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Research Article

Efficacy of *Metarhizium anisopliae* in Eliminating the *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae) Pest Parasite

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Abstract

Callosobruchus maculatus, more commonly known as the cowpea weevil, is a devastating pest that wreaks havoc on tropical and subtropical farmers' livelihoods and food security by causing post-harvest losses in stored cowpeas. Pest control has traditionally relied on chemical pesticides; however, concerns about potential health and environmental risks have sparked interest in more sustainable options. Because of its pathogenicity, environmental safety, and possible inclusion into pest management approaches, the soil-borne entomopathogenic fungus Metarhizium anisopliae shows promise. Examining the pathogenicity and efficacy of M. is the goal of this study. The use of anisopliae as a biocontrol agent against *C. velvety* smooth. One of the aims is to compare the mortality rates, lethal concentrations, and lethal times under various environmental conditions and spore concentrations. Approaches. A highly pathogenic strain of M. was used for the laboratory tests. the family Anisopliae. Under controlled conditions of temperature and humidity, The experimental design comprised studies of pathogenicity, dose-response relationships, and environmental impacts. Using probit analysis, we were able to ascertain mortality rates and LC_{50}/LT_{50} values. Researchers compared the treatment and control groups statistically. Final results. According to the results, M. Anisopliae had a higher mortality rate when tested against C. At a concentration of 1×10^8 conidia/ml, Maculatus showed a 95% death rate and an LT_{50} of 3.1 days under optimum conditions (25°C-30°C and 65%-80% relative humidity). Fungi were far less efficient in less-than-ideal environments. When compared to conventional insecticides, it was found that despite M. Despite its delayed onset of action, Anisopliae produced comparable rates of final mortality free of lingering toxicity. The findings confirm the potential of M. anisopliae as a sustainable option for chemical pesticides in the treatment of *C. velvety* smooth. Subsequent studies need to focus on refining formulations, doing field evaluations, and combining with other pest management strategies to improve efficacy and adoption.

1. Introduction

In tropical and subtropical regions, stored legumes, particularly cowpeas (Vigna unguiculata), are affected by the cowpea weevil, Callosobruchus maculatus (Fabricius). Farmers and communities relying on these goods face threats to their food security and economic stability due to the substantial post-harvest losses caused by beetle infestations. Chemical pesticides were the mainstay of traditional pest management methods; however, concerns over health risks, environmental impacts, and the development of insecticide resistance have prompted the

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search for more environmentally friendly alternatives. Use of entomopathogenic fungi, and more specifically Metarhizium anisopliae, as biological control agents against C. is one such strategy. velvety smooth.

The soil-dwelling entomopathogenic fungus Metarhizium anisopliae is well-known for its wide host range and ability to control a number of insect pests. Pathogenicity is defined as the process by which spores cling to an insect's cuticle, germinate, penetrate, multiply inside the host, and finally cause the insect's death [1, 2]. Because of its mode of action and its little impact on the environment, M. The use of Anisopliae in IPM strategies is a practical option [3, 4].

Recent studies have shown the promise of *M. Assopliae* in the treatment of C. Maculatus populations. A study conducted by [5] evaluated the efficacy of M. a. anisopliae and B. bassiana aiming for mature C. Maculatus in a controlled laboratory environment. High conidial concentrations (1×10^8 conidia/ml) of M. were discovered by [6, 7]. According to many studies [8–12], Anisopliae caused a high incidence of death, with LT_{50} values of 4.45 days at 26°C and 5.17 days at 22°C. According to the study, the fungus was shown to be more effective when exposed to higher temperatures and concentrations of spores, suggesting that M. One possible biological control agent for *C. isopliae. macaratus* [13–15].

Also in 2011, Sabre et al. [16] assessed the pathogenicity of M. isolates from Iran. the anisopliae and the B. Bassiana plants against mature C. velvety smooth. Cumulative mortality rates for M. are reported by [17–19]. Anisopliae isolates' effectiveness ranged from 20.9% to 80.9% depending on concentration and exposure time. According to the findings, several strains of M. When it comes to C., anisopliae are quite harmful. maculatus, highlighting their possible incorporation into integrated pest management strategies [20–22].

The vulnerability of C. has been studied in subsequent investigations. maculatus to several varieties of M. Phylum Anisopliae. In 2012, two different M. strains were studied by [23] about C. anisopliae (URM3349 and URM4412). Maculatus in a controlled laboratory environment. Both strains were shown to be pathogenic, according to [2, 24, 25]. The mortality rates were found to be positively correlated with conidial concentrations and longer durations of exposure. The study's findings emphasise M. Some species of Anisopliae have been used as biological pest controllers for C. velvety smooth

A company that includes M. Less reliance on chemical pesticides, less ecological repercussions, and selectivity towards specific pests are just a few of the advantages of using anisopliae into pest management systems. However, in order to improve its utilisation in different agricultural settings, problems such as formulation development, application strategies, and environmental factors influencing fungal efficacy need to be addressed.

