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RESEARCH ARTICLE Agricultural Science Digest

Unveiling the Entomopathogenic Potential of Beetle-associated Fungi for *Culex* Management in South-West Algeria

Nouha Halima Bendekhis[1], Ali Boulanouar[2], Lakhdar Mebarki[2]

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ABSTRACT

Background: The escalating threat of mosquito-borne diseases demands innovative control strategies. This study explored the potential of beetle-associated fungi as biological control agents against *Culex* mosquito larvae in southwestern Algeria.

Methods: Fungal strains were isolated from beetle carcasses collected from pesticide-free regions of Beni Abbès and Béchar using dilution plating techniques. Based on metabolic profiles, four strains were selected for bioassays: Three *Aspergillus* species and one *Penicillium* species. *Culex* larvae were exposed to fungal concentration of 10^6 conidia/ml in sterile rearing water. Larval mortality was monitored daily over seven days.

Result: The results demonstrated significant larval mortality across all fungal strains. *Aspergillus* species showed faster mortality rates within three to five days, while *Penicillium* sp took seven days to show complete death. The LT_{50} values also confirm the rapid action of *Aspergillus* strains with *Aspergillus* sp 1 showing the most potent activity (LT_{50}=1.25 days). On the other hand, *Penicillium* sp was slower with an LT_{50} value of 3 days, these findings highlight the power EPF associated with beetles to be an effective tool for mosquito management in the region.

Key words: Beetle, Biocontrol, *Culex*, Entomopathogenic fungi.

INTRODUCTION

Entomopathogenic fungi (EPFs) have become a promising biological control agents for managing insect pests. Their broad host range, endophytic capabilities and plant growth-promoting properties, the EPFs offer a number one environmentally friendly alternative to chemical insecticides (Panwar and Szczepaniec, 2024). Despite the ongoing danger posed by mosquito-borne diseases (Bursali *et al.,* 2024), that requires the creation of effective control strategies, the potential of EPFs in areas such as southwest Algeria remains poorly investigated (Zimowska and Krol, 2019). Due to the possibility of mosquitoes' resistance to the chemical pesticides EPFs can be a useful biological alternative (Pratibha *et al*., 2025; Ramirez *et al*., 2023).

However, the unique environmental conditions in southwest Algeria such as climate, local mosquito species and the availability of suitable fungal strains, influence the efficacy and sustainability of the EPFs (Li *et al.,* 2024). A lot of studies have shown that EPFs are so effective against a variety of mosquito species, however nothing is known about the entomopathogenic capacities of beetle associated fungi in controlling mosquito vectors.

This study evaluates the entomopathogenic capability of beetle-associated fungi against mosquitoes in southwest Algeria. By investigating this innovative approach, we seek to contribute to the creation of sustainable and region-specific mosquito control strategies.

MATERIALS AND METHODS

This study was conducted in laboratory for valorization of vegetal resource and food security in semi-arid areas at university Tahri Mohamed Bechar, Algeria during December 2021-March 2024.

Isolation

Fungal strains were isolated from beetle carcasses, that were collected in pesticide-free regions of Beni Abbes (Igli) and Bachar (Wakda). Using a dilution plating method, we aseptically homogenized 5 g of each carcass in 45 ml of sterile distilled water. Decimal dilutions of the resulting suspension were then inoculated onto chitinous Potato Dextrose Agar (PDA) for fungal cultivation. The isolated fungi were purified and identified using classic methods (Abdel-Raheem, 2019; Erick de Jesús de Luna-Santillana *et al.,* 2020).

[1]Division of Laboratory for Valorization of Vegetal Resource and Food Security in Semi-arid Areas University Tahri Mohamed Bechar, Bechar, Algeria.
[2]Division of University, Tahri Mohamed Bechar, Bechar, Algeria.

Corresponding Author: Nouha Halima Bendekhis, Division of Laboratory for Valorization of Vegetal Resource and Food Security in Semi-arid Areas University Tahri Mohamed Bechar, Bechar, Algeria. Email: bendekhis.nouha.h@univ-bechar.dz
ORCIDs: 0009-0009-0595-1669, 0000-0002-2464-2337, 0000-0003-2960-1996.

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Insect collection

Culex mosquito larvae were collected from Igli during April to July in both 2022 and 2023. Larvae were transported in containers containing the original wastewater while maintaining optimal temperature conditions. To ensure continuous feeding, algae and other organisms were included in the containers. Water parameters, specifically pH (5-8) and temperature (25±2°C) were monitored throughout.

