

Global Forum on Biological Control and Training Workshop on Biological Control

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Studies on non-target effects of biopesticides

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Introduction

❖ Agriculture partly depends on the beneficial services offered by nature:



- Over 75% of flowering crops depend on **insect pollinators**.
- Some pollinators (bees) provide hive products such as honey, wax, cerumen, bee bread, royal jelly, bee venom, and propolis.



- **Natural enemies** (parasitoids and predators) reduce pest populations.



- **Soil invertebrates** improve fertility.

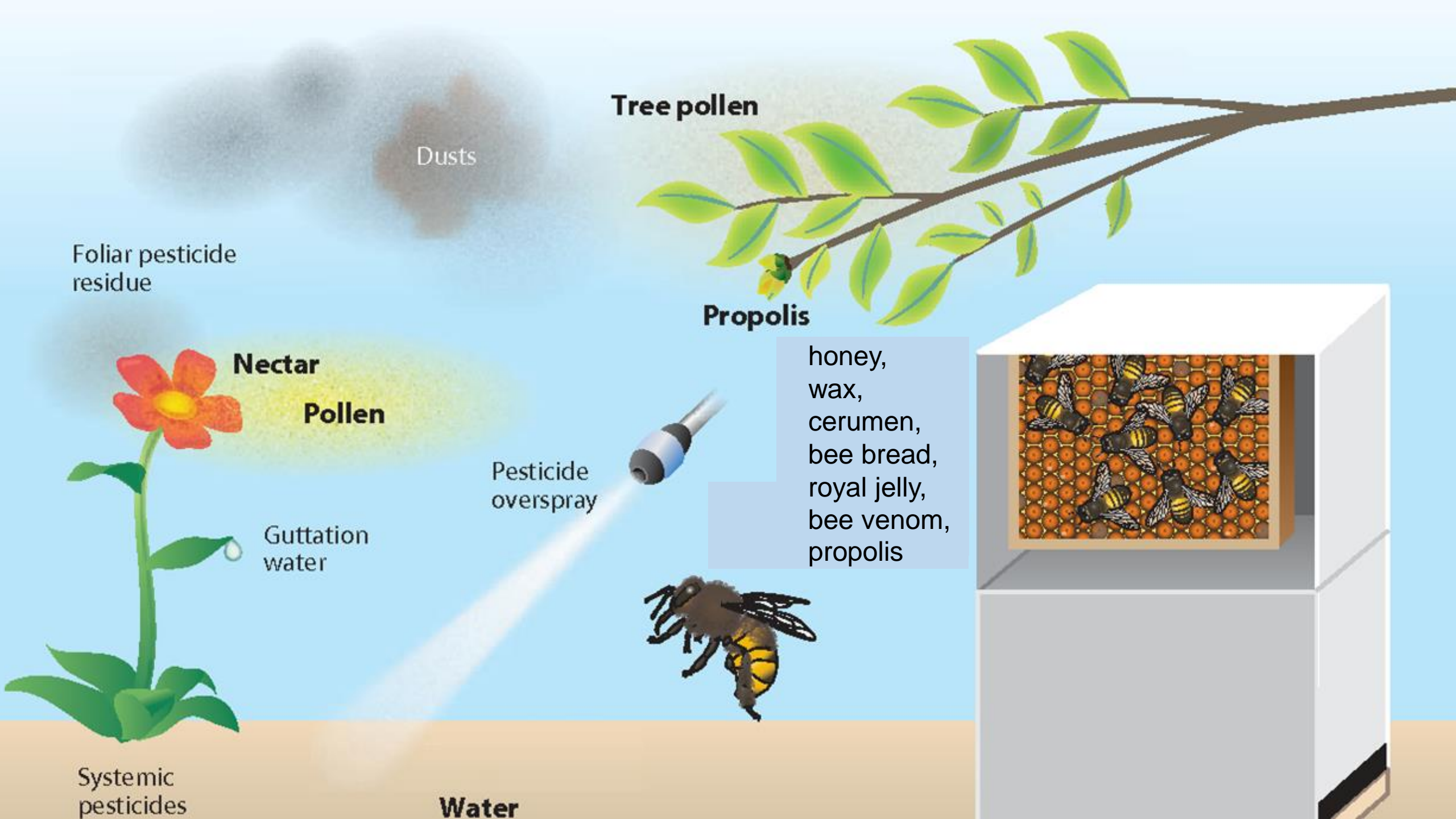
Chemical insecticides constitute the key drivers to the **alarming global decline** of pollinators.



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Tree pollen

Dusts

Foliar pesticide
residue

Nectar

Pollen

Guttation
water

Pesticide
overspray

Propolis

honey,
wax,
cerumen,
bee bread,
royal jelly,
bee venom,
propolis

Systemic
pesticides

Water



Do maize plants require insect pollination?

- Bees foraging on maize, other flowering crops (legumes, sunflowers) and wildflowers (weeds) on, and around maize field may be exposed to several chemical insecticides sprayed on fields (Fernandez-Cornejo et al. 2014; Long and Krupke 2016).
- To protect pollinators, biopesticides based on entomopathogenic fungi (EPF) can be used as alternatives to chemical insecticides.