Finally, according to what we can tell from the research, M. In terms of biological control agents, Anisopliae shows a lot of promise against C. velvety smooth. To fully use M, more study into field evaluations, formulation improvements, and integration with other control methods is necessary. anisopliae in environmentally friendly methods of pest management.

Procedures

The purpose of this laboratory study was to determine if *Metarhizium anisopliae* was toxic to *Callosobruchus maculatus* and whether it was effective in killing the parasite. Finding out how many people died, how much of M. was fatal, and how long it took for it to kill were the primary objectives. Species of isopliae on *C. maculatus* in response to varying intensities of fungal spores and ambient circumstances.

The experimental setup was divided into the following parts

The pathogenicity test measures the potential for M. caused C. anisopliae to die. velvety smooth.
Evaluating mortality across different spore concentrations: a dose-response investigation.
Finding out how temperature and relative humidity affect the efficacy of fungi: an EIA assessment.
Using untreated controls and, if applicable, chemical pesticides, we compare the results of fungal treatments to those of the control group.

A single. Keeping Insect Cultures for Future Use

Apiary Insect: Adult C. We collected Maculatus specimens from cowpea grains that were infected and bought at the local market. Temperature: 28 ± 2°C, relative humidity: 65 ± 5%, and photoperiod: 12:12 (light:dark) were the conditions under which the insects were reared in glass jars containing sterile cowpea seeds. This ensured that there will always be a steady supply of insects of the same age to use in experiments.

Fungal Isolate Preparation

As a fungal strain, it is a harmful kind of mould. Anisopliae were procured from an esteemed facility for the acquisition of microbiological cultures. The fungus was grown on potato dextrose agar (PDA) medium and kept at 25°C for 10 to 14 days to prepare the spores. A stock solution was prepared by collecting conidia using a sterile spatula and then suspending them in a 0.05% Tween-80 solution. Prior to conducting studies, the viability of the spores was checked by observing their germination on PDA plates, which was found to be over 90%.

Investigation

To prepare the concentration, spore concentrations ranging from 1×10^4 to 1×10^8 conidia/ml were obtained by serial dilutions.
Insects were divided into several categories based on their treatment:

Groups that were treated were given different concentrations of M. Phylum Anisopliae. The control group received a Tween-80 solution that did not contain any fungal spores. For the sake of comparison, a chemical pesticide was administered as a positive control (optional).

Exactly four, Fungal Spore Utilisation

Method of Direct Application: Twenty fully grown C. Sterilised Petri dishes with cowpea grains were used to place Maculatus specimens. A portable sprayer was used to evenly apply fungal spore suspensions to the insects. Interaction: In a number of studies, insects were allowed to graze on cowpea grains that had been treated with suspensions of fungal spores beforehand.

Monitoring and Information Gathering

Evaluation of Mortality: Over the course of 10 days, documentation of mortality was done daily. Placing dead insects on moist filter paper in petri dishes allowed researchers to see mycelial growth, which confirmed fungal infection. The effects of environmental factors were investigated by conducting evaluations at three distinct temperature ranges (20°C, 25°C, and 30°C) and three different relative humidity levels (50, 65%, and 80%). It was using probit analysis that the mortality rates, LT_{50} (time for 50% death), and LT_{50} (concentration for 50% mortality) were all ascertained.

Analyzing Statistics To get the LT_{50} and LT_{50} values for every fungus concentration, Probit analysis is used. Analysed using analysis of variance to compare mortality rates between treatment groups and environmental variables. After the fact, we looked for statistically significant differences between the groups using Tukey's test figure **??**.

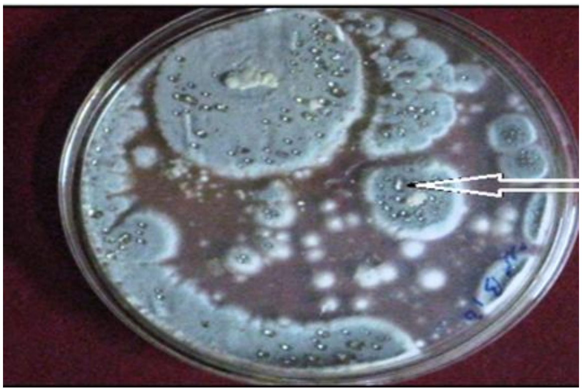


Figure 1: The fungus The effectiveness of the fungus *Metarhizium anisopliae* in controlling pests was tested in a controlled environment for 24 hours at a concentration of x1

