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Bioinsecticide effect of *Metarhizium anisopliae* on termite pests *Microtermes lepidus* and *Psammotermes hybostoma* in the laboratory

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Abstract

In Senegal, several studies have shown the economic importance of termite damage to crops. Farmers use chemical methods that are dangerous for humans, animals, crops, soil, and environmental health. Thus, the use of entomopathogenic fungi would be an effective and environmentally eco-friendly alternative to chemical pesticide use. The objectives of this study are to i) carry out infestations in the laboratory, ii) determine the lethal time (TL50) and iii) observe the germination of spores on the corpses of these species. The methodology is based on i) the infestation of 10 workers of *Microtermes lepidus* and *Psammotermes hybostoma* with Metarhizium solutions, ii) the monitoring of mortalities, iii) the determination of the lethal time 50 (LT50) and iv) germination of spores on corpses.

For the determination of LT50, the concentrations of 1 g/L and 1.5 g/L gave an LT50 of less than one day in workers of *Microtermes lepidus* and *Psammotermes hybostoma*. For the concentration of 0.5 g/l, the LT50 is 2.5 days for workers of *Psammotermes hybostoma* and less than one day for those of *Microtermes lepidus*. Incubation revealed the appearance of spores on the cadavers, which were given a concentration of 0.5 g/l. This study shows that mortality is related to the concentration of the solution. The higher the concentration, the shorter the LT50 time. The bioinsecticidal effect of *Metarhizium anisopliae* is not immediate, resulting in an LT50 of 1 to 2 days depending on the species.

Keywords: Bioinsecticide, *Metarhizium anisopliae*, termites, laboratory

Introduction

Termites have long been considered pests because of the damage they cause to crops and homes (Sib, 2022). Species of the genera Reticulitermes and Coptotermes (Family Rhinotermitidae) are the major termite pest species in the Americas, Europe and Asia (Pearce, 1997). While Macrotermitidae (especially *Odontotermes*, *Macrotermes* and *Microtermes*) are the predominant termite pest species in Africa and Asia (Pearce, 1997).

In West Africa, and more specifically in Côte d'Ivoire, termites are responsible for damage to rice and maize fields in the sub-Saharan savannah (Akpesse *et al*., 2008). Similarly, Coulibaly *et al.* (2014) were able to identify the species responsible for the damage to mango tree nurseries in northern Côte d'Ivoire and estimated that termite attacks can cause losses of up to 93.33%.

In Togo, in sugar cane plantations, losses on cuttings caused by termites are estimated at 51% for an economic loss of 29% at harvest (Kotoklo *et al*., 2011).

In Senegal, studies by Han and Ndiaye (1996) on termite attacks on 10 fruit species revealed attack rates of up to 76.2%. In the regions of Casamance, Saint-Louis, Thiès and Kaolack (Senegal), termites belonging to the trophic groups of lignivores and fungi have been the cause of serious attacks that can lead to the death of certain fruit species (Ndiaye and Han, 2000; 2002; 2006; 2007).

In the department of Tivaouane (Thiès region), the breadbasket of cassava production in Senegal, a study showed the susceptibility of 5 varieties of cassava to attacks by termites belonging to the genera *Macrotermes*, *Odontotermes*, *Amitermes*, *Psammotermes* and *Microtermes* (Faye *et al*., 2014) and (Faye, 2016).

Several methods of chemical management of these termite pests have been used without success. Thus, in the context of the agroecological

management of these pests, the use of entomopathogenic fungi is an effective and environmentally friendly alternative.

Entomopathogenic fungi have the power to kill or reduce the vigor of the host they infect. *Metarhizium anisopliae* is a mitosporic fungus with asexual reproduction. The classification of *Metarhizium anisopliae* has undergone several revisions in recent years (Benserradj 2014). There are two forms of *Metarhizium*: *Metarhizium flavoviride* and *Metarhizium anisopliae*. The objective of this study was to evaluate the bioinsecticidal effect of *Metarhizium anisoplea* in two species of termite pests. Specifically, it will be a question of carrying out infestations in the laboratory, determining the lethal time 50 (LT50) and noting the germination of the spores on the corpses. For the purposes of laboratory infestation tests, workers of *Microtermes lepidus* and *Psammotermes hybostoma* are the species selected. Their choice for testing is justified by the frequency of cassava attacks and the extent of the damage that follows.

Methodology

Laboratory environment

The tests were carried out at the Laboratory of Zoology of Terrestrial Invertebrates of the Institut fondamentale d'Afrique noire Ch. A. Diop (IFAN-Ch. A. Diop).

