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Research Article

Histopathological Effects of the Entomopathogenic Fungus *Metarhizium anisopliae* (Metschnikoff, Sorokin) on Adults of *Rastrococcus invadens*, (Williams, 1986)

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Abstract

The mealybug (*Rastrococcus invadens*), is a major pest of mango trees, causes extensive damage and significant yield losses in Senegal. The control methods used seem less effective. Mechanical control is the most widely used method in some regions of Senegal. However, the use of natural products has shown promising potential. The aim of this study was to investigate the pathogenicity of *Metarahizium* anisoliae on adults mealybugs (*Rastrococcus invadens*) and to assess the resulting histological damage. Under laboratory conditions (75% relative humidity and 27 ℃), we tested oil-based formulations of *Metarhizium anisopliae* conidia at doses of 10^3, 10^4, 10^5, 10^6, and 10^7 spores/ml to mango leaf discs infested with adult *Rastrococcus invadens*. Treated insects were examined using optical microscopy with histological techniques to observe tissue damage. The results revealed that mortality rates were higher 48 hours after application, with respective rates of 37.4±8.8%, 58.6±5.22%, 75.45±5.28%, 92.35±1.69%, and 100±0.01% compared to the control group at 2.4±1.2%. Histological sections showed a progressive invasion of fungal spores through the cuticle, leading to the destruction of fatty tissues, muscles, and wax glands, followed by internal proliferation. These findings suggest that entomopathogenic fungi such as *M. anisopliae* can be used and popularized as biopesticides in the management of *R. invadens*.

Keywords

Rastrococcus Invadens, Histopathology, *Metarhizium Anisopliae*

1. Introduction

Rastrococcus invadens was first identified in Dakar, Senegal in 1995 [1]. Since then, it has spread widely, causing damage to fruit trees, particularly mango trees, in regions with high production, especially Thiès and Casamance [2]. *R. invadens* is a polyphagous, bisexual species that attacks many citrus and mango species [3], and its female has a

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pre-reproductive period, producing up to 200 larvae in its lifetime [4]. Recent studies have shown morphometric [5] and genetic [6] characterization of *R. invadens* in these regions. Control methods practiced by producers are generally ineffective in controlling *R. invadens* infestation. Recent studies have shown the possibility of controlling this insect using parasitoids [7] or biopesticides such as plant extracts [8-10] or entomopathogenic fungi [11, 12]. Among entomopathogenic fungi, *M. anisopliae* is widely used against various aquatic [13, 14] and terrestrial insect species [15-17]. The pathogenicity of *M. anisopliae* strains has been demonstrated in various studies [18]. In most studies, it has been shown that the fungus acts through the insect cuticle [19]. Given the pathogenicity of *M. anisopliae* demonstrated in recent studies on *R. invadens* [11], we deemed it necessary to evaluate a local strain of *M. anisopliae* on *R. invadens* and show histological damage.

2. Materials and Methods

2.1. Animal Material

Given that male *Rastrococcus invadens* are winged, only females were used, which are fixed on mango leaves. They were sampled from mango leaves of the "Sewe" variety in the garden of the Baccalaureate Office of Cheikh Anta Diop University of Dakar (latitude 14 ° 41'16" N, longitude 17 °28'0") and transported directly to the laboratory for testing.

2.2. Plant Material

The Metarhizium (Metchnikoff Sorokin) strain was provided by the Plant Protection Directorate (DPV) in Dakar. The lyophilized spores in powder form were stored in aluminum packets under laboratory conditions and kept refrigerated (4 °C). Vegetable oil was used to formulate the spore oil suspension. Different concentrations were adjusted using a Malassez cell.

2.3. Toxicity Test

The applied doses were 10^3, 10^4, 10^5, 10^6, and 10^7 spores/ml. Spraying was done using ALFA 500 manual sprayer for horticultural sprayer directly on insects fixed on freshly harvested mango leaves. Vegetable oil was used only as a control. Mortality was assessed 24 and 48 hours after spraying. Mortality rates were corrected using Abbott's formula [20]. Three repetitions were performed for each test, and means were reported.

2.4. Histological Study

To show histological damage, treated adults were fixed with the highest dose that caused 100% mortality and those of the control group after 48 hours. Insects were randomly selected from the treated group and fixed in a Carnoy solution (60% ethanol + 30% chloroform + 10% acetic acid). The technique used to perform sections on *Rastrococcus invadens* adults was standardized and described [21]. Staining was done with Hematoxylin Eosin (HE). Optical microscope "Optika" was used with an integrated camera. Images were captured on a computer and recorded in JPEG format.