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Biopesticides developed at icipe



Ecotoxicological test of biopesticides



- Ecotoxicological dossiers are available for registered biopesticides.
- Ecotoxicological dossiers are based on Organisation for Economic Co-operation and Development (OECD, 1998) guidelines to test suboptimal doses ($<10^7$ conidia/mL) through oral exposure in short bioassays (<96 hr) with honeybees.
- Stingless bees have never been used as test insects.
- Long (10-days) bioassays with honeybees and stingless bees can result in variable toxicity levels depending on EPF isolates.
- International Organization of Biological Control (IOBC) classification:
 - ❖ Class 1: harmless ($<25\%$),
 - ❖ Class 2: slightly harmful ($25\%-50\%$),
 - ❖ Class 3: moderately harmful ($51\%-75\%$),
 - ❖ Class 4: harmful ($>75\%$)



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Selected biopesticides screened for non-target effect on pollinators (honeybees and stingless bees)



| Biopesticide isolate name | Trade Name | Target pests |
|--|---------------------------|--|
| <i>Metarhizium anisopliae</i> ICIPE 7 | Detain | Fall armyworm |
| <i>Metarhizium anisopliae</i> ICIPE 78 | Achieve, Mazao achieve | Fall armyworm, Spider mites, |
| <i>Metarhizium anisopliae</i> ICIPE 20 | - | Tuta absoluta, Fall armyworm, leafminers |
| <i>Metarhizium anisopliae</i> ICIPE 62 | Mazao supreme | Aphids |
| <i>Metarhizium anisopliae</i> ICIPE 69 | Campaign | Thrips, whiteflies, leafminers |
| <i>Beauveria bassiana</i> ICIPE 284 | - | Fall armyworm |

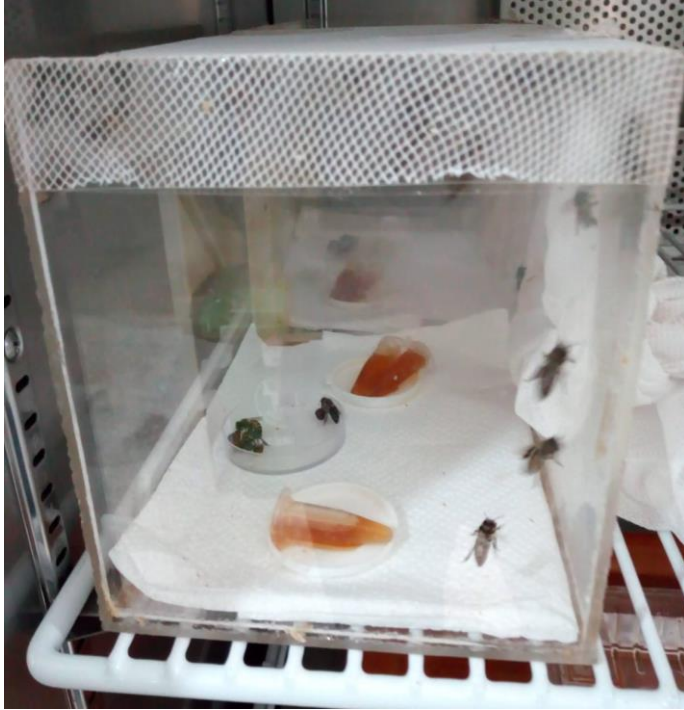
Akutse et al., 2020



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Objectives of the study



Effect biopesticides on
survival of bees in lab



Modelling performance of
the biopesticides in bee
colonies simulated
temperatures.



Effect of biopesticides on
bee pollination behaviour
and crop productivity in
semi-field trials



Effect of biopesticides on survival of bees

Honeybees
Apis mellifera



Stingless bees
Meliponula ferruginea



Spray tower



Fungal isolates

- Applying 10^8 conidia/mL or water on filter paper.
- Exposure of 25-30 bees/cage in four replicates.

