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Potential Efficiency of *Aspergillus chevalieri* Against *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) Larvae: Conidial Suspension and Ethanol Extract

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Abstract: In recent years, there has been a notable increase in research data supporting the use of fungal species from diverse genera such as *Penicillium, Aspergillus, Fusarium, Beauveria, Cordyceps, Metarhizium,* and *Purpureocillium* in biological control applications. The current study was conducted to identify *Aspergillus chevalieri* using morphological characteristics and molecular data, then to determine the potential efficiency of conidial suspension and ethanol extract against *Ephestia kuehniella* and to investigate its mycotoxin production potential and cytotoxicity. The identification was carried out using phenotypic characteristics and sequences of the internal transcribed spacer (ITS), beta-tubulin gene (benA), and RNA polymerase II second largest subunit (RPB2) loci. In developmental biology studies, it was determined that topically applied conidial suspensions and ethanol extracts at varying concentrations affected different life stages of the insect. In the conidial suspension treatments, the larval period (at 10^8 conidia/mL) and pupal period (at 10^6, 10^7, and 10^8 conidia/mL) were notably shortened compared to the control group. In ethanol extract applications, the adult emergence time was reduced at the lowest concentrations (0.5 mg/mL and 1 mg/mL). Furthermore, both conidial suspensions and ethanol extracts caused a significant decrease in the total number of eggs, depending on the concentration applied. In the cytotoxicity test, the ethanol extract of the fungus was found to be cytotoxic in the L929 mouse cell line (NCTC clone 929) at concentrations above 0.78 mg/mL. This study showed that the fungus does not produce aflatoxin and ochratoxin and provided the first information on its potential efficiency against *E. kuehniella* larvae. Based on the present findings, *A. chevalieri* can be considered a promising candidate for inclusion in biological control programs. To fully assess its potential, future studies should explore its efficacy against a broader range of pest species and conduct field trials under diverse environmental conditions to validate the laboratory results.

Keywords: Fungal identification, fungal pathogenicity, developmental biology, biological control, sustainable agriculture.

Aspergillus chevalieri'nin *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) Larvalarına Karşı Potansiyel Etkinliği: Konidial Süspansiyon ve Etanol Ekstraktı

Öz: Son yıllarda, *Penicillium*, *Aspergillus*, *Fusarium*, *Beauveria*, *Cordyceps*, *Metarhizium* ve *Purpureocillium* gibi çeşitli cinslere ait mantar türlerinin biyolojik mücadelede kullanılmasına yönelik araştırma verilerinde dikkate değer bir artış yaşanmıştır. Bu çalışma, *Aspergillus chevalieri*'nin morfolojik özellikleri ve moleküler veriler kullanılarak tanımlanması, ardından konidiyum süspansiyonu ve etanol ekstraktının *Ephestia kuehniella*'ya karşı potansiyel etkinliğinin belirlenmesi ve ayrıca mikotoksin üretim potansiyeli ile sitotoksisitesinin araştırılması amacıyla gerçekleştirilmiştir. Tanımlamada; fenotipik özelliklere ek olarak iç transkribe edilen aralık (ITS), beta-tübülin (benA) ve RNA polimeraz II'nin ikinci büyük alt birimi (RPB2) gen bölgelerinin dizileri kullanılmıştır. Gelişim biyolojisi çalışmalarında, topikal olarak uygulanan farklı dozlardaki konidiyal süspansiyonların ve etanol ekstraktlarının böceğin farklı gelişim evrelerinde etkili olduğu belirlendi. Konidiyal süspansiyon uygulamalarında özellikle kontrole göre larval periyodun (10^8 konidia/mL) ve pupal periyodun (10^6, 10^7, 10^8 konidia/mL) kısaldığı görülürken, etanol ekstresi uygulamalarında ise en düşük dozlarda ergin çıkış süresinin (0.5 mg/mL ve 1 mg/mL) kısaldığı görülmüştür. Ayrıca, hem konidial süspansiyonların hem de etanol ekstraktının konsantrasyonuna bağlı olarak kontrole göre yumurta sayısında önemli azalmalar görülmüştür. Sitotoksisite testinde ise fungusun etanol ekstraktı, 0.78 mg/mL üzerindeki konsantrasyonlarda L929 fare hücre hattı (NCTC clone 929) üzerinde sitotoksik etki göstermiştir. Çalışma ayrıca, bu mantarın aflatoksin ve okratoksin üretmediğini ortaya koymuş ve *E. kuehniella* larvalarına karşı potansiyel etkinliği konusunda ilk bilgileri sunmuştur. Elde edilen bulgulara dayanarak, *A. chevalieri* biyolojik mücadele programlarına dahil edilebilecek umut verici bir aday olarak değerlendirilebilir. Bu potansiyelin tam olarak anlaşılabilmesi için, gelecekte yapılacak araştırmalarda *A. chevalieri'nin* farklı zararlı türleri üzerindeki etkisinin değerlendirilmesi ve çeşitli çevresel koşullarda saha denemelerinin gerçekleştirilmesi gerekmektedir.