3. Results

3.1. Toxicity

At 24 hours, mortality was observed for all doses. However, mortality rates were only high 48 hours after application (Figure 1), with successive rates of 37.4 ±8.8%, 58.6 ±5.22%, 75.45 ±5.28%, 92.35 ±1.69%, and 100 ±0.01% compared to the control group at 2.4 ±1.2%.

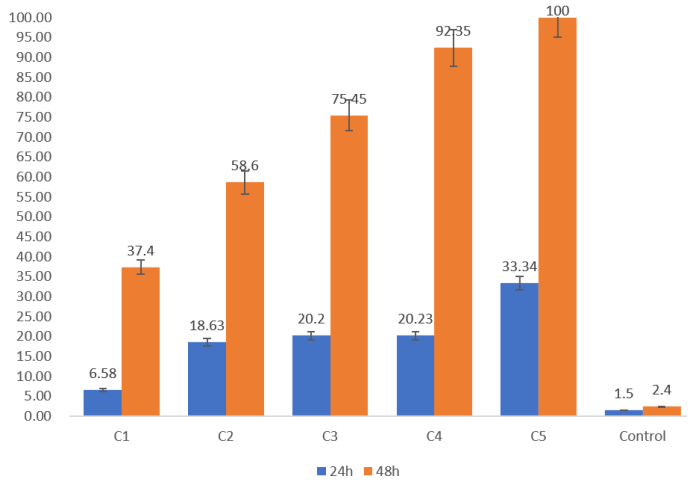

Figure 1. *Mortality of Rastrococcus invadens adults treated with a Metarhizium anisopliae oil solution (C1=10^3, C2=10^4, C3=10^5, C4=10^6, and C5=10^7 spores/ml) 24 and 48 hours after treatment. Values are mean corrected percent mortality of four replicates with standard error (SE).*

3.2. Histopathological Effects

Histological examination reveals a degradation of internal tissues. A comparison with the control sample shows significant disintegration of internal tissues in the treated samples. At the level of the overall view, we observe in longitudinal section a degradation of internal tissues of *R. invadens* within 24 hours (Figure 2b) compared to the control (Figure 2a).

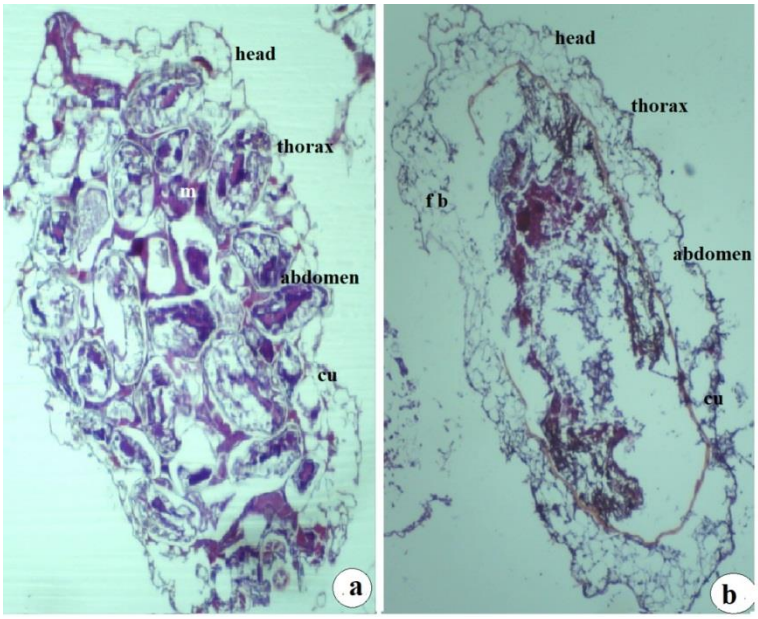

Figure 2. *Longitudinal sections of Rastrococcus invadens adults untreated (figure 2a) and treated with Metarhizium anisoplaie oil formulated (Figure 2b) 48 hours after treatment (X40). m= muscle, fb= fat body, cu= cuticle (X40), coloration HE.*