Bioassay

We selected four fungal strains based on their metabolic profiles observed on agar plates. Then, a standardized concentration of 10^6 conidia/ml was added into 50 ml of sterile rearing water containing 10 third-instar mosquito larvae. A drop of Tween 80 was added to each treatment to ensure homogeneous spore distribution. We included control groups containing only sterile rearing water and Tween 80. All setups were maintained in the dark at 25°C± 2°C. Larval mortality was recorded daily for seven days (Jaber *et al.,* 2016). The experiment was replicated three times. Lethal Time 50 (LT_{50}) values were calculated using Microsoft Excel.

RESULTS AND DISCUSSION

Isolation

The isolation study revealed that *Aspergillus* species (Fig 1 a, b and c) constituted the dominant fungal flora, comprising 75% of the isolated chitinolytic fungi, while *Penicillium* species (Fig 1d) accounted for the remaining 25%. Three distinct *Aspergillus* species and one *Penicillium* species were selected for bioassay experiments. Fig 1 illustrates the macroscopic morphological variations, including texture and colour, among these strains. Additionally, microscopic characteristics were observed using microculture techniques.

Bioassay results

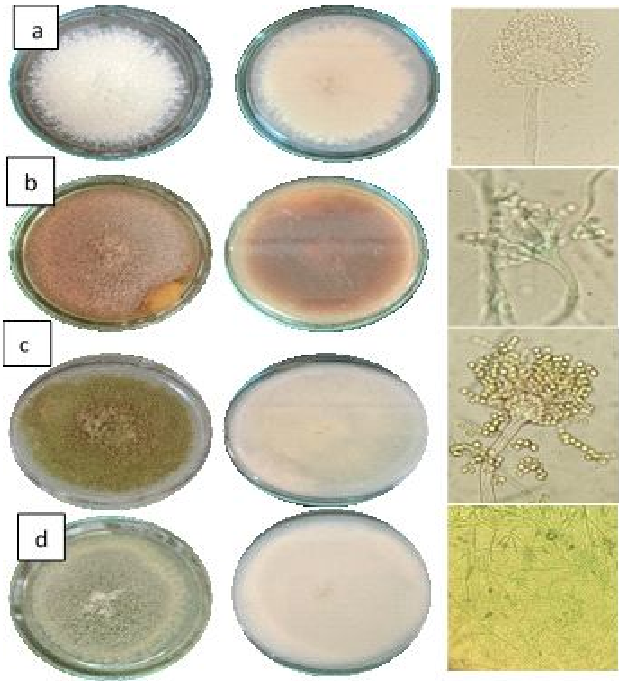
Experimental conditions were standardised by exposing 10 third-instar mosquito larvae to a consistent concentration of each fungal strain under controlled temperature, light and pH parameters. All fungal strains induced significant mosquito mortality, although with varying temporal dynamics. Strains *Aspergillus* sp 1, *Aspergillus* sp 2 and *Aspergillus* sp 3 exhibited rapid mortality rates, reaching 100% within five, four and three-days post-infection, respectively (Fig 2). In contrast, *Penicillium* sp demonstrated a slower progression, achieving complete mortality by the seventh day.

Strain *Aspergillus* sp 1 consistently induced high mortality rates, with a rapid escalation from 40% on day one to 100% by day five. *Aspergillus* sp 2 also demonstrated potent larvicidal activity, reaching 100% mortality by day four. Similar to *Aspergillus* sp 1, the strain *Aspergillus* sp 3 rapidly induced mortality, achieving 100% within three days. Conversely, *Penicillium* sp exhibited a more gradual mortality curve, culminating in complete larval death by day seven. *Aspergillus* strains exhibited a broad spectrum of pathogenicity, affecting both larval and adult stages of *Culex* mosquitoes. While all strains induced mortality in larval stages, primarily targeting the gut and siphon, as evidenced in Fig 3, their impact on adults was also pronounced. Adult mosquitoes exposed to these fungi displayed fungal accumulation in various body segments, leading to mortality shortly post-metamorphosis. In some cases, only the exoskeleton remained.