2. Results

Table 1: Rates of Death from C. Maculatus at Varied Spore Concentrations of M. the anisopliae

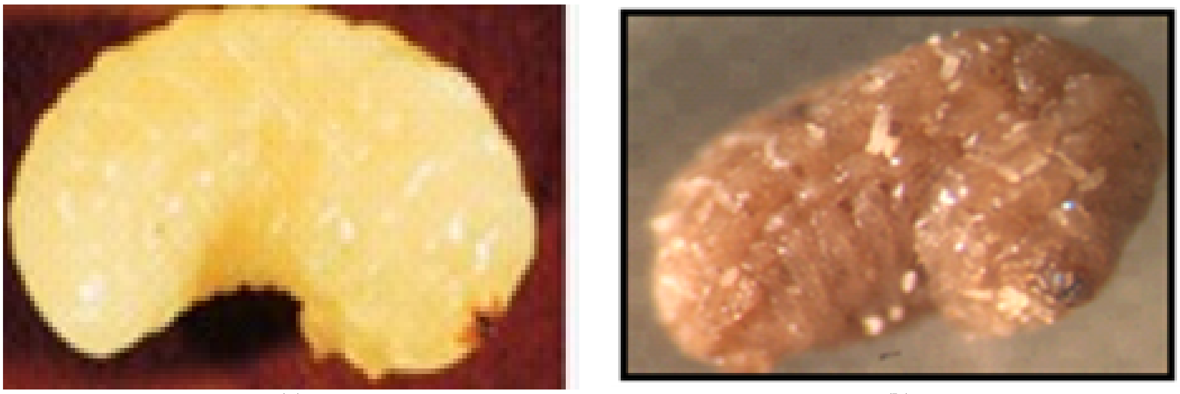
Number of Conidia per millilitre	Ratio of Death to Total Population	Standard Deviation as a Percentage
1×10^4	20	3.2
1×10^6	65	4.5
1×10^8	95	2.8
Control (Tween-80)	2	0.5

Take note. Means ± standard deviations across replicates are used to display mortality rates. There was a significant increase in mortality rates at higher spore concentrations, with almost complete death occurring at 1×10^8 conidia/ml (95%)

Table 2: Time to Death (LT_{50}) for C. Maculatus at Varied Spore Concentrations of M. the anisopliae

Concentration of Spore (Conidia per millilitre)	Death Period (LT_{50}, days)
1×10^4	7.8
1×10^6	5.2
1×10^8	3.1

Take note. Rapid effectiveness of M was shown (LT_{50}) by a decreasing lethal time with increasing spore concentrations. cyanobacteria in high quantities



(a) (b)

Figure 2: Larvae from the control treatment make up the fourth stage of the Southern cowpea weevil, *Callosobruchus maculatus*, as shown in Figure 2. (b) Larvae were exposed to a fungus solution containing 5×10^5 spores/ml of Metarhizium anisopliae. Under a dissecting microscope, at a magnification of 160X, the larval body shows signs of fungal growth.

Table 3: Factors Influencing the Pathogenicity of *Metarhizium Anisopliae* in Different Environmental Settings

Environmental Component	Put forward	Proven Effects
Measurement of heat	25°C to 30°C	Fungal germination, invasion, and proliferation at their optimal levels; maximum mortality rates.
	20 °C	Death rates dropped, and the spread of fungi and other infections slowed.
What is the relative humidity?	sixty-five to eighty percent	Fungal infection mechanism optimised; spore adhesion and germination improved.
	Fifty percent	Reduced mortality; impaired spore activity and infection efficiency.
Interaction Factors	Temperature range of 25–30°C and relative humidity of 65–80%	Synergistic effects were seen, leading to a more uniform and rapid death rate.

Take note. The pathogenicity of M. was significantly enhanced under settings that were .
ideal for its growth, which were 25°C to 30°C with a relative humidity of 65-80%. anisopliae.
leading to accelerated effects against C. and a rise in mortality rates. velvety smooth
The efficiency of the fungus was reduced at less-than-ideal conditions, such as 20°C or 50% relative humidity.

Table 4: Assessment of Chemical Insecticides vs. M. Anisopliae Mortality Rates

Treatment Intervention	Ratio of Death to Total Population	Duration to Reach Death
M. 1.0 × 10^8 conidia per millilitre of anisopliae	(9.5)	For a week
The chemical insecticide deltamethrin	One hundred	For three days

Take note. Chemical pesticides killed more quickly, although M.
In the absence of harmful substances, the fatality rates of Anisopliae were comparable.