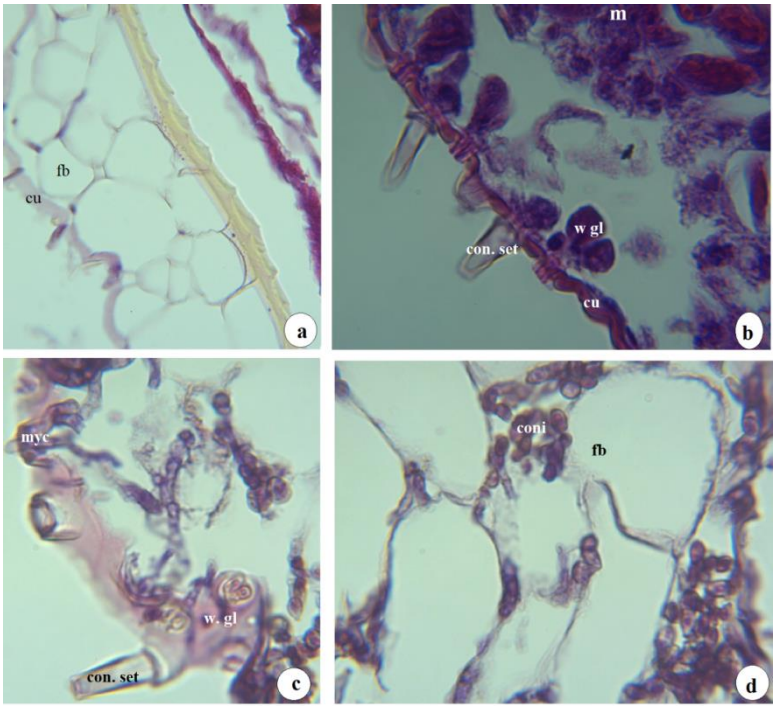

Figure 3. *Histological section of the peripheral zone (cuticle and fat body) of untreated Rastrococcus invadens adults (Figure 3a, 3b) and treated with a Metarhizium anisopliae oil suspention (Figure 3c and 3d) 48 hours after. Cer. gl= wax gland, fb= fat body, cut= cuticle, con. set= conical setae, coni.= conidia (X400).*

At high magnification (X400), we observe destruction of the wax glands near the cuticle of treated *R. invadens* adults (figure 3c) compared to the intact glands of untreated adults (figure 3b). Mycelium develops through the cuticle (Figure 3c) and invades the adipose tissue (Figure 3c and 3d).