Incubators

Honeybees



Stingless bees



10-days survival bioassays

Results

Kaplan-Meier survival curves of fungus-exposed bees

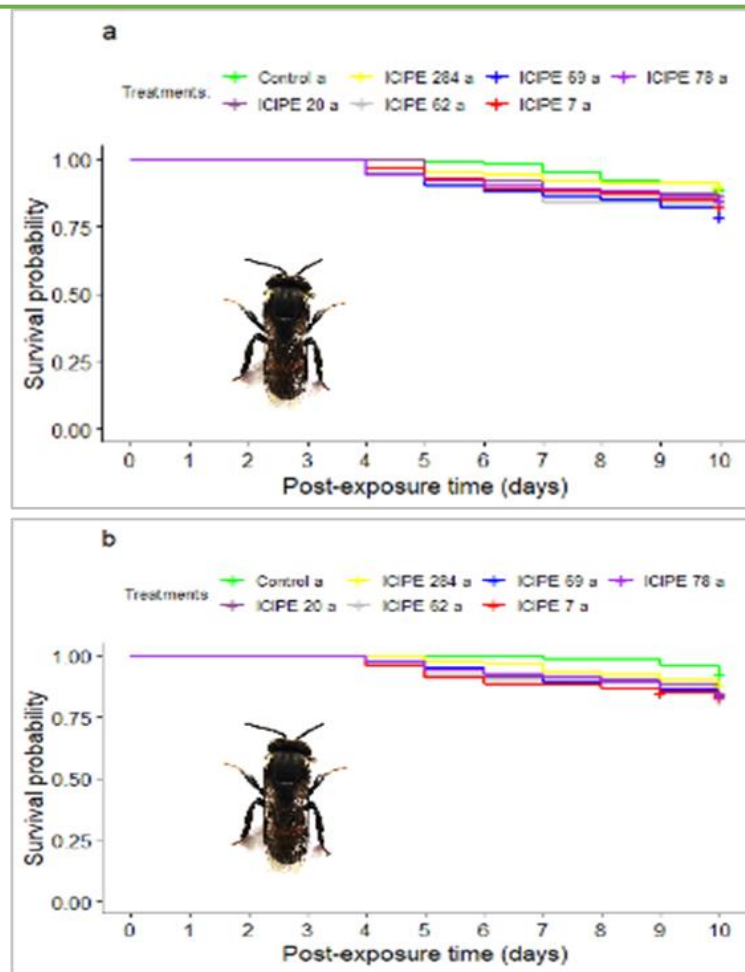
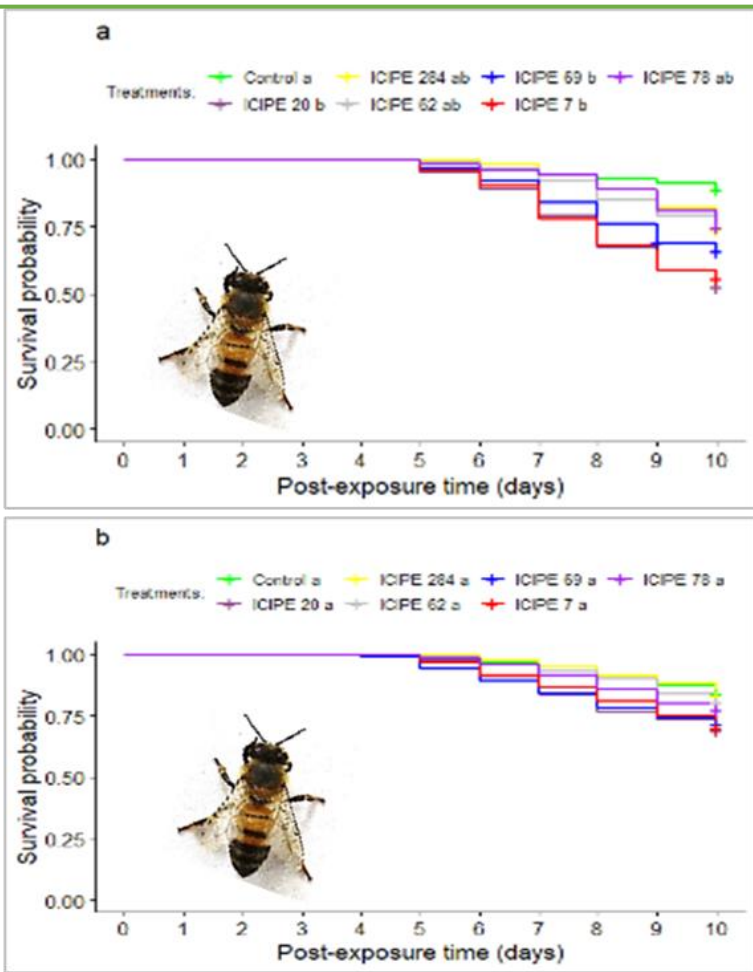
Honeybees

Stingless bees



1st experiment

2nd experiment



The tested isolates were moderately and **nontoxic** to bees according to the **International Organization of Biological Control (IOBC)** classification



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Research



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Biological and Microbial Control

Susceptibility of the Western Honey Bee *Apis mellifera* and the African Stingless Bee *Meliponula ferruginea* (Hymenoptera: Apidae) to the Entomopathogenic Fungi *Metarhizium anisopliae* and *Beauveria bassiana*

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Modelling the performance of the biopesticide isolates under bee colonies' temperatures



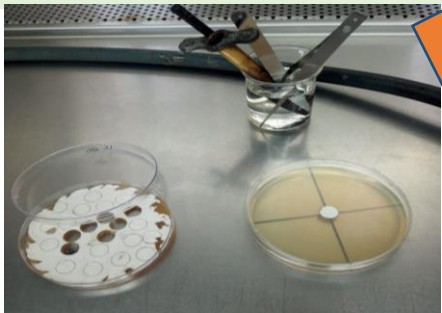
Conidial germination



Fungal suspension
(10^6 conidia/mL)

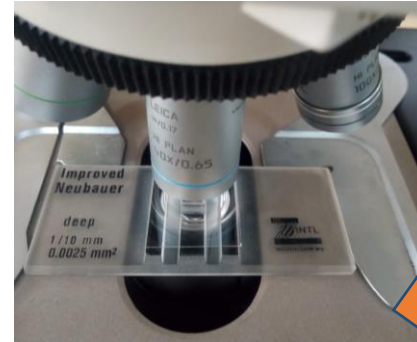


Mycelial growth



Mycelial mat

Incubation at
12, 16, 20, 24,
28, 32, 36°C



Observation of
conidial germ tubes



Measurement of
radial growth

Model selection

Eight models from
literature

Model validation

Output

Model
comparison

- Goodness-of-fit
- Adjusted R-squared

**Thermal requirements
for isolates:**
Tmin (°C), Topt (°C),
Tmax (°C), Pmax
(%germination, mm/day)



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Temperature dependent models tested