Lethal time 50

The LT_{50} values provide a quantitative measure of treatment efficacy and speed of action. The strain *Aspergillus* sp 1 exhibited the shortest LT_{50} of approximately 1.25 days (Fig 4), demonstrating the most rapid and potent insecticidal activity. *Aspergillus* sp 3 followed with an LT_{50} of roughly 1.4 days, while the strain *Aspergillus* sp 2 displayed a slightly longer LT_{50} of 1.67 days. In contrast, *Penicillium* sp demonstrated the longest LT_{50} of 3 days, indicating a slower onset of action and reduced efficacy compared to the other strains.

This study aimed to evaluate the potential of entomopathogenic fungi isolated from scarab cadavers as biocontrol agents against *Culex* mosquitoes. Dominant fungal isolates recovered from scarab cadavers in southwestern Algeria were *Aspergillus* species,



1a: *Aspergillus* sp 2; 1b: *Aspergillus* sp 1; 1c: *Aspegillus* sp 3; 1d: *Penicillium* sp.

Fig 1: Pictures of some fungi isolated for use in the bioassay, depict the macroscopic and microscopic morphology of the isolated fungi.

corroborating previous findings on the prevalence of *Aspergillus* in *Coleoptera* and from arthropod *Penicillium* and *Cladosporium* (Jaber *et al*., 2016). *Aspergillus* and *Penicillium* species are common within the microbiome of scarab cadavers and it's compatible with their terrestrial habitat, especially in arid settings (Alfiky, 2022). According to Gebremariam (2021) high temperatures, ultraviolet rays, low humidity and sandy soils are all features that encouraged the proliferation of these fungi. The ubiquitous nature of *Aspergillus* species explains its dominance in our study, aligning with the work of Tekaia *et al.* (2005).

All entomopathogenic fungi employed in this study showed a chitinolytic activity and achieved 100% mortality against *Culex* mosquitoes. This is explained by their natural

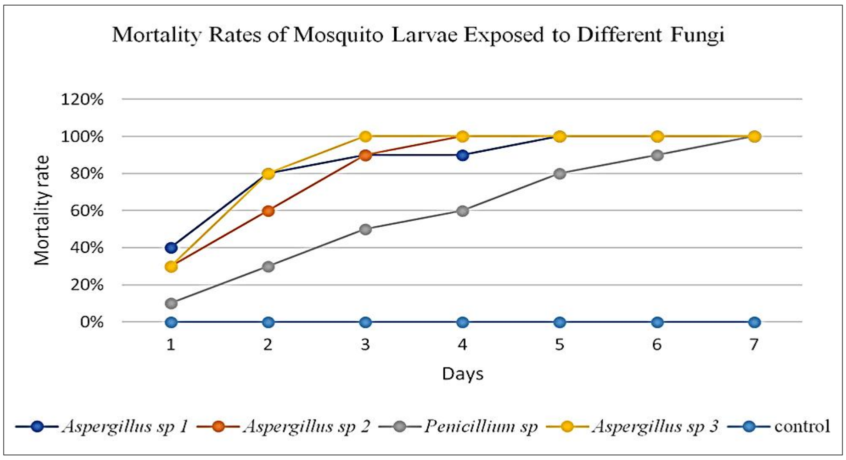


Fig 2: Mortality rates of *Culex* mosquito larvae exposed to *Aspergillus* and *Penicillium* species.

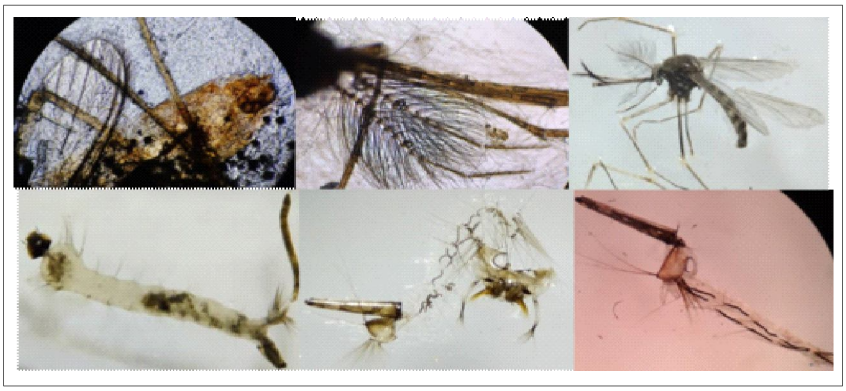


Fig 3: Fungal **e**ffects on *Culex* mosquitoes and larvae.