Anahtar kelimeler: Fungal tanılama, fungal patojenite, gelişim biyolojisi, biyolojik kontrol, sürdürülebilir tarım.

1. Introduction

In addition to abiotic factors, biotic factors such as oomycetes, fungi, viruses, bacteria, nematodes, and viroids also play a significant role in product losses within ecological systems (Shahrajabian et al., 2023). Research indicates that fungal infections account for 10–23% of crop losses, 20–40% of bacterial contamination, and approximately 18% of the damage caused by herbivorous insects (Spescha et al., 2023). While chemical-based

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protection methods have traditionally been used, they are increasingly recognized as unsustainable and harmful to the environment (Şahin Taylan & Er, 2024). Biological control strategies, particularly those involving entomopathogenic fungi (EPFs), have emerged as promising eco-friendly solutions (Sani et al., 2020; Güner et al., 2025). EPFs are microorganisms capable of infecting and killing insects and other arthropods with over 60% of natural insect infections attributed to these fungi (Deka et al., 2021; Güner et al., 2023). Important EPFs include *Metarhizium anisopliae*, *Beauveria bassiana*, *Nomuraea rileyi*, *Cordyceps fumosorosea*, *Penicillium citrinum*, *Lecanicillium* spp., and *Aspergillus* spp. (Marcinkevicius et al., 2017; Diao et al., 2022; Liu et al., 2023; Nguyen et al., 2023; Song et al., 2023). Numerous studies have examined the pathogenicity of *Aspergillus* species using a variety of insect models, revealing their considerable potential as biological control agents. (Zhang et al., 2015; Lin et al., 2021).

Aspergillus chevalieri (L. Mangin) Thom and Church (Eurotiales: Aspergillaceae), previously referred to as *Eurotium chevalieri* (L. Mangin, 1909) (Eurotiales: Aspergillaceae). *A. chevaliari* was isolated from the sea (Ningsih et al., 2023), fermented foods (Wang et al., 2022), and soil (de Oliveira et al., 2022). Apart from this, Zhang et al. (2024a) isolated it for the first time from the guts of termites. Insect-associated microorganisms have been identified as resources to discover metabolites with important biological properties and to improve their potential use (Chevrette et al., 2019; Zhang et al., 2015). As demonstrated in the research by Zin et al. (2017) and Bovio et al. (2019), the metabolites of *A. chevalieri* have been shown to inhibit the growth of bacterial and fungal plant pathogens in biological control. In addition to this, these compounds have been found to possess antioxidant, antimicrobial, anti-malarial, anti-inflammatory, anti-tumor, and insecticidal properties (Zin et al., 2017; Lin et al., 2021). The biological control effects of certain *Aspergillus* species, such as *Aspergillus flavus*, *Aspergillus nomius, Aspergillus fijiensis,* and *Aspergillus oryzae* on insect pests have been reported and these species can be used to control *Locusta migratoria*, *Spodoptera litura, Diaphorina citri*, and *Dolichoderus thoracicus* (Zhang et al., 2015; Karthi et al., 2018; Lin et al., 2021; Yan et al., 2022). Khan & Khan (2023) demonstrated the efficacy of *Aspergillus nidulans* and *Aspergillus fumigatus* against *Rhyzopertha dominica*, *Sitophilus zeamais,* and *Trogoderma granarium*. Zhang et al. (2024b) were the first to report that an *Aspergillus nomiae* isolate exhibits entomopathogenic activity against insect pests from the orders Lepidoptera and Hemiptera. Notably, this isolate functions as a dual biocontrol agent by simultaneously exerting direct insecticidal effects and enhancing plant resistance to phytopathogens through endophytic colonization. In addition, Mensah & Young (2017) found that oil-based extracts from *Aspergillus* species are toxic to adult *Bemisia tabaci*. In their study, Kaur et al. (2016) demonstrated that the EtOAc extract of *Aspergillus niger* negatively impacts survival and developmental processes of *S. litura*. Furthermore, the authors showed that metabolites derived from *A. niger* exert antifeedant and toxic effects.

