



# Advancing Sustainable Pest Management in Rice Storage: Behavioural Insights, Infestation Detection, and Essential Oil Effects on *Sitophilus* spp.

TRACING RICE AND VALORIZING SIDE STREAMS ALONG  
MEDITERRANEAN BLOCKCHAIN

28.10.2024



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Instituto Nacional de  
Investigação Agrária e  
Veterinária, I.P.

# Importance of Pest Management in Rice Storage

- Effective pest management is crucial to prevent infestations that compromise rice quality, leading to substantial losses and reduced market value.
- Key Focus: *Sitophilus* spp. (rice weevils) due to their significant impact on rice quality, reducing grain value through contamination and physical damage.
- Challenges with conventional insect management and the extensive use of chemical methods have led to resistance development within insect populations; and environmental and health concerns due to pesticide use.
- Environmentally friendly insect management techniques have been researched and published (2023) offering alternative strategies for sustainable rice storage.



Open Access Review

## Advances in Environmentally Friendly Techniques and Circular Economy Approaches for Insect Infestation Management in Stored Rice Grains

by Inês Gonçalves de Sousa <sup>1,2</sup>, Jorge Oliveira <sup>3,4</sup>, António Mexia <sup>2,5</sup>, Graça Barros <sup>5</sup>, Carina Almeida <sup>1</sup>, Carla Brazinha <sup>6</sup>, Anna Vega <sup>7</sup> and Carla Brites <sup>1,8,\*</sup>



# Relevance of the Study



Preventing infestations from the field to industrial processing and retail.

Chemical solutions raise concerns about environmental impact and human health.



**new alternatives  
and  
approaches**

Insects reveal resistance mechanisms.

# Main Objective

Test the effectiveness of physical and biological-based solutions to eradicate the use of synthetic chemicals and ensure the elimination of insect infestation in stored rice.

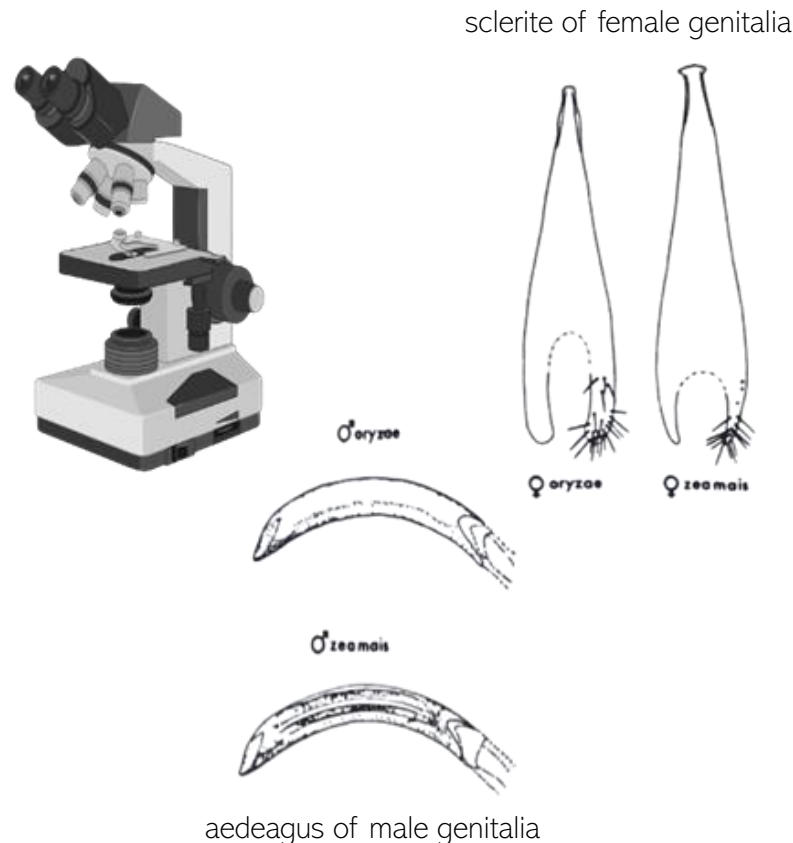
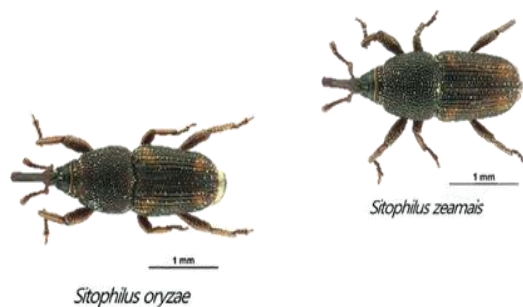
# Key Research Tasks