Model tested

1. Briere 1
2. Briere 2
3. Ratkowsky 2
4. Ratkowsky 3
5. Lactin 1
6. Van Der Heide
7. CTMI (Cardinal temperature model with Inflection)
8. Generalized beta function

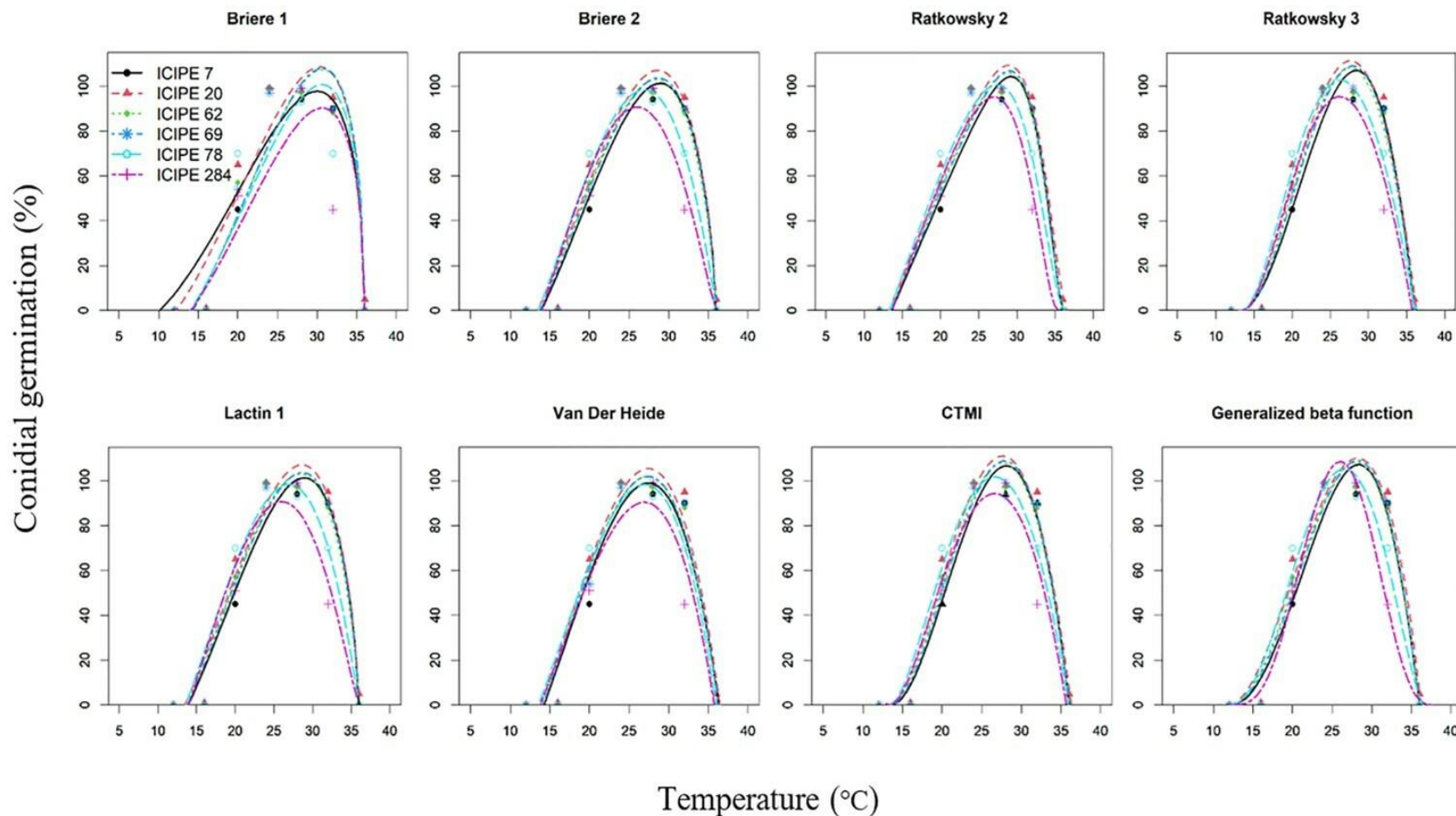


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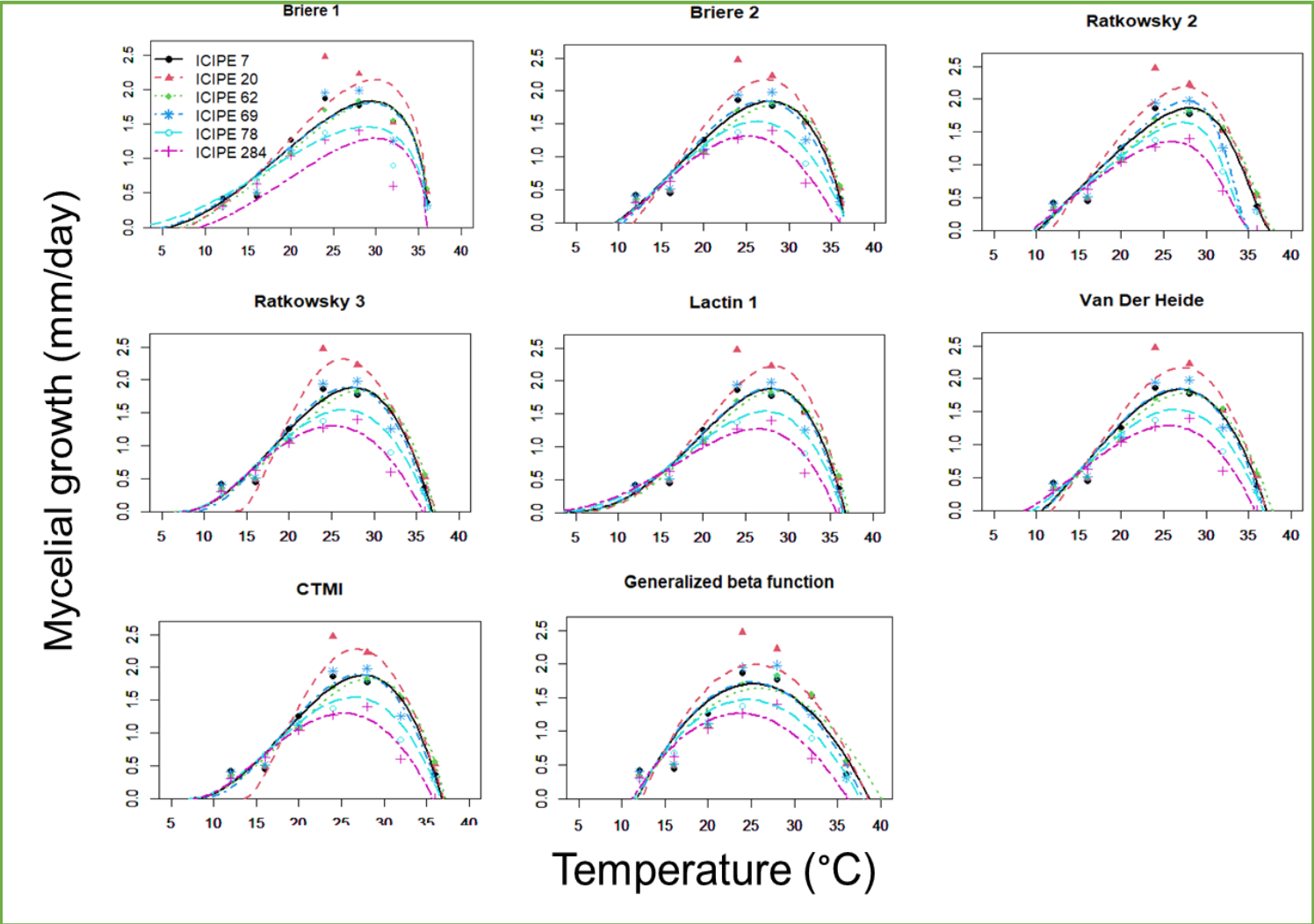
Results

Conidial germination curves by temperature-dependent models



Conidial germination **started** from 10–14°C and reached **a peak** at 25–30°C, followed by a precipitous decline reaching **upper thresholds** at 35–38°C.

Growth curves as predicted by 8 temperature-dependent models



| Isolate | Tmin (°C) | Topt (°C) | Tmax (°C) | Pmax (mm/day) |
|-----------|-----------|-----------|-----------|---------------|
| ICIZE 7 | 7.4 | 27.6 | 36.8 | 1.89 |
| ICIZE 20 | 13.5 | 26.8 | 37.0 | 2.28 |
| ICIZE 62 | 3.7 | 28.4 | 37.2 | 1.85 |
| ICIZE 69 | 8.7 | 27.1 | 36.6 | 1.89 |
| ICIZE 78 | 7.3 | 26.5 | 36.7 | 1.54 |
| ICIZE 284 | 9.5 | 25.9 | 35.4 | 1.36 |



Biocontrol Science and Technology



ISSN: (Print) (Online) Journal homepage: <https://www.tandfonline.com/loi/cbst20>

Suitable models to describe the effect of temperature on conidial germination and mycelial growth of *Metarhizium anisopliae* and *Beauveria bassiana*

Evanson R. Omuse, Saliou Niassy, John M. Wagacha, George O. Ong'amo, Abdelmutalab G. A. Azrag & Thomas Dubois

