



# 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.

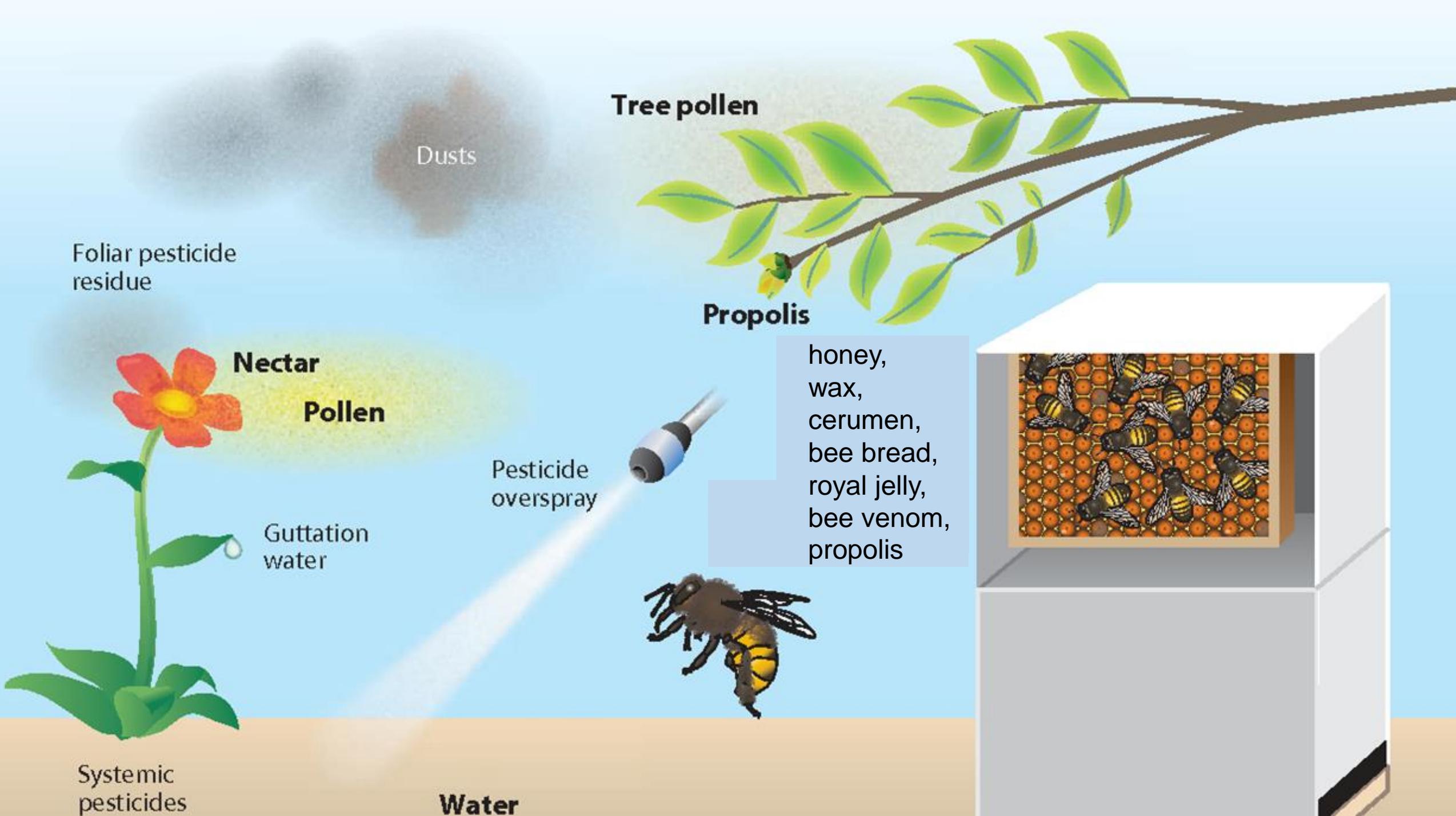
**Chemical insecticides** constitute the key drivers to the **alarming global decline** of pollinators.