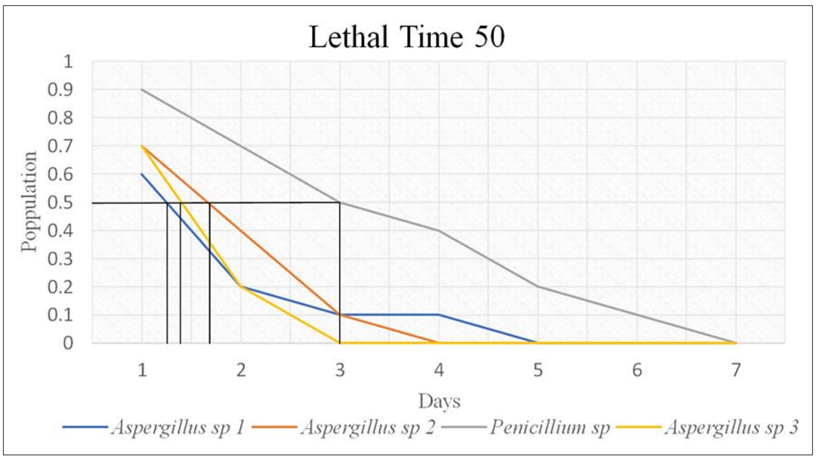


Fig 4: Lethal time 50 of *Culex* mosquitoes population exposed.

entomopathogenicity and ability to degrade chitin which is a vital component of insect cuticles.

The observed variations in mortality rates among fungal treatments might be caused by the differences in vegetative growth rates, though Parveen and jeyarani (2023) research results confirmed that the optimum temperature for entomopathogenic fungi growth and toxicity is 25°C to 30°C. The slow and gradual increase in mortality caused by *Penicillium* sp reaching 100% by day seven (Fig 2) indicates a delayed release of endotoxins and delay the onset of death (Sun *et al*., 2002). Additionally, the thicker cuticle and decreased food intake of later larval stages may have reduced spore penetration, further influencing mortality rates (Lord and Fukuda, 1990; Apperson *et al*., 1992).

Aspergillus species, on the other hand, show greater larvicidal activity against *Culex*, achieving total mortality within 3-5 days, indicating their potential for practical applications. These results align with previous studies showing that *Aspergillus niger* has a powerful larvicidal (Abideen *et al*., 2021; Rai *et al*., 2023) and poricidal properties, especially when paired with silver nanoparticles (Awad *et al*., 2022). *Aspergillus parasiticus* has also strong entomopathogenic properties (Abrar *et al.,* 2022). Other studies have demonstrated that *Aspergillus* germination is effective against various mosquito species and its metabolism has proven to be effective (Ragavendran and Natarajan, 2015; Ragavendran *et al*., 2018; Balumahendhiran *et al.,* 2019).

Aspergillus species isolated from scarab, often considered environmental contaminants especially soil (Beemrote *et al*., 2024), have shown unexpected potential as entomopathogens. They were as pathogenic as *Beauveria bassiana* against *Aedes* and *Culex* mosquitoes. (Jaber *et al*., 2016). Mosquitoes' mortality caused by EPF is a complex process influenced by multiple factors. Host characteristics, including species, age and population density, significantly impact disease progression and mortality rates in addition to fungal virulence. Consequently, attributing mortality solely to fungal dosage is an oversimplification (Batta, 2005; Mantzoukas *et al.,* 2019; Mantzoukas and Grammatikopoulos, 2020; Mantzoukas *et al.,* 2022).

EPF spores infect insects with both hard and soft exoskeletons (Sharma *et al.,* 2023) and function as midgut toxins just like *Nerium oleander* leaf extracts (Boulkenafet *et al.,* 2023). Fig 3 illustrates spore accumulation in siphons, guts and adult mosquito articulations. Primarily ingested through the mouth, spores readily infect mosquito larvae when applied to the water surface (Bukhari *et al.,* 2010). Once inside the host, spores obstruct feeding structures, colonize internal tissues and release toxins, causing damage to larval and mosquito guts as depicted in Fig 3 (Hegedus and Khachatourians, 1995).