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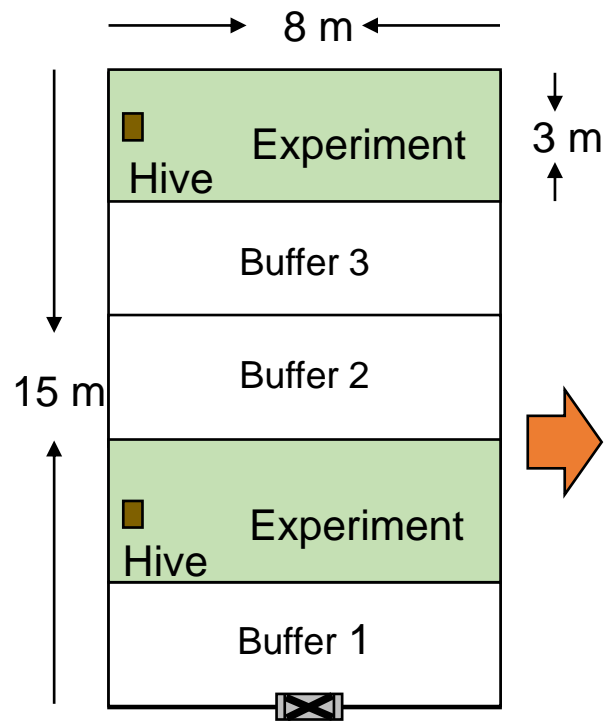
To link to this article: <https://doi.org/10.1080/09583157.2021.1993133>



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Effect of a biopesticide on survival, pollination behaviour and success of the stingless bee



Cultivation of cucumber
in screenhouses



Introduction of bee
colonies and application of
biopesticide (ICIPE 69) or
distilled water (control)



Flight activity



Foraging activity,
survival

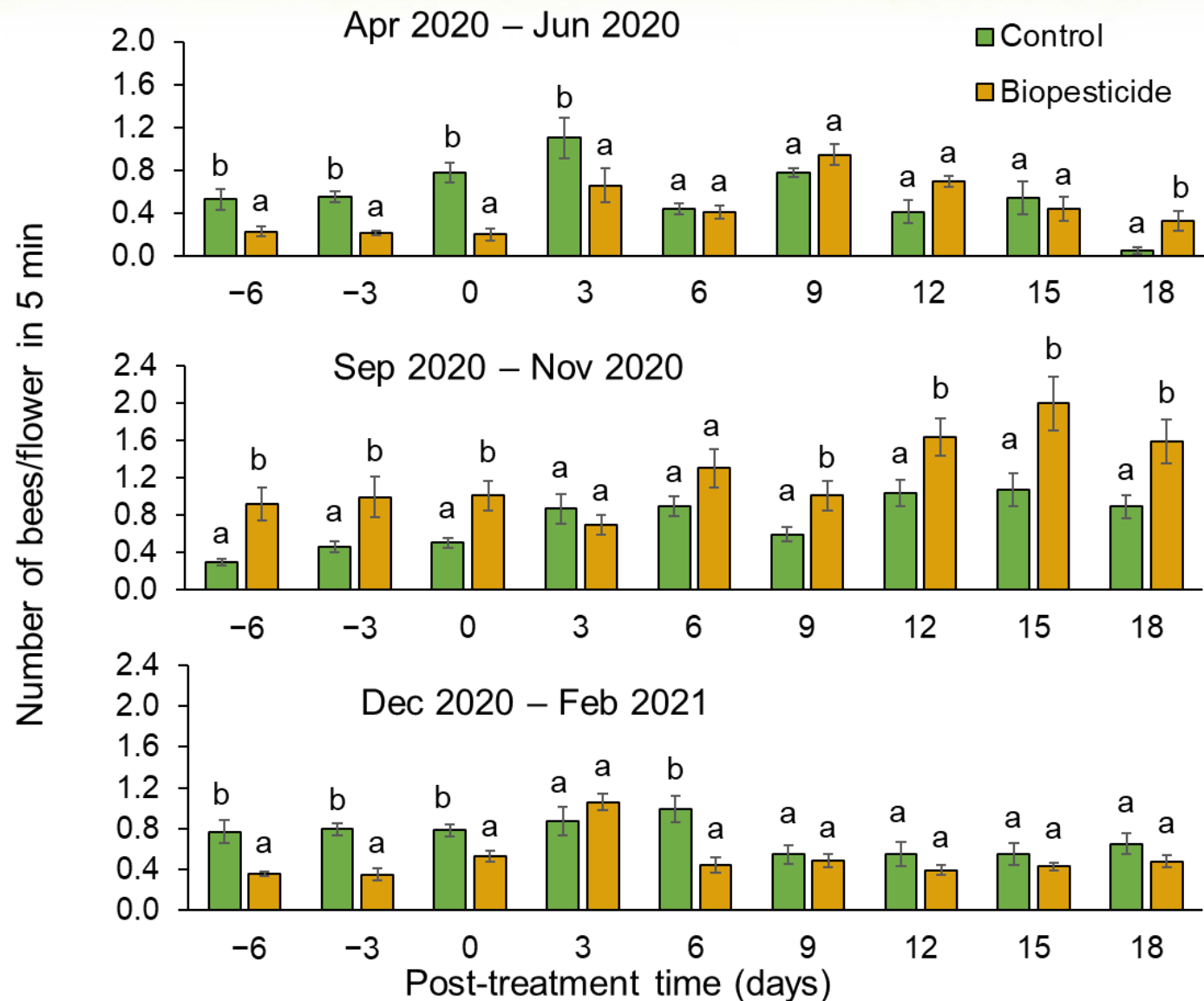


Fruit set,
maturation and
yield)