1. Reproduction of insect biomass - *Sitophilus oryzae* and *Sitophilus zeamais*.
2. Testing fast and accurate methods to detect hidden insect infestations in rice.
3. Evaluating the repellent activity of several volatile substances.
4. Testing the efficiency of physical and biological solutions to control infestations in rice.
5. Evaluating the effect of treatments on the physicochemical and organoleptic quality of rice.

# Reproduction of Insect Biomass

## *Sitophilus oryzae* and *Sitophilus zeamais*



Total insects	Total larvae/pupae	Identified insects	
		male	female
~5000	~600	~260	~200

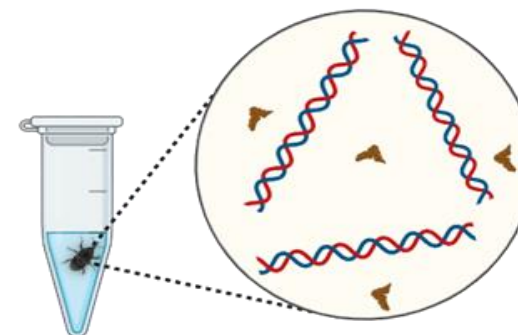


# Testing fast and accurate methods to detect **hidden infestations** in rice

Improved carbon dioxide production detection (portable sensor)



Quantitative real-time polymerase chain reaction (qRT-PCR)