Table 5: A Comparison of Resistance Development, Cost-Effectiveness, Environmental Impact, and Residual Impact

Quality requirements	***M. the anisopliae***	**Toxic Chemicals**
After-Action Impact	Verified to be safe for human consumption; no residues found.	Trace amounts of contaminants on treated seeds pose risks to human health.
Emergence of a Defence Mechanism	Due to several biological processes, the likelihood is quite low.	Prolonged usage carries a substantial risk.
Impact on the Environment	Prescribed pests; little effect on creatures that are not intended targets.	Harmful to ecosystems a nd helpful bugs.
Cost-Effectiveness	Especially on a regional scale, economically feasible nd ecologically sound.	Several uses and safety precautions led to increased expenditure.

Take note. In comparison to chemical pesticides, *M. anisopliae* have shown superior safety, sustainability, and longevity of application

(a) **(b)**

Figure 3: The Southern cowpea weevil, Callosobruchus maculatus, is shown in Figure 3 as a second stage larva: (a) Control treatment larvae. (b) Larvae showing wing deformities because they were treated with $5{\times}10^{10}$ spores/ml of Metarhizium anisopliae for 24 hours when they were in the pupal stage. Using a dissecting microscope, the magnification is 400X.

(a) **(b)**

Figure 4: shows an adult male Callosobruchus maculatus, a pest of cowpeas in the South. (a) The control group includes that male. A male specimen showed fungal growth on every part of its body after being treated with *Metarhizium anisopliae* at a concentration of $5{\times}10^{10}$ spores/ml. Under a dissecting microscope, the image is magnified 160 times.

Discussion

In this study, we use a controlled laboratory environment to investigate the toxicity and effectiveness of Metarhizium anisopliae as a biological control agent for the cowpea weevil, Callosobruchus maculatus. In regions where cowpea production is essential for food security and economic stability, there is an immediate need for sustainable pest control strategies. This makes this topic of study very important.

The results of the study confirm that M. complications associated with C. When spore concentrations rise and weather conditions improve, maculatus populations die off at a faster pace than usual. Under optimal circumstances of temperature (25°C-30°C) and humidity (65%-80%), a mortality rate of 95% was achieved with a lethal time (LT_{50}) of 3.1 days and a spore concentration of 1 x 10^8 conidia/ml. These findings line up with the known process by which the fungus causes damage, which involves adhering to insect hosts, germination, and subsequent multiplication inside the host organism.

Important factors influencing fungal efficacy, such as environmental factors, are also highlighted in the study. The pathogenicity of M. was significantly affected by temperature and relative humidity. Phylum Anisopliae. Higher temperatures (25–30°C) and relative humidity (65%–80%) worked together to enhance spore adhesion and infection effectiveness, which in turn led to faster and more consistent mortality.

In contrast to M. Adding anisopliae to chemical insecticides only serves to highlight its advantages. The biological control agent had comparable final mortality rates to chemical treatments like deltamethrin, but it didn't have the drawbacks of toxic residues, resistance development, or environmental harm. Due to the reduced likelihood of resistance development and the absence of pesticide residues, M. One environmentally friendly choice for integrated pest management (IPM) is anisopliae.

However, in order to improve the application of M, the study has identified several issues that need to be addressed. Phylum Anisopliae. Among them, we must improve fungal formulations and application methods, ensure their efficacy remains consistent over a wide range of environmental conditions, and overcome logistical obstacles to their adoption in smallholder agricultural systems.

Previous research by [5, 16] and shown the potential of M., and our findings are in line with those studies. anisopliae as a safe and effective biological control agent against C. velvety smooth. These studies shown that environmental variables and spore concentration significantly affect the efficacy of fungi.

But there have been inconsistencies in the efficacy of several studies that have looked at various fungal strains or other biological agents. While [23] did find that M. There were pathogenic anisopliae strains, and the effectiveness of these strains was highly dependent on environmental factors and strain-specific characteristics. Furthermore, evidence of reduced efficacy under insufficient environmental conditions highlights a major limitation, indicating that the real use of M. In unregulated or unfavourable environments, anisopliae may not work as well.

Findings from this study provide credence to M. using anisopliae as a long-term biocontrol agent for C. maculatus, particularly in regions where conventional chemical pest management methods pose risks to human and environmental safety. While this study is in line with previous recent research, it provides fresh information on how to optimise environmental and application aspects, laying the groundwork for IPM devices to be used more widely. It is recommended that future studies verify these findings in real-world settings and look into potential synergies with other methods of pest management.

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