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## 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



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# 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 ( $>75\%$ )



# 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

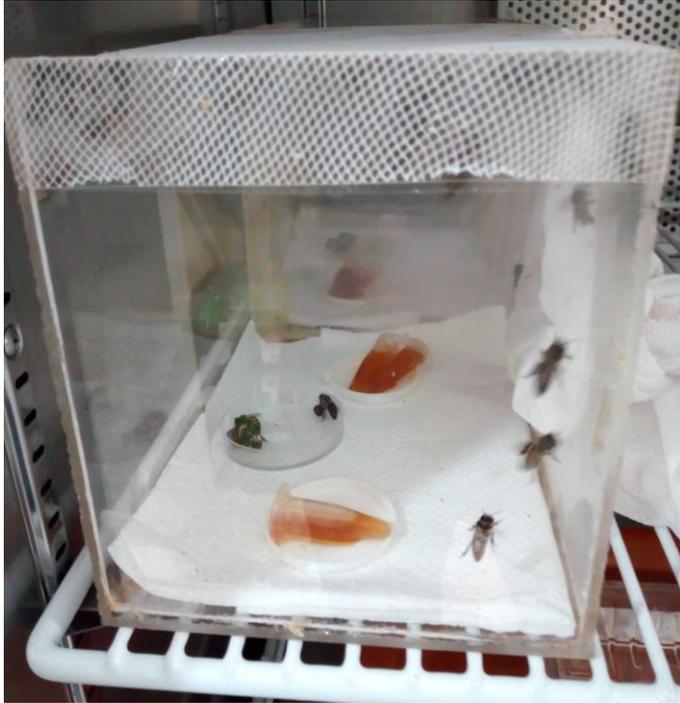
Akutse et al., 2020



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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