Ephestia kuehniella Zeller, 1879 (Lepidoptera: Pyralidae), also known as the Mediterranean flour moth, is a cosmopolitan pest that affects stored products in both industrial and domestic environments, representing a significant economic burden (Jacob & Cox 1977). Although several *Aspergillus* species are known for their effectiveness against insect pests, the potential of *A. chevalieri,* especially against storage pests, has not been adequately explored.

This study hypothesizes that both the conidial suspensions and ethanol extracts of *A. chevalieri* exhibit potential effects against *E. kuehniella*. The primary objective is to evaluate the bioefficacy of different concentrations of these formulations under laboratory conditions. Additionally, the study aims to identify the fungal strain through morphological and molecular characterization (ITS, benA, and RPB2 regions) and assess its mycotoxin production and cytotoxic potential to evaluate its safety for biocontrol applications.

2. Materials & Methods

2.1. Rearing of *E. kuehniella*

The maintenance of stock cultures of *E. kuehniella* was conducted at a temperature of 26±2°C and a relative humidity of 60±5% in conjunction with a photoperiod of 12:12 h (L:D) at the Animal Physiology Research Laboratory at Balıkesir University in Türkiye. The larvae were cultivated on a standardized artificial diet, prepared in accordance with the methodology previously outlined by Shakarami et al. (2015). The diet consisted of 400 grams of wheat flour, 200 grams of corn flour, 200 grams of barley flour, and 200 grams of fine bran, with each 1 kg of diet containing an equal proportion of these ingredients.

2.2. Description of Fungi

2.2.1. Fungal isolation and culture

The fungus examined in this study was initially isolated in 2015 from soil collected in Balıkesir, Türkiye, employing the standard soil dilution method. The obtained isolate is currently maintained in the Fungal Culture Collection of the Microbiology Research Laboratory at Balıkesir University. For fungal isolation, soil profiles were first excavated with surface layers carefully removed. Using a sterilized spatula, vertical samples were collected from a depth of 10 cm with the tool inserted perpendicularly to the profile face. The collected samples were stored in large, sterile, and cooled thermos flasks to preserve their integrity during their transport to the laboratory. The moisture content of the soil was determined and the amount of fresh soil per 25 g of oven-dried soil was calculated. Subsequently, serial dilutions of 1:10,000 were prepared following the protocol by Warcup (1955). One milliliter of each dilution was transferred into sterile 9 cm Petri dishes. Rose Bengal Chloramphenicol Agar (RBCA, Merck 1.00467), pre-cooled to approximately 45°C, was poured into the dishes. After ensuring even distribution and complete solidification, the plates were incubated at 28°C for 5–7 days. Distinct fungal colonies that developed were subcultured onto Malt Extract Agar (MEA, Oxoid, LP0039B) to obtain pure isolates, which were subsequently stored at -20°C.

2.2.2. Morphological characterization

The fungal strain (F3-2) used in this study was selected from the Fungal Culture Collection. For identification purposes, four different culture media were utilized: Czapek-Dox-Agar (CDA, Oxoid, CM0097), Sabouraud

Dextrose Agar (SDA, Oxoid, CM0041), Potato Dextrose Agar (PDA, Oxoid, CM0139B), and MEA. Petri dishes were employed to observe the fungi's microscopic and macroscopic characteristics and to obtain conidial suspensions (Klich, 2002). The media were inoculated using the three-point method and incubated at 28°C for two weeks. After incubation, macroscopic observations focused on key morphological features, including colony diameter, upper and lower surface color, surface texture, and odor (exudate zonation). Microscopic analysis involved examining spores, hyphae, conidiaophores, conidia, and cleistothecia, using preparations made with the Scotch tape method and the lamellar culture technique (Fujita, 2013). Lactophenol Cotton Blue (Sigma, Aldrich) solution was used for slide preparations during the Scotch tape method. The morphological features were examined using a binocular research microscope (OPTIKA 1000PH; Olympus CX23).