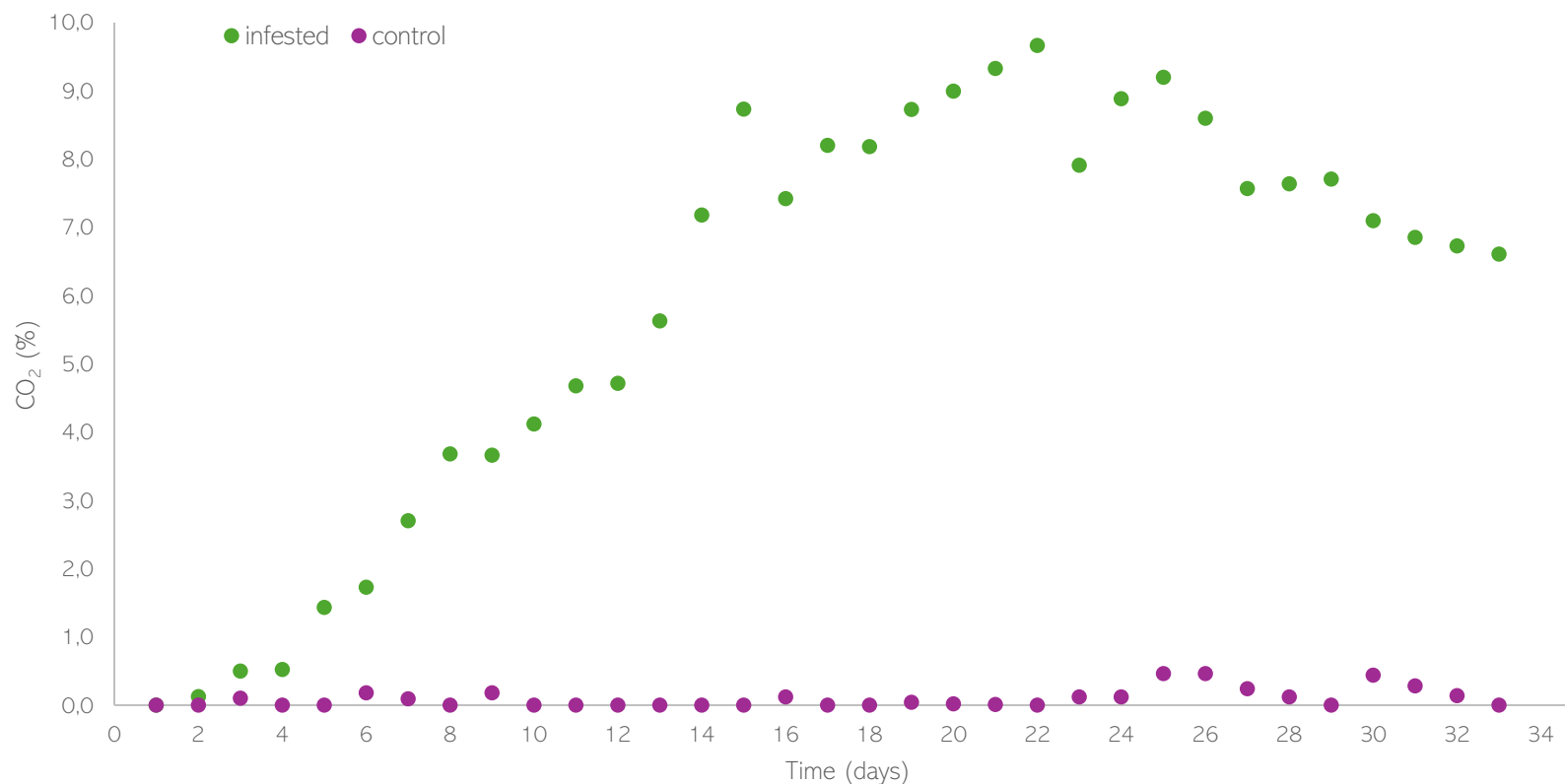




# Testing fast and accurate methods to detect **hidden infestations** in rice

Evolution of the carbon dioxide levels throughout the insect's life cycle.

The test ends when adult insects are detected.



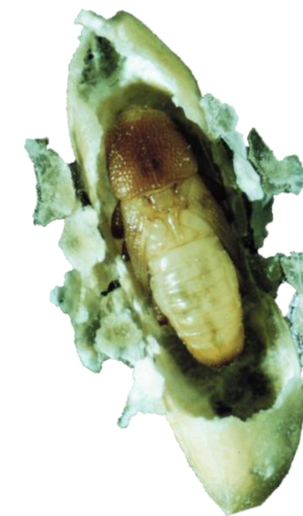
Mean CO<sub>2</sub> values (%) throughout the life cycle of the insects, determined on previously infested rice samples.