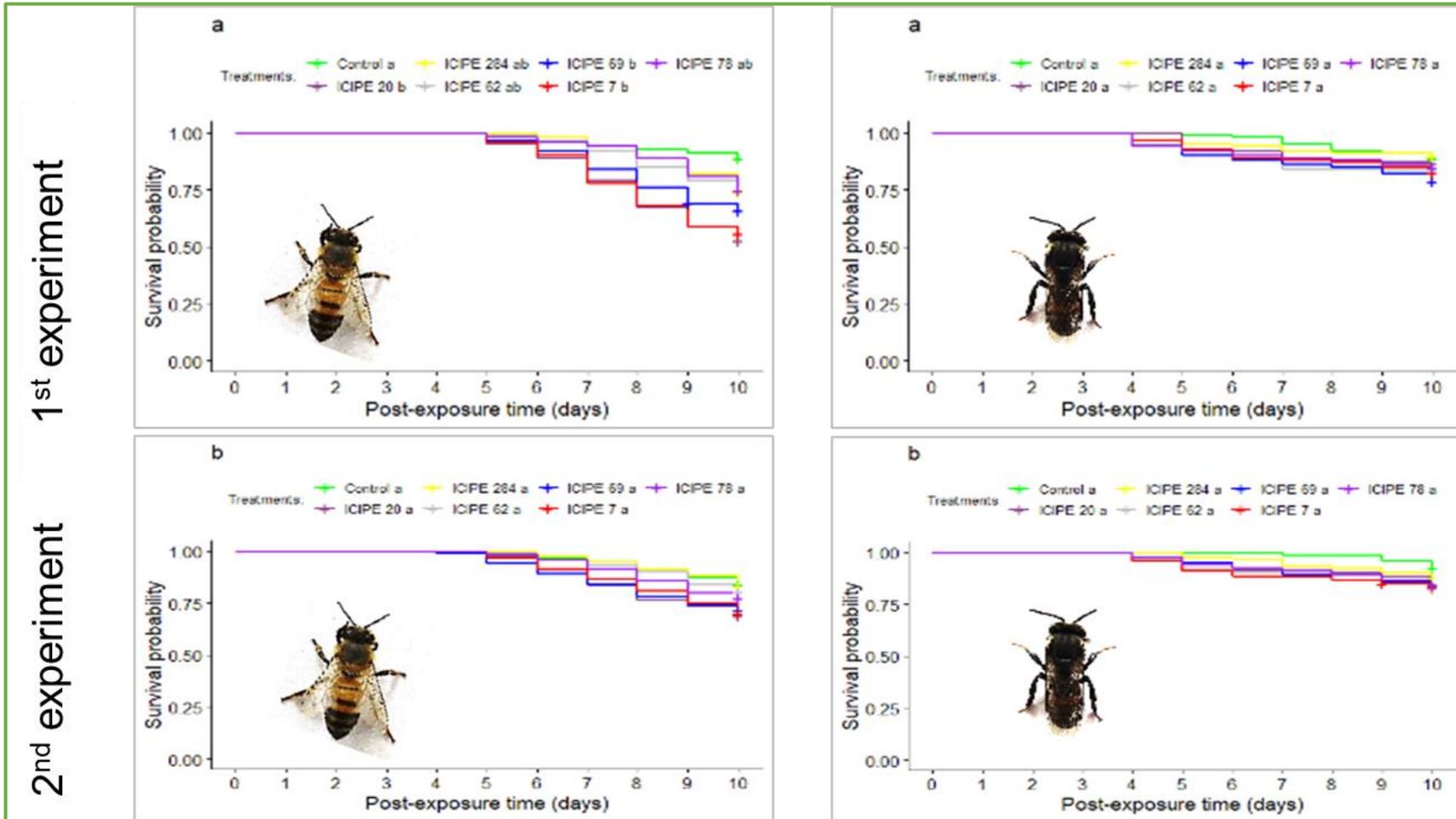
# Results

Kaplan-Meier survival curves of fungus-exposed bees



Honeybees

Stingless bees



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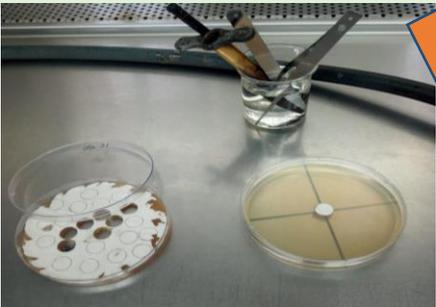
# Modelling the performance of the biopesticide isolates under bee colonies' temperatures

## Conidial germination



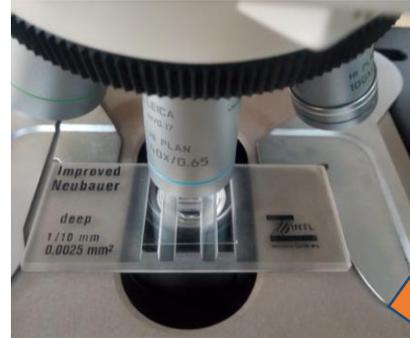
Fungal suspension ( $10^6$  conidia/mL)

## Mycelial growth

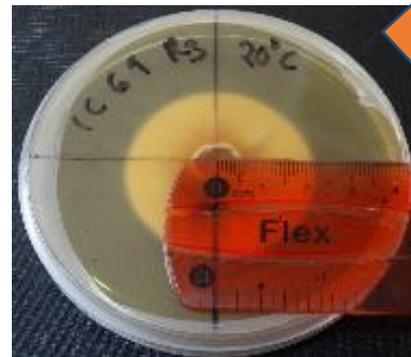


Mycelial mat

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)