2.2.3. Molecular analysis

In this study, the extraction of genomic DNA from mycelium that had been cultivated on PDA at 28°C for a period of seven days was conducted. The DNeasy Plant Mini Kit (Qiagen) was utilized in this process. PCR amplification of the target genes was conducted using primers that were specific to ITS, benA, and RPB2 (Table 1). The agarose gel electrophoresis results were visualized using a Quantum ST5 gel documentation system. The high-quality PCR products were sequenced commercially by Macrogen (Seoul, Korea) using both forward and reverse primers. The DNA sequences were analyzed using the BioEdit biological sequence alignment editor (Hall, 1999) in order to obtain contigs from bidirectional sequencing. The sequences from the three regions were aligned using MAFFT v7, which is available at https://mafft.cbrc.jp/alignment/server/. Each aligned Fasta file was subjected to a Gblocks 0.91b (Talavera & Castresana, 2007) alignment process, whereby less stringent selection criteria were applied to smaller end blocks, gap positions within end blocks, and less stringent side positions. The trimmed Fasta files were then concatenated in the following manner: beta_tub (1-362), ITS (363-865), and RPB2 (866-1173). A maximum likelihood tree was constructed using the IQ-TREE software (Nguyen et al., 2015) with the evolutionary model for each region determined automatically by the same program. The models employed were as follows: K2P+G4 for beta-tubulin, K2P for ITS, and TN+G4 for RPB2. Branch support values were calculated with 1000 ultrafast bootstrap replicates. The resulting tree was visualized using iTOL v6 (Letunic & Bork, 2024).

Table 1. Primers used for the amplification.

Locus	Primer name	Primer sequence (5′–3′)	Length (bp)	References
ITS	ITS5m	TCC GTA GGT GAA CCT GCG G	~700	(White et al., 1990)
	ITS4	TCC TCC GCT TAT TGA TAT GC		
BenA	Bt2a	GGT AAC CAA ATC GGT GCT GCT TTC	~500	(Glass & Donaldson, 1995)
	Bt2b	ACC CTC AGT GTA GTG ACC CTT GGC		
RPB2	5F	GAY GAY MGW GAT CAY TTY GG	~700	(Liu et al., 1999)
	7CR	CCC ATR GCT TGY TTR CCC AT		

2.3. Preparation Conidial Suspensions

The fungus was cultured in the PDA for 14 days at 28°C in darkness to prepare the conidial suspension. Subsequent to the conclusion of the incubation period and colony development, 10 mL of sterile water containing 0.01% Tween 20 was added to the Petri dishes and the spores were removed using a glass spatula. The resulting suspension was then filtered through a 4-layered gauze in order to remove mycelium and agar pieces. The final solution was transferred into sterile tubes. The suspension was counted using a Thoma slide to obtain 10^9 conidia/mL and appropriate dilutions were prepared to achieve the desired working concentrations. (Fancelli et al., 2013; Güner et al., 2025).

2.4. Preparation of Ethanol Extract

The ethanol extract was prepared using Petri dishes containing PDA. Fungal cultures were incubated at 28 °C for 14 days to promote metabolite production. Following the incubation period and colony development, 2 mL of Tween 80 was added to each plate to facilitate spore removal. The agar medium, once cleared of spores, was cut into small fragments. Subsequently, ethanol (1:2 w/v) was added to 100 g of these agar pieces in an Erlenmeyer flask and the mixture was shaken at 160 rpm for 72 hours at 26°C using a shaker (ZHWY-211D) to extract the metabolites into the ethanol. The resulting solution was then filtered sequentially through 0.45 µm and 0.22 µm Minisart filters (Supelco). The solvent was evaporated using a rotary evaporator (IKA RV 10 basic) to obtain a concentrated extract. Finally, lyophilization was performed using a freeze-dryer (CHRIST ALPHA 1–2 LD) (Güner et al., 2025). After lyophilization, 0.5 g of the dried ethanol extract was weighed and solved in 10 mL of phosphate-buffered saline (PBS, Sigma-Aldrich P4417) to prepare a stock solution at 50 mg/mL. From this stock, working solutions were prepared in the concentration range of 0.5 to 10 mg/mL. All extract solutions were stored in a deep freeze at − 20°C.

