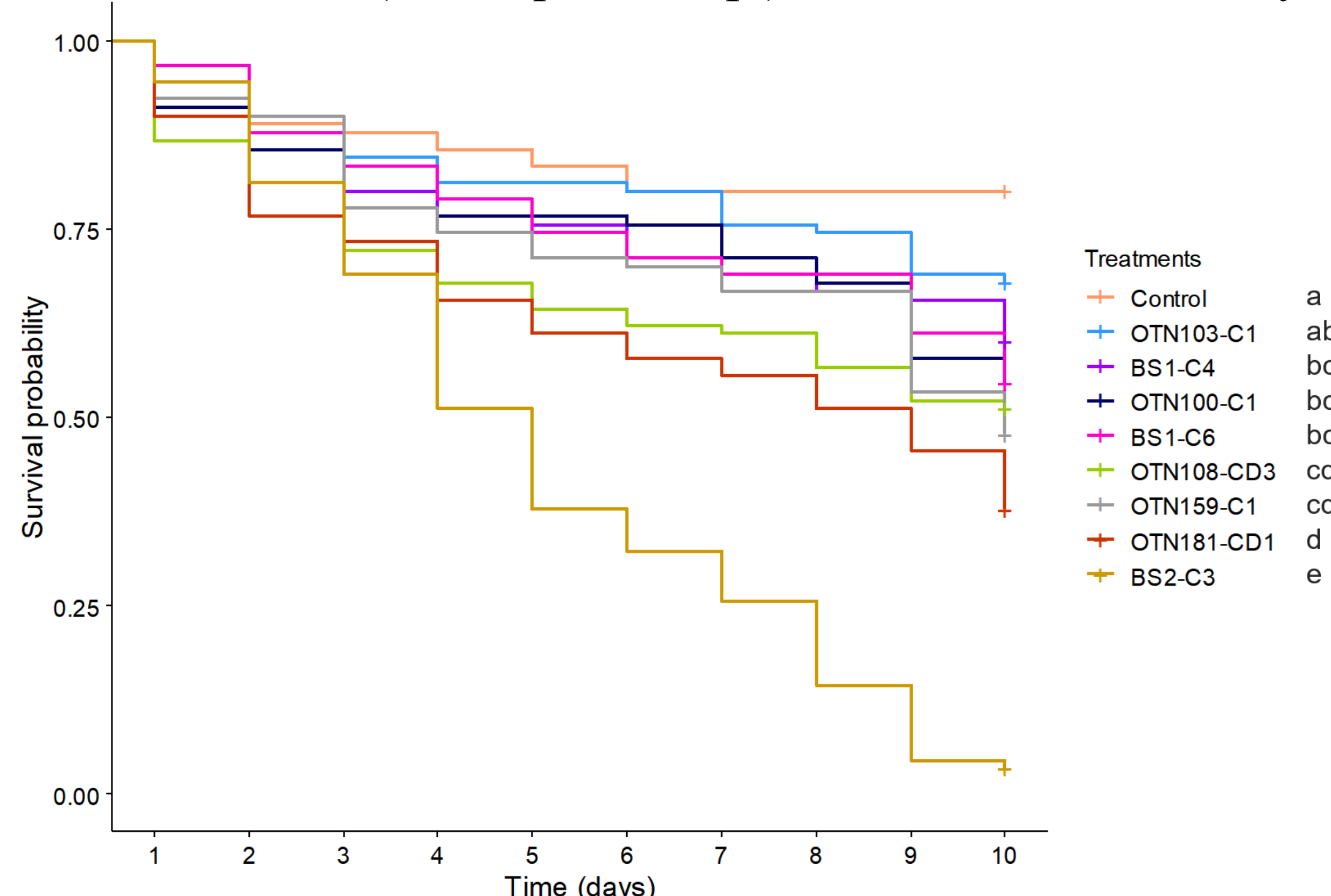


Figure 3. Daily cumulative survival probability (Kaplan-Meier) of *Culex pipiens molestus* adults exposed to conidial suspensions ( $10^8$  conidia/mL) of different genera of fungi. Treatments followed by different letters are significantly different (log-rank test,  $\alpha = 5\%$ ).

## CONCLUSION

The eight selected isolates showed different degrees of activity against mosquito larvae and adults, with BS2-C3 (*Metarhizium* sp.) showing the greatest entomopathogenic potential against *C. pipiens molestus*. Bioassays also revealed that isolates of *Penicillium*, *Purpureocillium* and *Cladosporium* had activity against larvae or adults, despite their biocontrol potential remaining largely underexplored.

## ONGOING & FUTURE STUDIES

Our research is focused on further characterizing these isolates, assessing their host range and optimizing culturing and formulation to maximize insecticidal activity, to develop effective, safe, and environmentally-sustainable alternatives to chemical insecticides for integrated vector management programs.

## ACKNOWLEDGEMENT

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