# 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

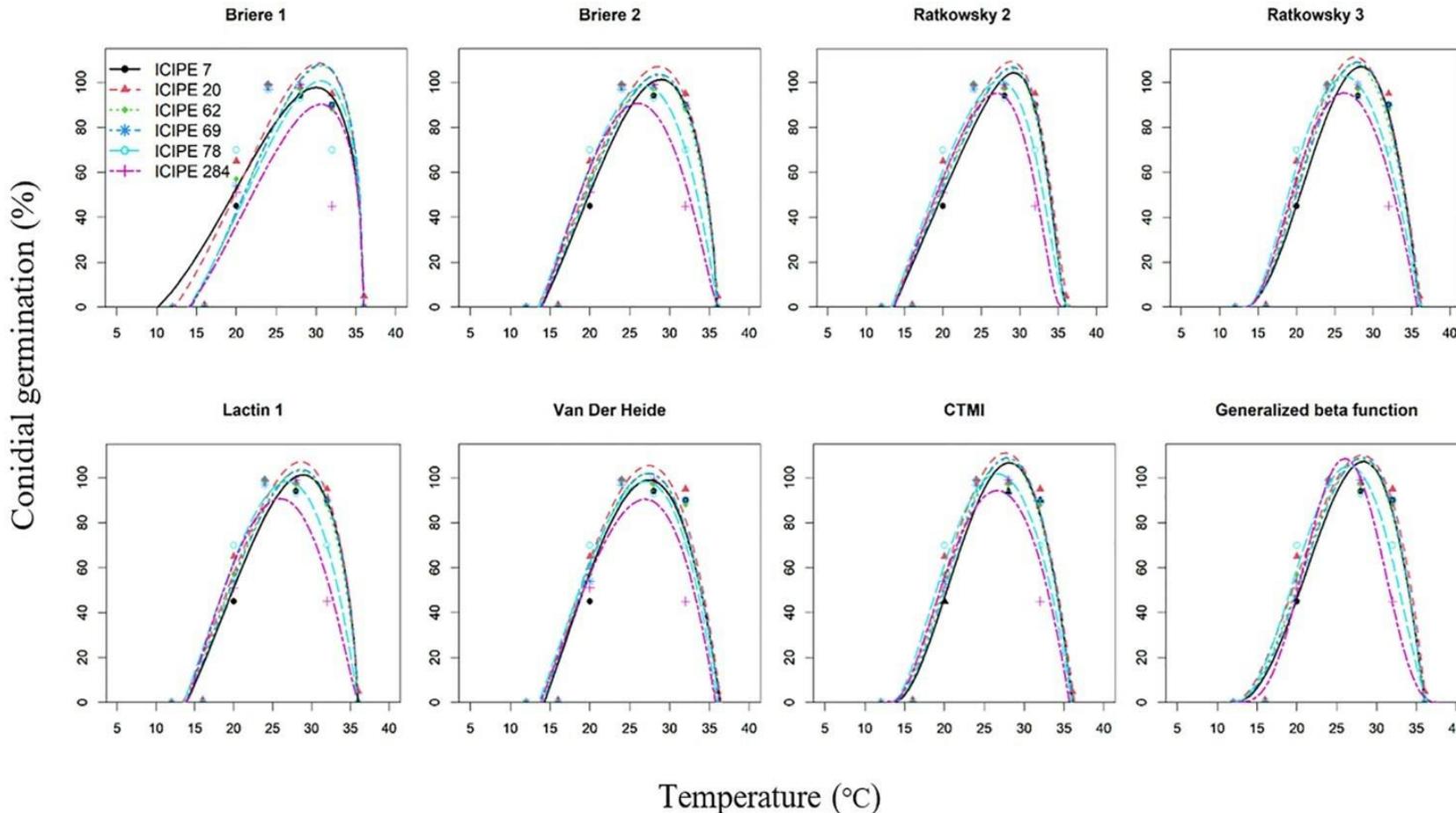


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# Results

## Conidial germination curves by temperature-dependent models

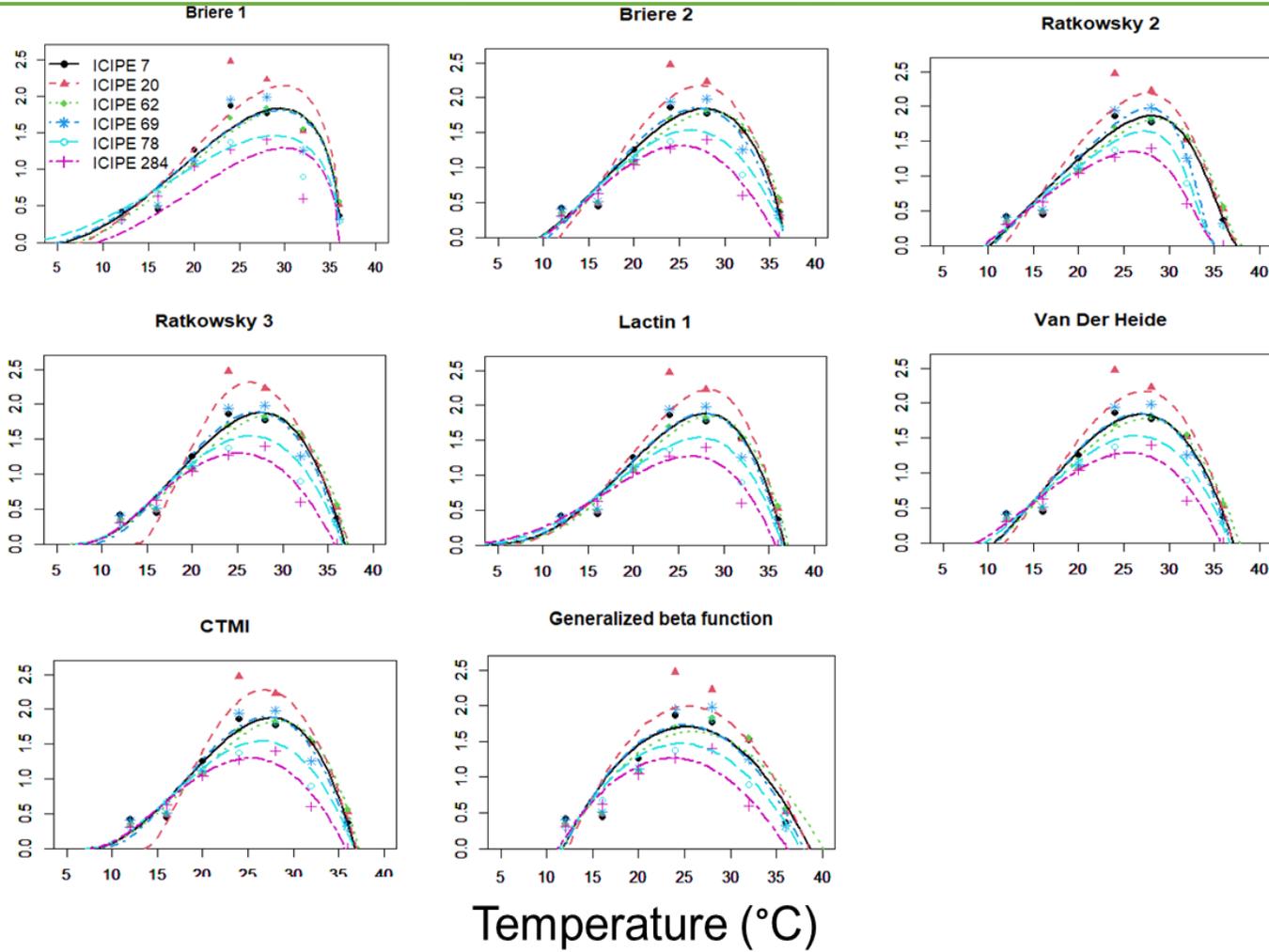


Conidial germination **started** from 10–14°C and reached a **peak** at 25–30°C, followed by a precipitous decline reaching **upper thresholds** at 35–38°C.

# Growth curves as predicted by 8 temperature-dependent models



Mycelial growth (mm/day)