In the laboratory, the tests were carried out under conditions of an average temperature of 25.5 °C and an average relative humidity of 56.5%.

Preparation of the fungal solution

The fungal solution was prepared with a strain of *Metarhizium anisopliae* provided by the Directorate of Plant Protection (DPV) of Senegal. The viability of the strain is 82.33% (Diatta, 2018).

The solution was prepared from 1g of *Metarhizium anisopliae*, 20 ml of distilled water and 2 drops of tween 80. By adding 250 ml of distilled water, the solution is brought to a concentration of 4 g/l.

From this solution, 1 ml was taken and then added to 9 ml of distilled water to evaluate the concentration of conidia in the solution. By depositing 50 microliters of the solution on a Malassez cell, the observation under the LEICA DME microscope at magnification 40 made it possible to evaluate the concentration of fungal spores in the solution, which is 1.19,106 spores/ml using the following formula.

The concentration **C** of conidia per ml is obtained by the following formula:

$$c = X \times 5.10e^{4} c = X \times 5.10e^{4}$$

X, the average number of conidia counted is obtained by the formula:

$$X = \frac{(a + b + c + d)}{4}$$

a, **b**, **c** and **d** being respectively the number of repetitions performed, corresponding to the number of slides prepared.

Termite infestation tests in the laboratory

Termites

The termites used in the tests were obtained by a bait trap containing cardboard. The traps were buried about 20 cm above the ground.

In the laboratory, petri dishes lined with blotting paper soaked in distilled water were prepared to accommodate the specimens to be tested.

Fungal inocula

Inocula are made up of three concentrations: 0.5 g/l, 1 g/l and 1.5 g/l. The 10 termite workers placed in petri dishes were contaminated by spraying with a micropipette. Each termite received a drop of inoculum. The control boxes contain individuals that have been treated with water. For each treatment, three replicates were performed. The Petri dishes were then placed in a wooden oven.

Test Tracking

Termite monitoring is carried out every day. Dead termites are counted and removed from the boxes and then incubated to control the sporulation of the fungus. The corpses are placed in petri dishes lined with blotting paper moistened with distilled water. After 72 hours of incubation in an oven, the boxes are removed for the observation of termites under the binocular magnifying glass.

Termite mortality rate during testing

The termite mortality rate (TM) corresponding to the ratio is obtained by:

$$TM = \frac{Number\ of\ dead\ individuals}{Total\ number\ of\ individuals} x\ 100$$

Lethal Time 50 (TL50)

The LT50 corresponds to the time needed to obtain 50% mortality of individuals. After plotting the mortality curve as a function of time, it is obtained by projecting 50% mortality on the x-axis (time).

Statistical analyses

The table of cumulative numbers is used to get an idea of the daily evolution of the number of dead individuals. An ANOVA test with XLSTAT version 2014 is used at the 5% threshold to compare the termite mortality rate between the different treatments used.

Results

Mortality of workers of *Microtermes lepidus*

Statistical tests performed show that there is no significant difference between control individuals treated with water and those treated with the 0.5 g/L concentration (Figure 1). Similarly, no difference was observed between individuals treated with the 1 g/l and 1.5 g/l concentrations. On the other hand, a significant difference was noted in the evolution of the mortality rate of control individuals treated with 0.5 g/l compared to those treated with 1 g/l and 1.5 g/l concentrations.