Studies have confirmed that mechanical obstruction of tracheal trunks and larval siphons by fungi is a primary factor contributing to larval mortality (Daniel *et al.,* 2017; Amobonye *et al.,* 2020). The interplay between insects and their associated microbial communities, both on the cuticle and within the gut, significantly impacts the efficacy of entomopathogenic fungi. Mosquitoes harbouring gut microbiota exhibit accelerated mortality rates when exposed to the studied fungi compared to their microbiota-depleted counterparts (Liu *et al.,* 2023). Notably, the presence of *Wolbachia*, an endosymbiotic bacterium commonly found in *Culex* mosquitoes, does not provide a protective advantage against fungal infections (Ramirez *et al.,* 2021). The conclusions drawn from these studies align closely with our own research results.

Larval immune systems face a multifaceted challenge in combating EPF infections, potentially reducing the likelihood of developing resistance to these biological control agents (Mulla *et al.,* 2003). While studies indicate increased antibacterial defences during metamorphosis, antifungal immunity remains relatively unchanged (Kokoska *et al*., 2005; Meylaers *et al.,* 2007) which explains the mortality of adult mosquitoes (Fig 3). Studies like Shoukat *et al*., (2020) confirm that prolonged exposure to EPFs can compromise immune function and other physiological processes, as evidenced by continued mortality rates, melanisation responses in larval siphons and tearing of midguts.

Beyond their efficacy against mosquitoes, the EPFs employed in this study also exhibit potential for controlling scarab populations. Research indicates that EPF strains adapted to specific environments demonstrate enhanced effectiveness against local pests (Liu *et al.,* 2021). However, this localised adaptability requires careful evaluation of the wider pest control implications. Although using a single fungal pathogen to target multiple insect pests has benefits, it also raises the risk of non-target organism mortality, demanding careful administration methods (Ortiz-Urquiza *et al.,* 2015).

This study also revealed notable differences in the efficacy of different entomopathogenic fungi against *Culex* mosquitoes, as determined by LT_{50} values. These findings highlight the complex interplay between fungal species, mosquito hosts and environmental factors influencing disease progression and mortality rates.

As observed with strains *Aspergillus* sp 1, *Aspergillus* sp 2 and *Aspergillus* sp 3, rapid mortality rates suggest powerful insecticidal properties. As opposed to *Penicillium* sp that showed a slower killing rate, implying a potentially less virulent or less adaptable isolate.

Our results exceeded those of previous studies highlighting the efficacy of certain fungal species, such as *Beauveria bassiana,* which had LT_{50} values from 2 to 5 days against *Culex pipiens* larvae and 3.68 days against *Aedes albopictus* larvae (Kirsch *et al.,* 2022; Istabraq *et al.,* 2023; Waheeb, 2023; Renuka *et al.,* 2023). And *Metarhizium anisopliae,* that had LT_{50} values from 3.2 to 4.7 days against *Culex* genera (Choi *et al.,* 2020).

In contrast, the LT_{50} of entomopathogenic fungi against *Aedes aegypti* ranged from 6.4 to 16.3 days, with *Aspergillus tamarii* scored the highest virulence and *Trichoderma euskadiense* the lowest (Aguilar-Durán *et al.,* 2023). Another study highlighted that the LT_{50} values were approximately 2 days for *Aedes aegypti* and 2.5 days for *Culex quinquefasciatus*, underscoring the effectiveness of *Aspergillus tamarii* extracts as larvicides for these species (Baskar *et al.,* 2020).

However, the observed variations in LT_{50} values across different mosquito species emphasise the need for a targeted approach to fungal-based mosquito control, considering the specific ecological and epidemiological context. The differential susceptibility of mosquito species to entomopathogenic fungi may be attributed to a combination of factors, including variations in cuticle composition, immune response and behavioural patterns. Further research is warranted to elucidate the underlying mechanisms contributing to these observed differences.

CONCLUSION

Overall, the *Aspergillus* species have shown to be highly effective against not only *Culex* instar but also adults, especially *Aspergillus* 1 which showed the lowest LT50 value proving its rapid action compared to the others. Whereas *Penicillium* showed the slowest and less effective insecticidal activity. Future research should focus on characterising the specific bioactive compounds responsible for its insecticidal activity and investigate the environmental stability.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All experiments involving mosquitoes were conducted following institutional and national guidelines for the care and use of invertebrates in research. As mosquitoes are invertebrates, no specific ethical approval was required under current animal welfare regulations.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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