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



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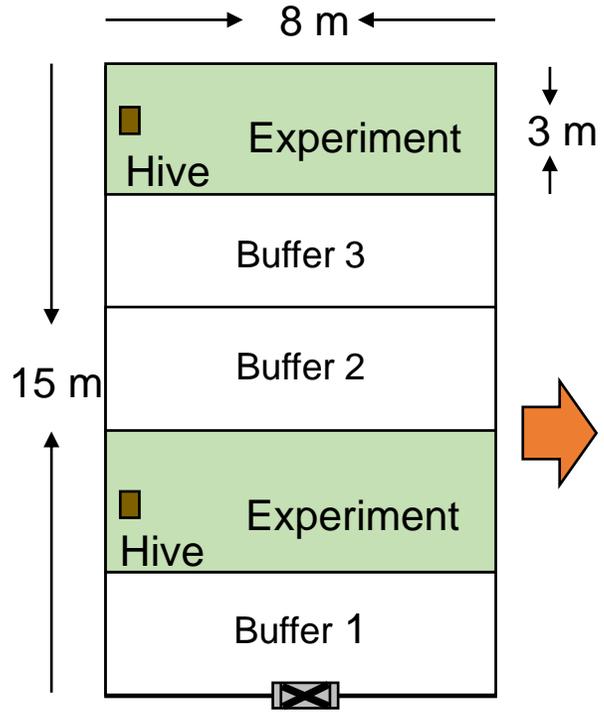
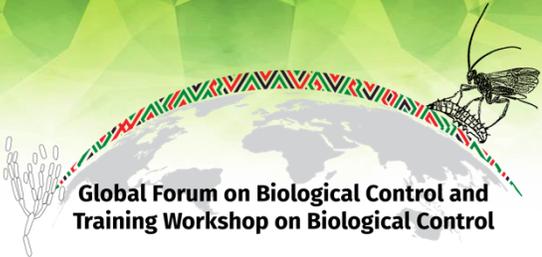
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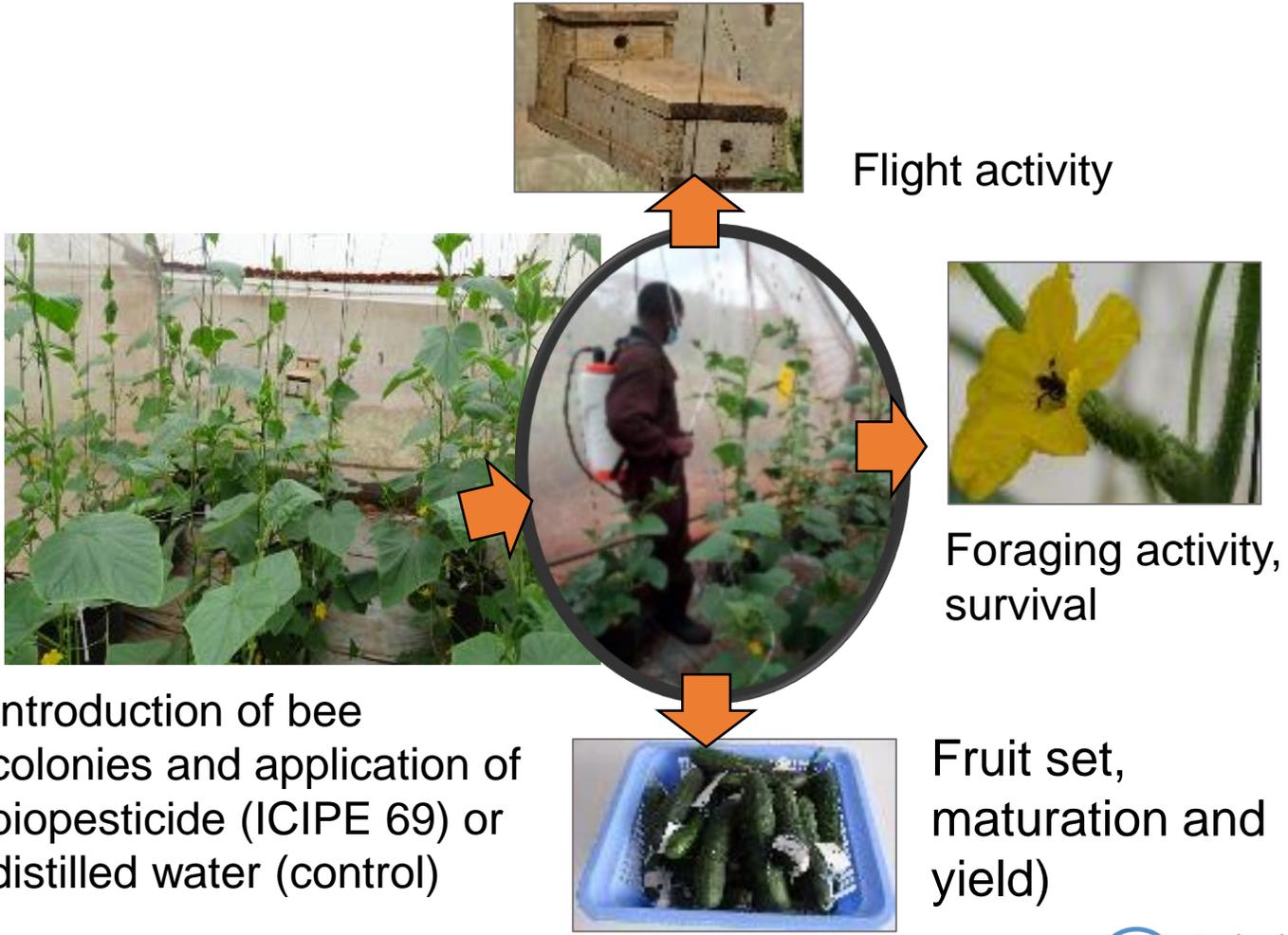
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# Effect of a biopesticide on survival, pollination behaviour and success of the stingless bee