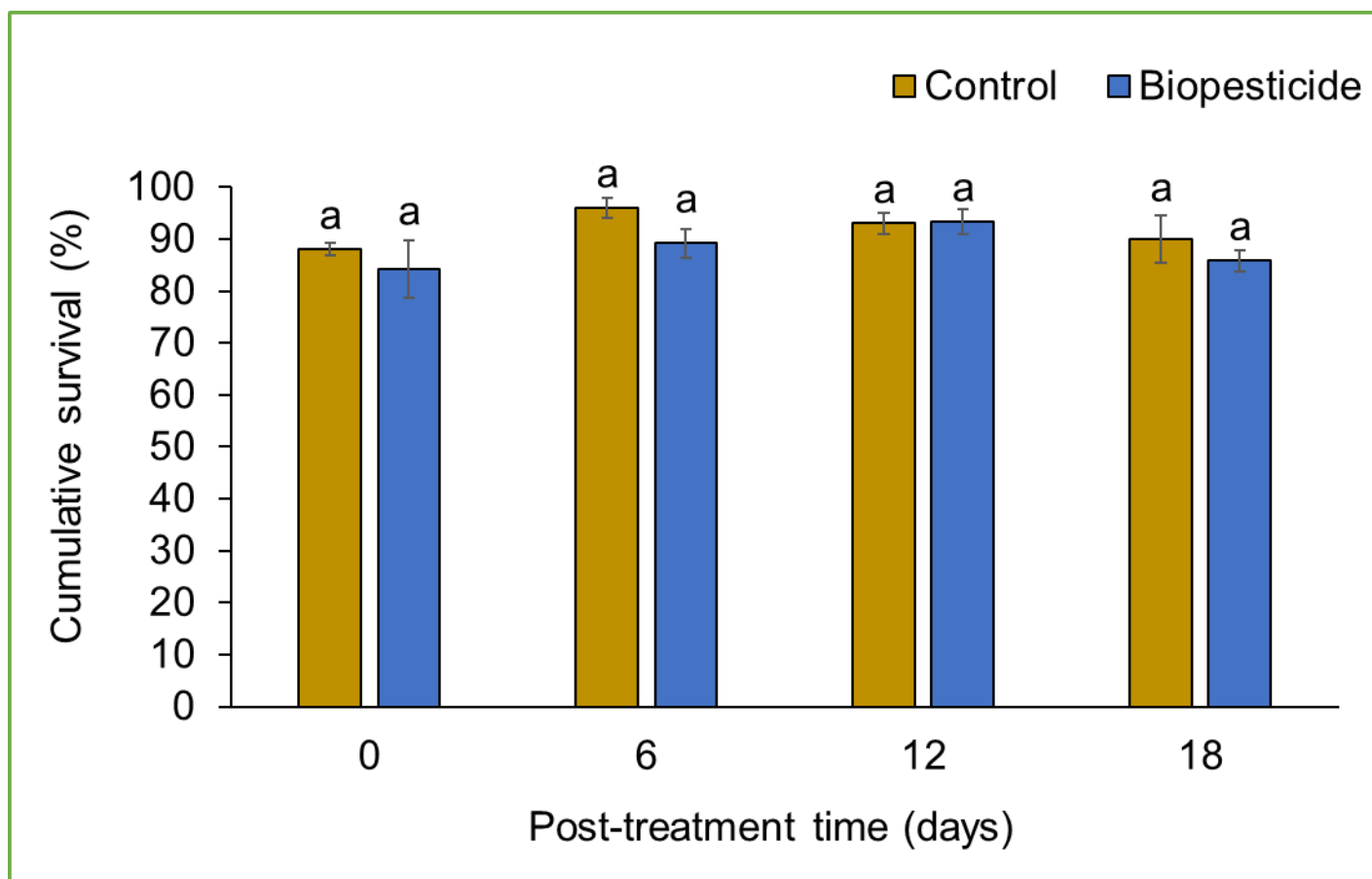
Experiment was
repeated three times
in Apr 2020 – Jun
2020, Sep 2020 –
Nov 2020, and Dec
2020 – Feb 2021

Results

Foraging
activity

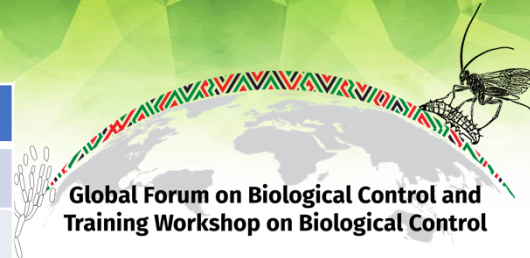


Survival of forager stingless bees



Fruit set and
maturation (%)

| Days | Fruit set (%) | | | Mature fruits (%) | |
|---------|---------------|--------------|--|-------------------|--------------|
| | Control | Biopesticide | | Control | Biopesticide |
| -9 – -7 | 88 ± 2 | 89 ± 3 | | 80 ± 4 | 80 ± 4 |
| -6 – -4 | 94 ± 1 | 91 ± 2 | | 88 ± 2 | 87 ± 2 |
| -3 – -1 | 94 ± 1 | 89 ± 5 | | 90 ± 2 | 87 ± 2 |
| 0 – 2 | 94 ± 2 | 96 ± 3 | | 84 ± 5 | 88 ± 5 |
| 3 – 5 | 94 ± 1 | 92 ± 2 | | 89 ± 2 | 88 ± 2 |
| 6 – 8 | 88 ± 1 | 86 ± 6 | | 81 ± 2 | 76 ± 2 |