# Testing fast and accurate methods to detect **hidden infestations** in rice

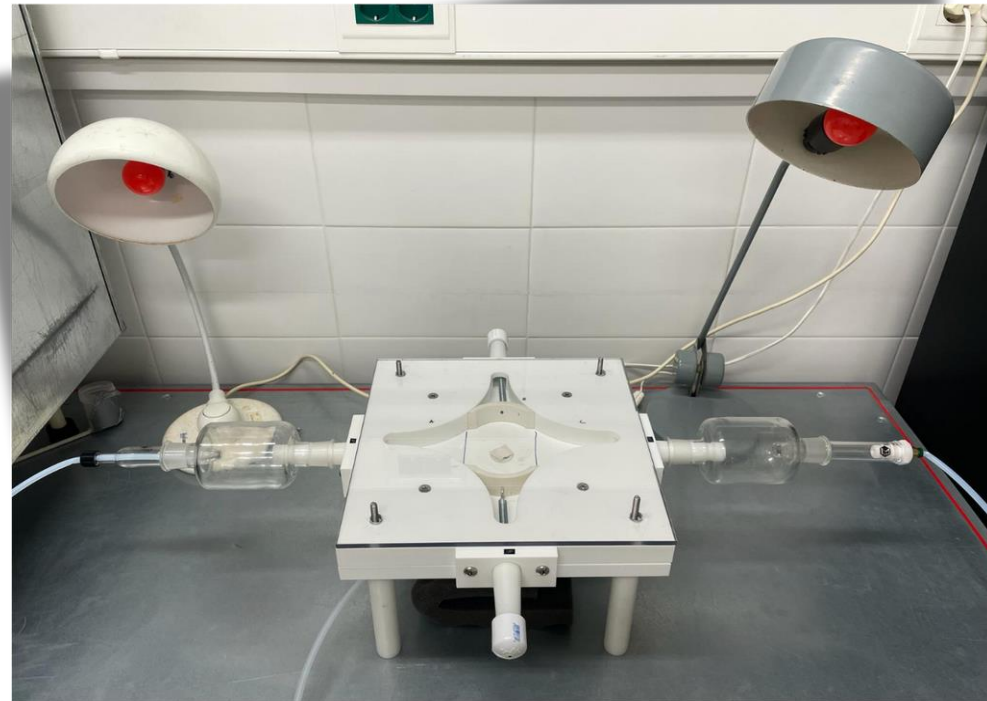
CO <sub>2</sub> (%)	qRT-PCR results
0,2	<i>S. zeamais</i> positive <i>S. oryzae</i> positive
0,5	<i>S. zeamais</i> positive <i>S. oryzae</i> positive
2	<i>S. zeamais</i> positive



# Evaluating the repellent activity of compounds from essential oils

→ Understand the behaviour of *Sitophilus* towards substances with possible repellent activity.

Eugenol  
Eucalyptol  
(S)-(-)-Limonene  
L-menthol  
Thymol



# Evaluating the repellent activity of compounds from essential oils

200, 100 and 50  $\mu$ L

Mean Time (min.)

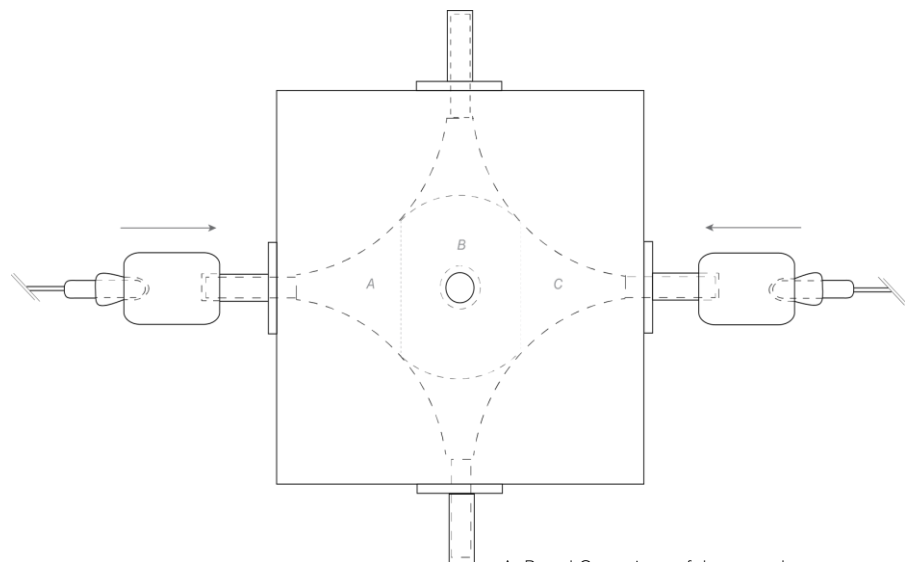
source	near	central	far
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Cotton Disc

not significantly different

Rice Flour  
(200 mg)

$4,92 \pm 2,72^b$      $2,95 \pm 1,89^a$      $1,71 \pm 1,76^a$



A, B and C - regions of the central arena;  
analysed whether the insects were near or far from the stimulus source

Mean Time (min.)