Cultivation of cucumber in screenhouses



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

Flight activity

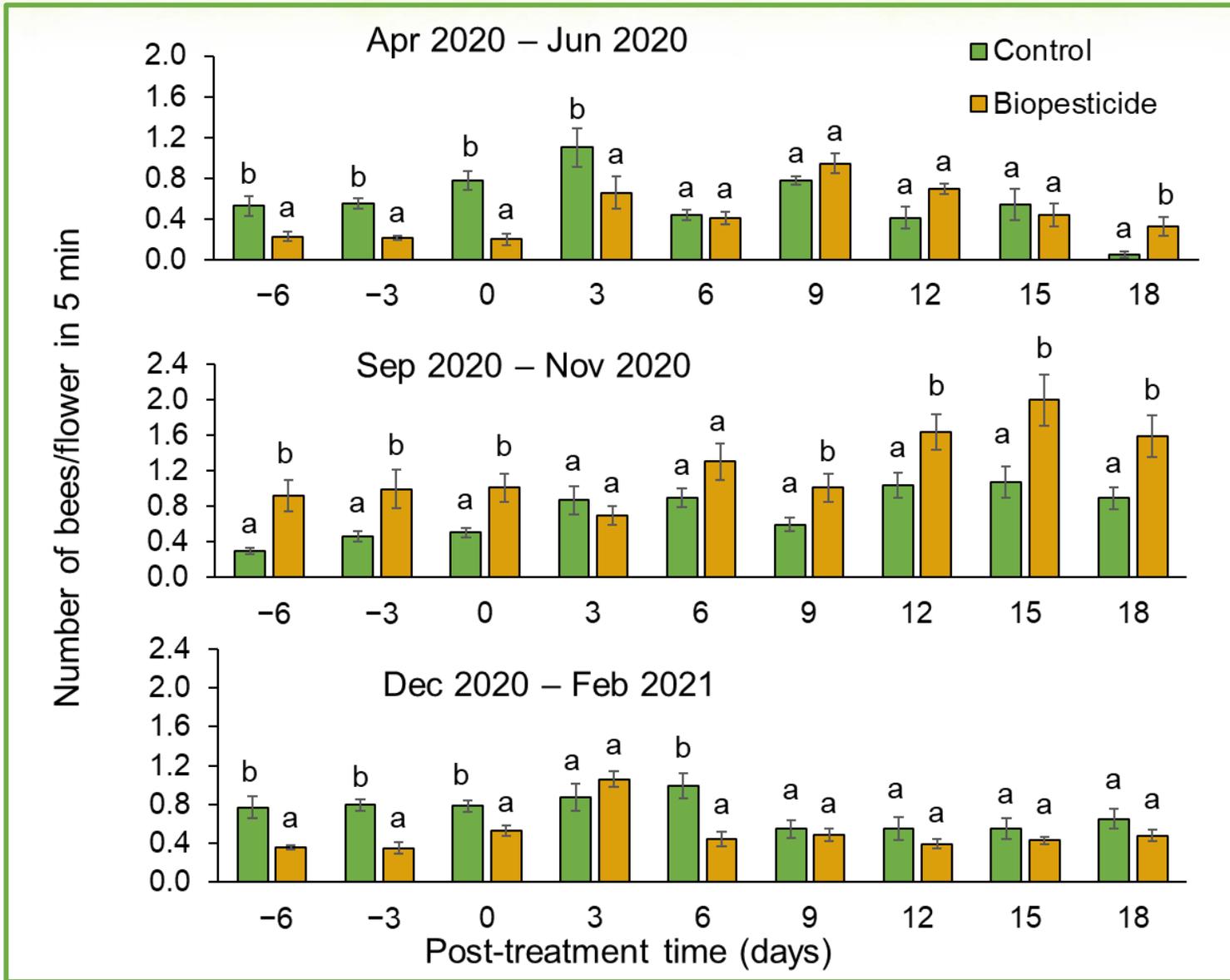
Foraging activity, survival

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

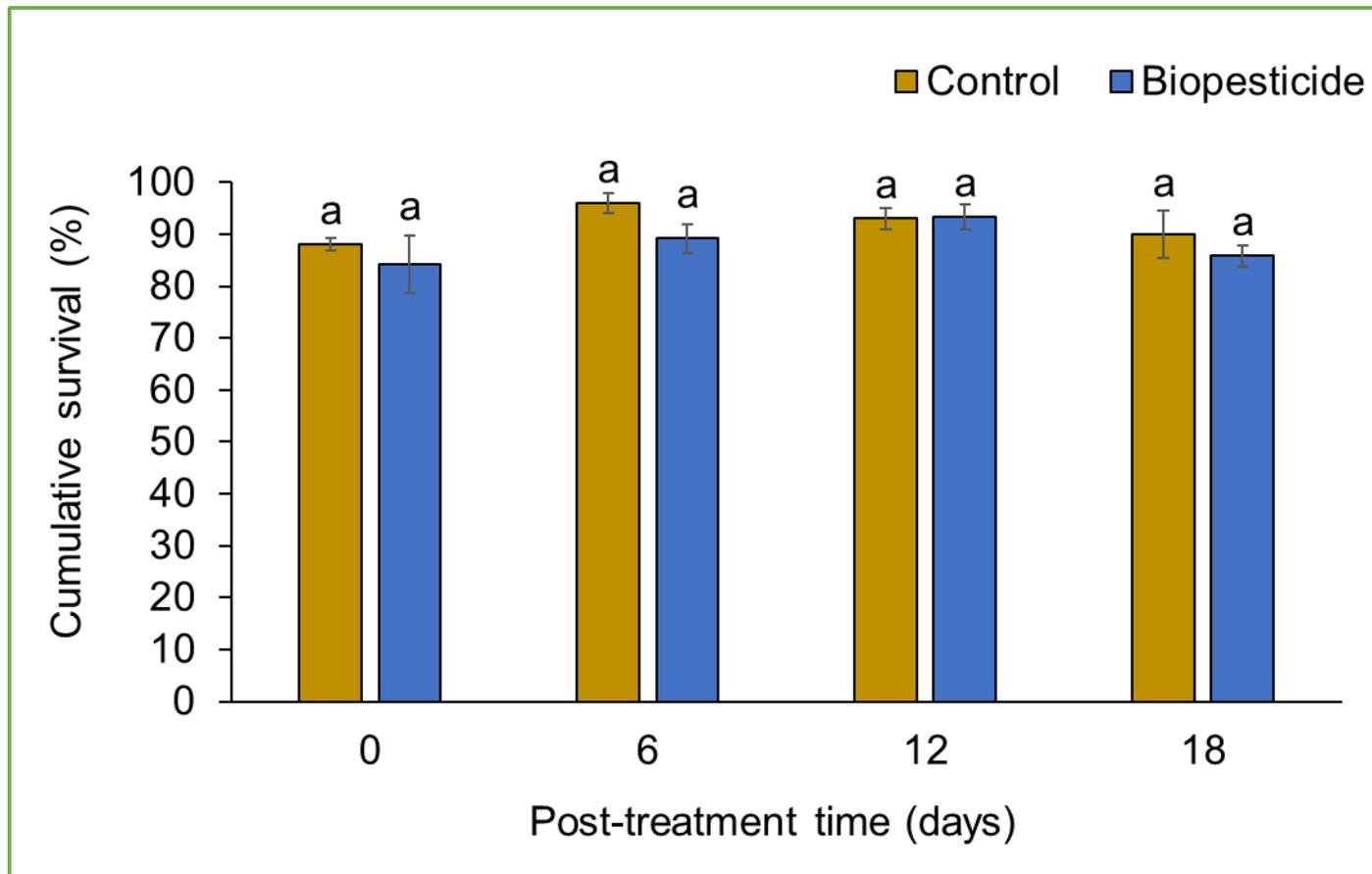
Foraging activity



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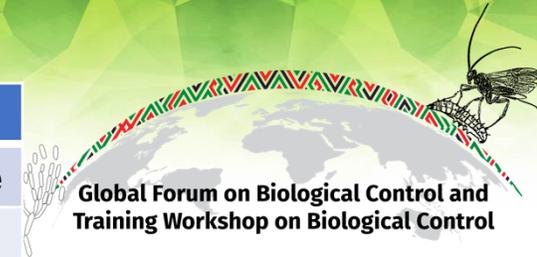


# Survival of forager stingless bees



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



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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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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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# 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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