Fruit weight (g)

| Days | Apr 2020 – Jun 2020 | | Sep 2020 – Nov 2020 | | Dec 2020 – Feb 2021 | |
|---------|---------------------|--------------|---------------------|--------------|---------------------|--------------|
| | Control | Biopesticide | Control | Biopesticide | Control | Biopesticide |
| -9 – -7 | 296 ± 15 | 257 ± 22 | 354 ± 13 | 358 ± 27 | 338 ± 15 | 371 ± 29 |
| -6 – -4 | 289 ± 22 | 272 ± 22 | 366 ± 15 | 376 ± 21 | 363 ± 18 | 366 ± 21 |
| -3 – -1 | 246 ± 22 | 234 ± 28 | 362 ± 36 | 359 ± 14 | 379 ± 42 | 372 ± 22 |
| 0 – 2 | 304 ± 21 | 242 ± 26 | 379 ± 19 | 353 ± 15 | 376 ± 24 | 380 ± 27 |
| 3 – 5 | 248 ± 20 | 253 ± 37 | 385 ± 83 | 336 ± 24 | 372 ± 22 | 378 ± 20 |
| 6 – 8 | 234 ± 24 | 196 ± 16 | 346 ± 31 | 311 ± 20 | 364 ± 20 | 376 ± 27 |



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Apidologie (2022) 53:28







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<https://doi.org/10.1007/s13592-022-00938-1>

Original article



A fungal-based pesticide does not harm pollination service provided by the African stingless bee *Meliponula ferruginea* on cucumber (*Cucumis sativus*)

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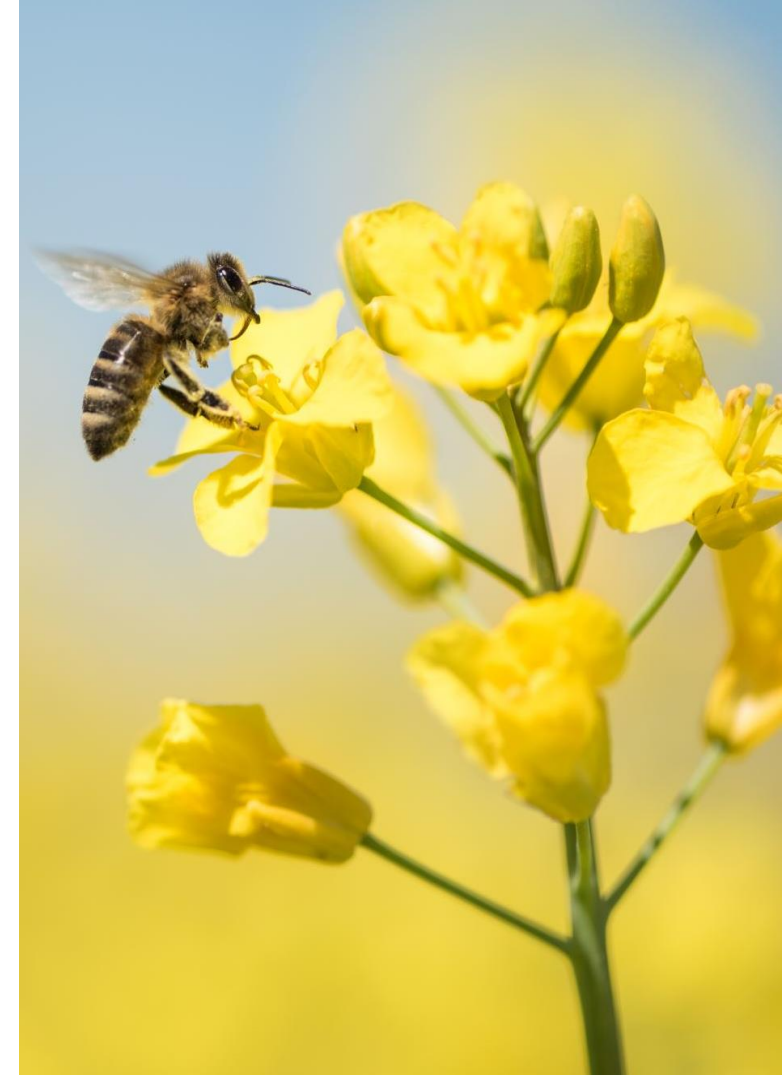


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Conclusions

- Mortality due to biopesticides did not exceed 17.4% for honey bees or 11.0% for stingless bees.
- Biopesticides can germinate and grow optimally in beehive conditions.
- Biopesticide did not hamper bee pollination behaviour and success.
- The tested biopesticides can be safely used as to control insect pests in bee-resourced crop systems.
- The interactions of bee pollinators and biopesticides can also be limited by:
 1. careful timing of biopesticide application to avoid peak foraging periods.
 2. improving 'lure and infect' application techniques.



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- Dr Thomas Dubois, Dr. Saliou Niassy, Dr. Subramanian Sevgan, Dr. Samira Mohamed.
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