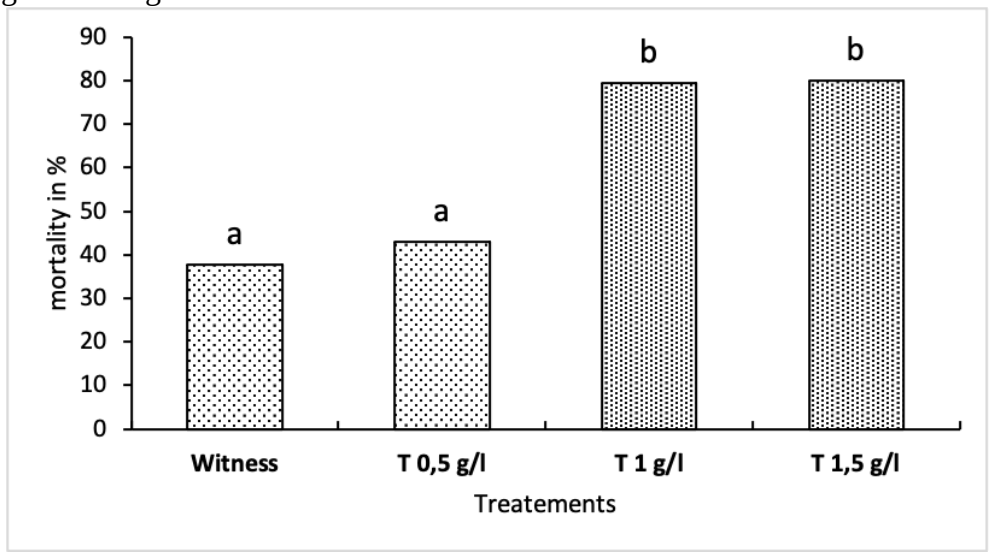

Figure 1: Mortality during worker testing of Microtermes lepidus

Individuals treated with 1 g/L and 1.5 g/L did not spore. Sporulation of fungal spores was noted on individuals treated with a concentration of 0.5 g/l.

Lethal Time (LT50) for *Microtermes lepidus Workers*

Depending on the concentration, the number of dead individuals varies over time. Thus, for individuals treated with concentrations of 1 g/l and 1.5 g/l, the LT50 is less than one day. For the 0.5 g/L concentration, the LT50 is 2.5 days (Figure 2).

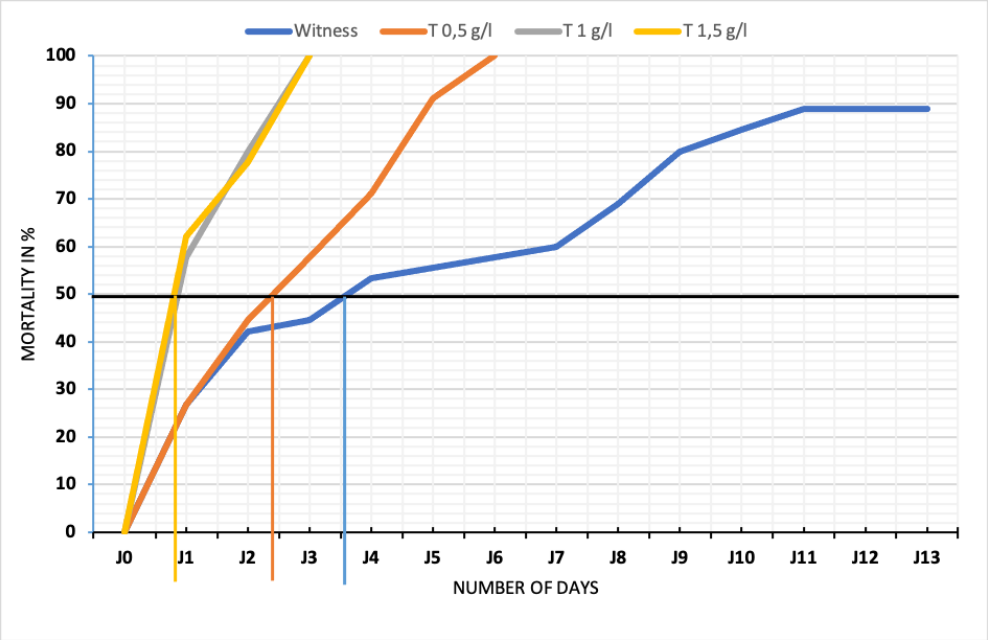

Figure 2: Evaluation of LT_{50} of different concentrations of Metarhizium anisopliae on Microtermes lepidus workers in the laboratory

The sporulation of the fungus on the corpses of *M. Lepidus*

Sporulations of the fungus, *Metarhizium anisopliae*, are observed on the corpses of individuals treated with the concentration of 0.5 g/l. The insects' corpses are colonized by the greenish spore's characteristic of *M. anisopliae* after 11 days of incubation (Figure 3).