source	near	central	far
--------	------	---------	-----

EC200

not significantly different

EC100

$2,98 \pm 1,33^a$      $5,17 \pm 1,62^b$      $1,85 \pm 1,71^a$

EC50

$3,08 \pm 1,57^a$      $4,95 \pm 1,96^b$      $1,97 \pm 1,42^a$

L200

$2,86 \pm 2,55^a$      $2,54 \pm 1,51^a$      $4,60 \pm 2,86^b$

L100

not significantly different

L50

$2,78 \pm 2,19^a$      $2,74 \pm 1,05^a$      $4,48 \pm 1,94^b$

EG200

not significantly different

EG100

$2,84 \pm 1,84^a$      $3,13 \pm 2,13^a$      $4,03 \pm 2,16^b$

EG50

$2,93 \pm 2,10^a$      $5,19 \pm 2,37^b$      $1,88 \pm 2,46^a$

EC – Eucalyptol  
L – (S)-(-)-Limonene  
EG – Eugenol

T  
A  
S  
K  
1

T  
A  
S  
K  
2

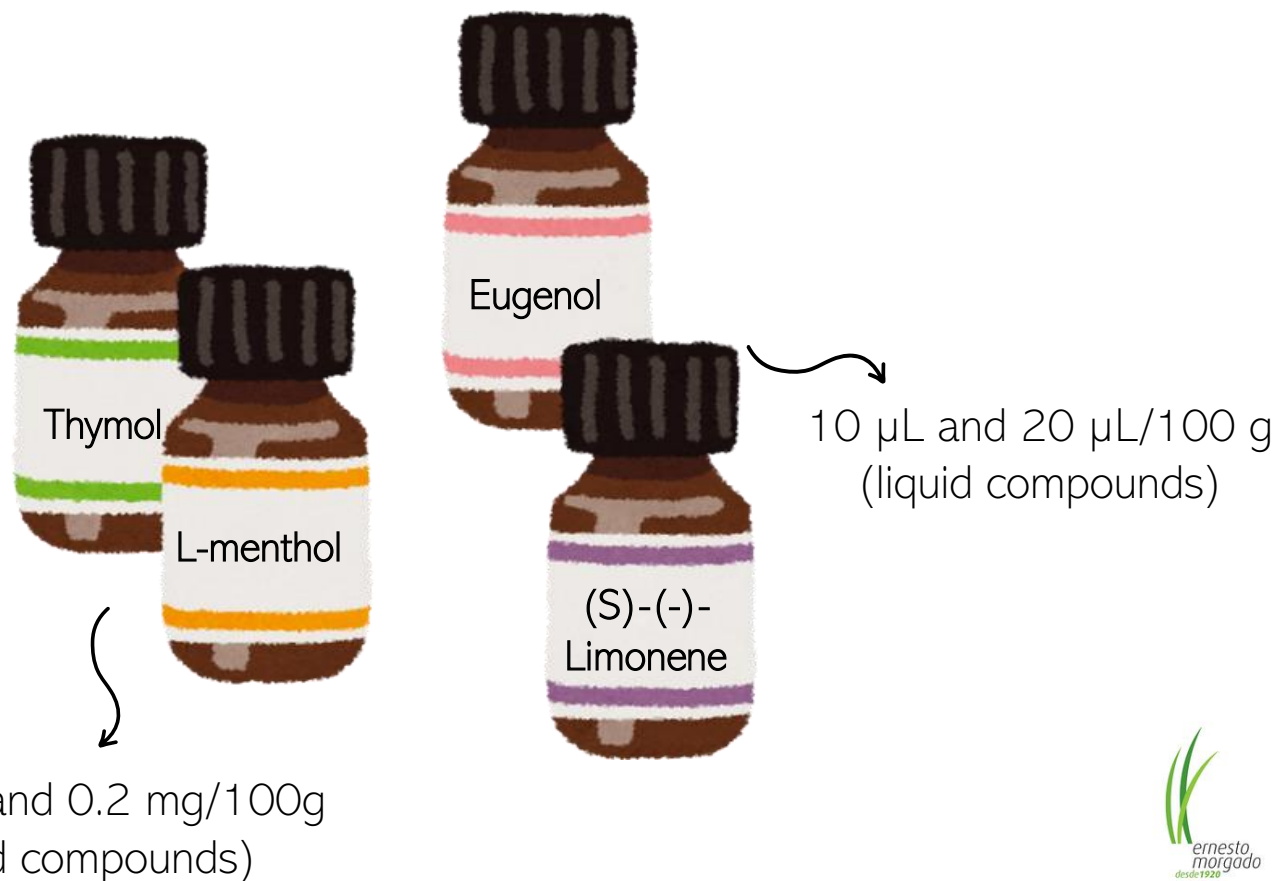
T  
A  
S  
K  
3

T  
A  
S  
K  
4

T  
A  
S  
K  
5

# Testing the application of compounds from essential oils in rice

Application of food-grade compounds from essential oils to prevent weevil infestations in stored rice.



# Testing the efficiency of ultraviolet light (UV-C) to control infestations in rice



3 g of infested rice treated with UV-C 280nm



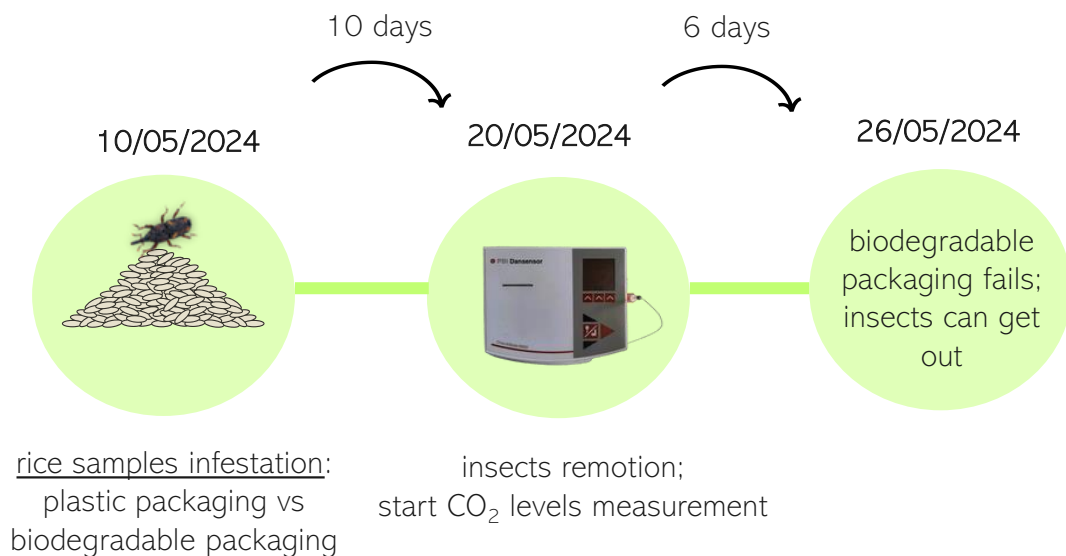
UV-C 280nm directly in larvae



Ultraviolet equipment tested



# Testing the use of biodegradable packaging



A – biodegradable packaging; Mater-Bi (EN 13432 standard)  
B – traditional petrochemical plastic (BOPP with a 30 µm transparent layer and a 70 µm transparent PE layer)

# Evaluating the effect of treatments on the physicochemical and organoleptic quality of rice



Analyses carried out to evaluate the effect of the selected treatments on the physicochemical quality characteristics of the rice.

Analysis	Method
basic chemical composition	Near Infrared Spectroscopy (NIR)
pasting properties gelatinization temperature	Rapid Viscoanalyser (RVA) AACC 61-02.01 AACC 61-04.01
colour	Colourimeter Minolta CR300
moisture	ISO 712:2009
amylose	ISO 6647-2:2020
resistance to extrusion	ISO 11747:2012

# Conclusions and Future Directions

- Field-Based Prevention: Implement strategies to reduce weevil infestations originating from the field.
- Facility Hygiene and Temperature Control: Prioritize thorough cleaning and maintain optimal temperature control in storage facilities to reduce infestation risks, though complete prevention may be challenging.
- Advanced Sensor Technology: Modern sensors show promise for early detection and prediction of hidden infestations, supporting proactive pest management.