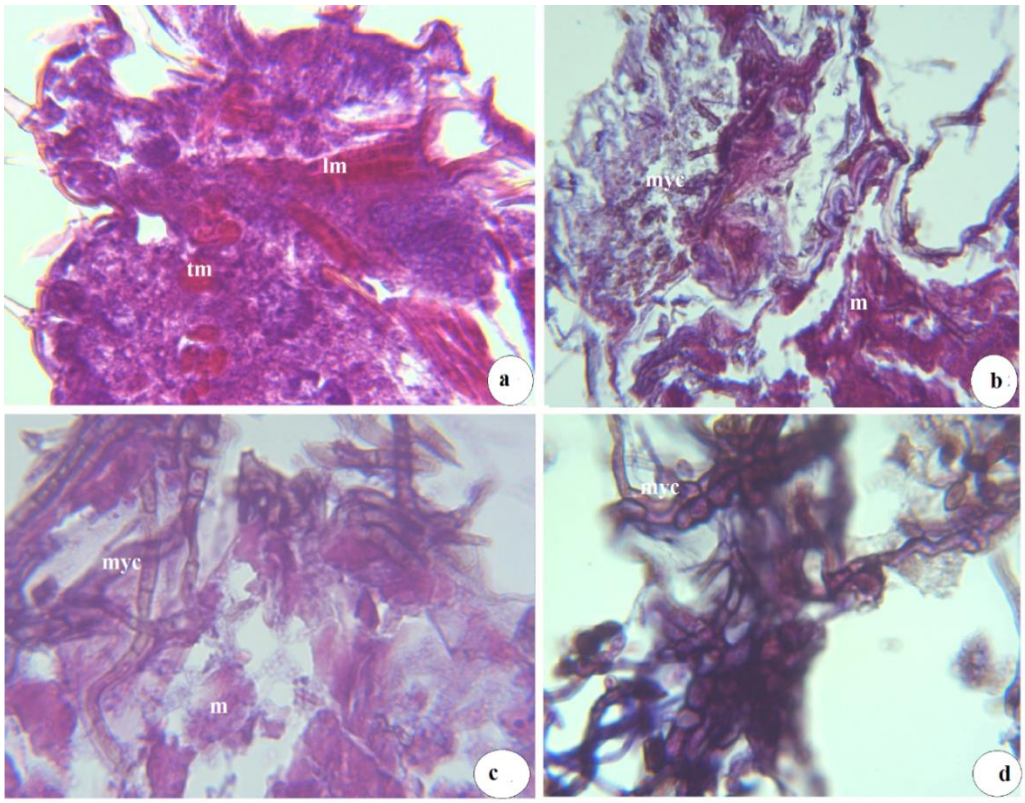

Figure 4. *Histological section of untreated Rastrococcus invadens adult (Figure 4a) and treated (Figure 4b, 4c, 4d) with Metarhizium anisopliae oil formulation 48 hours after. tm = transversal muscle, lm= longitudinal muscle, myc = mycelium (X400).*

Compared to the control (Figure 4a), we observe at high magnification a development of mycelium in the muscle tissue in many areas (Figure 4b, 4c, 4d). This development is accompanied by massive and almost complete destruction of the muscle tissue in treated adults. Microscopic examination shows fungal growth in the insect's internal cavity, leading to disintegration of internal tissues. The fungus colonizes the internal cavity, causing extensive tissue damage and disruption of vital organs, ultimately contributing to the insect's mortality.

4. Discussion

The application of the *Metarhizium anisopliae* oil formulation showed increasing mortality rates of 37.4±8.8%, 58.6±5.22%, 75.45±5.28%, 92.35±1.69%, and 100±0.01% for doses ranging from 10^3 to 10^7 compared to the control group at 2.4±1.2% within 48 hours. The formulation of spores with vegetable oil seems to facilitate adhesion of conidia and the infestation time. This leading to high mortality rates within 48 hours compared to other study [18]. Indeed, these authors observed mortality rates between 48 and 96 hours after applying *M. anisopliae* spores in aqueous suspension. This suggests that spores adhere more easily in large numbers with an oil formulation than with an aqueous formulation. In fact, our previous work has shown that oil formulations of entomopathogenic fungi can increase the effectiveness of spores on insects [14, 22, 23]. However, in this study, we did not evaluate the germination capacity of spores on the insect beyond 48 hours.

However, through optical microscopy, the sections performed showed that *M. anisopliae* spores caused destruction of internal structures of R. *invadens*. The spores were able to penetrate through the cuticle before developing in the hemocoel and fat body. Spore development was also observed in muscle tissue and exocrine glands such as wax glands. These microscopic observations demonstrate a mechanism of action of *M. anisopliae* on insects, confirming several studes [16, 24]. In fact, studies have shown that after penetration through the cuticle or after ingestion, the spores begin their development by excreting secondary metabolites, particularly toxins that disorganize and destroy the surrounding tissues [24, 25]. Since *R. invadens* is a sap-feeding insect, the spores could only penetrate through the cuticle and not through the digestive tract.

This led us to focus on tissues other than the digestive tract, unlike other insects that feed only by ingestion, such as mosquito larvae [21, 24, 25] or locusts [26].

Furthermore, we were unable to identify and examine other tissues such as those of the digestive system, nervous system, and reproductive organs. However, the proliferation of mycelium in the internal structures of *R. invadens* would likely not leave other internal tissues intact.

5. Conclusion

This study highlights the importance of *Metarhizium anisopliae* oil formulated againts pests as *Rastrococus invadens*. The vegetable oil enhances the effectiveness of the fungus by facilitating the adhesion of conidia that penetrate through the cuticle before destroying internal tissues. further histological studies on the effects of the fungus on the reproduction or biology of *R. invadens* is necessary to assess its pathogenicity on development.

Abbreviations

HE	Hematoxylin Eosin
DPV	Plant Protection Directorate
SE	Standard Error

Author Contributions

Fawrou Seye: Conceptualization, Methodology, Writing - original draft

Mamour Toure: Writing - original draft

Amadou Fall: Conceptualization

Fatou Kamby: Methodology

Mady Ndiaye: Supervision

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Conflicts of Interest

The authors declare no conflicts of interest.

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