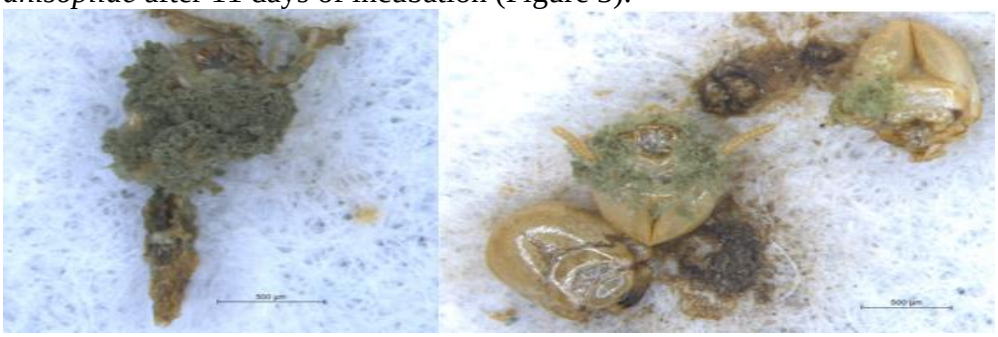

Figure 3: Sporulation of Metarhizium anisopliae on cadavers

Given the desired effect in the fungus, the transmission of the infestation to the colony by workers, the 0.5 g/L dose which takes longer to kill termites and which gives evidence of the development of the fungus with sporulation should be used in termite control tests.

The mortality of workers of *Psammotermes hybostoma*

The control individuals all died after 5 days. Statistically, there were no significant differences between the different concentrations tested (Figure 4).

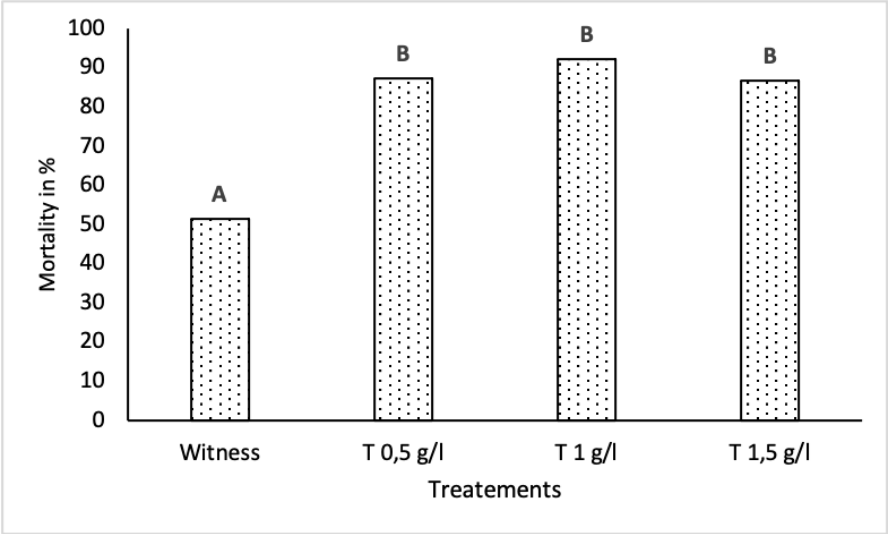

Figure 4: Mortality during testing of workers of Psammotermes hybostoma infested with different concentrations of Metarhizium anisopliae

Lethal Time (TL50) for workers of *Psammotermes hybostoma*

For all three concentrations, the LT50 is less than 24 hours. In addition, it is shorter for the 1g/l and 1.5 g/l concentrations of the LT50 than for the 0.5 g/l concentration (Figure 5).

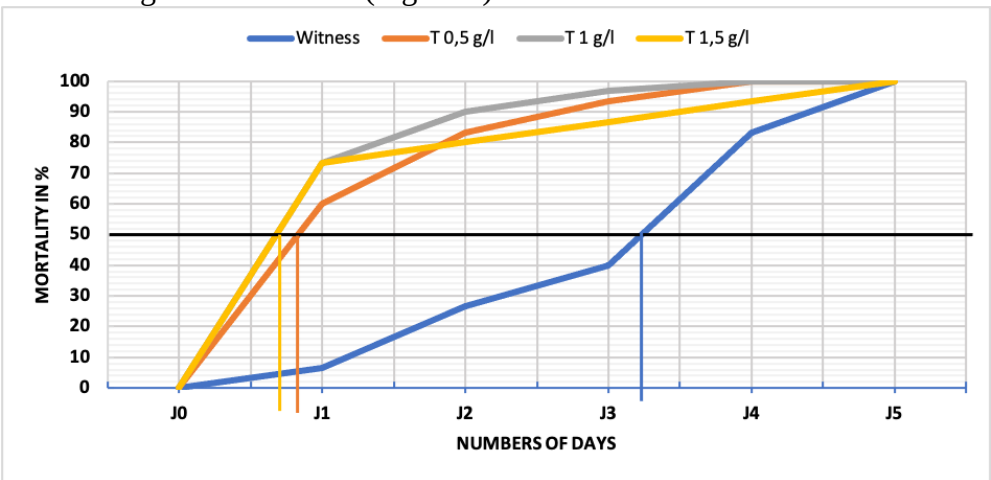

Figure 5: Evaluation of LT_{50} of different concentrations of Metarhizium anisopliae

Sporulation of the fungus on the corpses of *P hybostoma*

Sporulation on cadavers is observed after 11 days of incubation only on individuals treated with the concentration 0.5 g/l. Metarhizium spores cover the entire exoskeleton of the dead insect (Figure 6).