## TRACE-RICE Project Innovations:

Trialled chemical-free solutions to prevent egg hatching and control infestations in stored rice.

Aimed to reduce reliance on conventional chemical treatments in the industry.

## Promising Approaches for Validation:

Essential Oil Compounds: Impregnation of rice with Limonene, Eugenol, and Eucalyptol.

UV-C Application: Tested as an effective non-chemical control method.



# Posters

**STATE OF THE ART OF NON-CONVENTIONAL TREATMENTS FOR INSECT CONTROL IN RICE STORAGE**

Inês Sousa<sup>1,2</sup>, Jorge Oliveira<sup>3</sup>, António Mexia<sup>2,4</sup>, Graça Barros<sup>5</sup>, Carla Brites<sup>1,2</sup>

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*most relevant species*

**Development of a rapid molecular methodology for the detection of hidden insect infestation in rice**

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**ENHANCING MOLECULAR SCREENING OF HIDDEN INSECT INFESTATION IN RICE GRAINS BY COI BARCODING: PRIMER PERFORMANCE AND LIMIT OF DETECTION**

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**MOLECULAR PROFILING OF HIDDEN INSECT INFESTATION IN STORED RICE GRAINS: A Comprehensive RT-PCR Approach for Species Discrimination and Limit of Detection Assessment**

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# Publications

**CIÊNCIA 2023**

**HOW TO DETECT HIDDEN INSECT INFESTATION IN RICE GRAINS?**

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**Dare 2 Change**

**Identification of molecular rapid methods for hidden insect infestation determination in rice**

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**International Trainee Symposium in Agri-Food, Nutrition and Health**

**Assessment of physical and biological solutions to prevent weevil infestation in stored rice**

Inês Sousa, Graça Barros, António Mexia and Carla Brites

**INTERNATIONAL CONFERENCE ON SUSTAINABLE FOODS**

**ESTIMATING HIDDEN INFESTATIONS IN RICE BY MEASURING CARBON DIOXIDE LEVELS**

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**EFFECTS OF COMPOUNDS FROM ESSENTIAL OILS ON SITOPHILUS SPP. DEVELOPMENT AND RICE QUALITY**

Inês Sousa<sup>1,2,3</sup>, Andreia Soares<sup>4</sup>, Graça Barros<sup>5</sup>, António Mexia<sup>6</sup>, Carla Brites<sup>1,3</sup>

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# Oral Communications

**7º Simpósio | 29 de Maio de 2024**

**PRODUÇÃO E TRANSFORMAÇÃO DE ALIMENTOS EM AMBIENTE SUSTENTÁVEL**

Instituto Nacional de Investigação Agrária e Veterinária

**Explorar o comportamento de *Sitophilus* spp.:**

**UM PASSO PARA A GESTÃO SUSTENTÁVEL DE PRAGAS NO ARMAZENAMENTO DO ARROZ**

I. Sousa, P. Naves, G. Barros, A. Mexia, C. Brites

# Posters

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**ReadyToPub Award**

Awarded to the best student poster communication presented at the **International Conference on Sustainable Foods** (July 24-25, 2024, Bragança - Portugal)

**Inês Sousa**

**Estimating Hidden Infestations in Rice by Measuring Carbon Dioxide Levels**

**INTERNATIONAL CONFERENCE ON SUSTAINABLE FOODS**  
 Achieving the Sustainable Development Goals

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# Awards

**Prémio ReadyToPub**

Melhor póster de um estudante na temática "Sustentabilidade no Sistema Alimentar"

**Inês Sousa**

**150€**

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Inês Sousa & Carla Brites

thank you!



PhD Grant 2023.02433.BDANA

Collaborations:  
INIAV - Pedro Naves, Carina Almeida, Ana Maria Campos  
UL/ISA - António Mexia, Graça Barros  
Ernesto Morgado S.A. – Jorge Oliveira, João Simões  
iBET - Vanessa Pereira, Nicole Ferreira