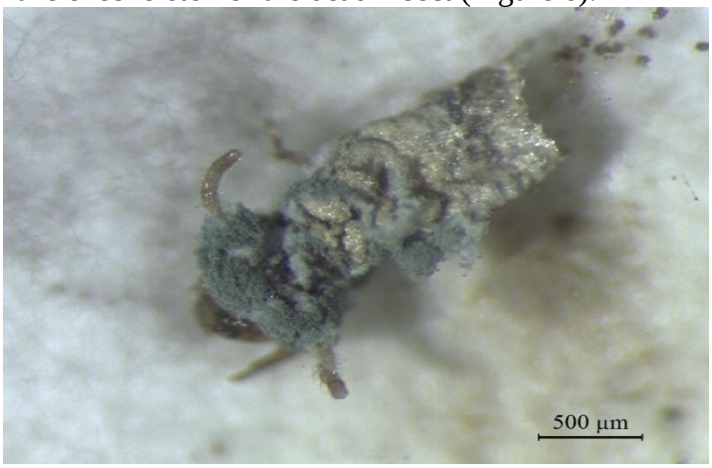

Figure 6: Sporulation of Metarhizium anisopliae on a cadaver

Discussion

The *Metarhizium anisopliae* infestation of termite workers carried out in the laboratory showed that the LT50 varies according to the species. For workers of *Microtermes lepidus* and *Psammotermes hybostoma* exposed to concentrations of 0.5, 1 and 1.5 g/l of LT50, the short lethal time could be explained by several parameters related to the insect, in particular size, but also to the dose received. In this laboratory work, under the same incubation conditions, Diatta (2018) had obtained a 4-day LT50 for the concentrations of 1 and 2 g/l on workers of *Macrotermes subhyalinus* and *Amitermes evuncifer*. The results show that the time taken by *Metharizium* to kill the insect is a function of the dose applied, as Kpindou *et al*. had pointed out. (2012) but also according to the size of the insect. The smaller the insect, the faster the lethal time and vice versa. The same remarks were made by Kpindou *et al*. (2012) on the different larval stages of *Helicoverpa armigera*, the more advanced the stage, the higher the dose to be applied. Statistical analyses showed that the mortality rates recorded for the 1g/l and 1.5 g/l concentrations are significantly higher than those recorded for the 0.5 g/l concentration and the control. The higher the dose of *Metarhizium anisopliae,* the shorter the lethal time and the higher the mortality rate. These results are similar to those of Diatta (2018) who had higher mortality rates with the high doses and those of Kpindou *et al*. (2012) who also had low levels of pupae and adults of *Helicoverpa armigera for high concentrations*.

During the incubation of cadavers, sporulation was observed on individuals tested at the lowest concentration (0.5 g/l) on *Macrotermes*

subhyalinus and *Amitermes evancifer*. These observations were made by Diatta (2018) who recorded sporulations at the lowest concentrations. The strain of *Metarhizium anisopliae* is the same as that used by Diatta (2018). The sporulation of *Metarhizium anisopliae* on cadavers is closely related to the concentration of conidia received by the insect during infection.

Conclusion

Evaluation of the insecticidal effect of *Metarhizium anisopliae* in the laboratory has made it possible to understand that mortality is strongly related to the concentration of the solution. For a higher concentration, the mortality rate is higher. The LT50 has made it possible to understand that the higher the concentration, the shorter the time. Thus, the bioinsecticidal effect of *Metarhizium anisopliae* is not immediate, which gave a LT50 of 1 to 2 days depending on the species. The lethal time 50 (LT50) for a concentration of 1 g/l on a target such as *Macrotermes subhyalinus*, which is a large termite, is longer than that of a target such as *Microtermes lepidus* or *Psammotermes hybostoma* which are small species. For the germination of the fungus on the corpse, the lower the concentration of spores, the more the spores have the opportunity to germinate on the corpse.

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Data Availability: All data are included in the content of the paper.

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