2.5. Pathogenicity of *A. chevalieri*

The pathogenicity of the *A. chevalieri* was determined in accordance with Koch's postulates. *A. chevalieri* conidial suspension (10^7 conidia/mL/ 2 µL) was applied topically to the dorsal thorax of each third instar larvae (25±5 mg). Throughout the experiment, all groups were maintained under controlled laboratory conditions with a temperature of 26±2°C and relative humidity of 60±5%. In the pathogenicity tests, 10 out of the 15 larvae used died. A total of ten *A. chevalieri*-treated *E. kuehniella* cadavers were obtained. Prior to the appearance of the fungal development in the deceased larvae, a modified trap, known as the White Trap, was prepared. The trap was

created by placing a small Petri dish (60 mm) inside a larger Petri dish (90 mm) with a 5 cm diameter filter paper placed on top. Sterile distilled water (15 mL) was added to the larger Petri dishes, after which the dead larvae were placed on the filter paper and maintained under the conditions determined throughout the experiment for a period of 5–10 days (Orozco et al., 2014). The presence of sporulated dead larvae was documented through photographic evidence captured using a stereo microscope (Olympus SZ51, Japan). The microorganisms isolated from these larvae were cultured on MEA plates to examine micro and macromorphological characteristics, followed by DNA extraction (Gradmann, 2008).

2.6. Developmental Biology Studies

Three independent replicates were conducted on separate dates, each involving the topical application of 2 µL of conidial suspensions at concentrations of 10^5, 10^6, 10^7, and 10^8 conidia/mL in PBS to groups of five healthy third-instar *E. kuehniella* larvae. Similarly, five third-instar larvae were treated topically with 2 µL of ethanol extract at concentrations of 10, 5, 2, 1, and 0.5 mg/mL (dissolved in PBS), in groups of five. The control group consisted of three replicates, each containing five larvae, and received 2 µL of PBS. All experimental groups were maintained under controlled conditions at 26±2 °C, 65±5% relative humidity and a 12:12 h (L:D) photoperiod. The effects of the conidial suspensions and ethanol extract at different concentrations were evaluated based on the developmental parameters of *E. kuehniella*, including larval and pupal periods, adult emergence time, adult longevity, weight, and total number of eggs.

2.7. Cytotoxicity Test and Mycotoxin Analyses

Cytotoxicity tests and mycotoxin analyses performed as a paid external service by the Scientific and Technological Research Council of Turkey Marmara Research Center (TUBITAK-MRC). The cytotoxicity assessment followed the OECD Guidance Document No. 129 on Using Cytotoxicity Tests to Estimate Starting Doses for Acute Oral Systemic Toxicity. The test protocol was validated both by the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the European Centre for the Validation of Alternative Methods (ECVAM). The L929 mouse fibroblast cell line (NCTC clone 929), recommended by ISO 10993-5:2009, was used to represent the mammalian system. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Sigma Cat# D0547, lot# SLBH5487), supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco #10,500-064), 1% antibiotic–antimycotic solution (Gibco #15,240-062), 1% GlutaMAX™ (Gibco #35,050,061), and 1% sodium pyruvate (Gibco #11,360,070). A stock solution was prepared by dissolving 200 mg of the extract in 1 mL of DMEM. Serial dilutions were made in a 1:2 ratio, ranging from 200 mg/mL to 0.2 mg/mL. L929 cells were seeded at a density of 1.2×10^4 cells/well in 96-well plates and incubated at 37 °C with 5% CO_2 for 24 hours. After incubation, various concentrations of the ethanol extract were added and incubated under the same conditions for an additional 24 hours. Subsequently, 5 mg/mL of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma #5655) was added to each well and incubated for 4 hours. Then, 100 µL of DMSO was added to each well to dissolve the resulting formazan crystals and the plate was placed on a shaker for 2 hours. Cell viability was determined by measuring absorbance at 570 nm with a reference wavelength of 650 nm using a microplate reader.

In mycotoxin analyses, Aflatoxin B1 was analyzed using the AOAC 999.07 method, aflatoxin B2 using the AOAC 999.31 method, and aflatoxin G1 and G2 using the D.05.G116 method. Furthermore, the R Hone-Diagnostics OTA method was used to analyse ochratoxin A (OTA). The standards were identified through an in-house protocol employing high-performance liquid chromatography coupled with fluorescence detection (HPLC-FLD).

2.8. Statistical Analysis

In statistical analysis, Levene's test (for normality of data distribution) was used to test the mortality values derived from the conidial suspensions and ethanol extract treatments and one-way analyses of variance (ANOVA) were used to assess significance differences. Differences were separated by Tukey's honestly significant post hoc tests (HSD). When using SPSS software (SPSS 18.0 for Windows) for data analysis, differences were found to be statistically significant when $P < 0.05$ in all tests.

3. Results

3.1. Morphological Identification

Colonies in CDA incubated at 28°C for one week were 3.5-4 cm in diameter, wrinkled at the edges, in shades of gray green, with a lighter zone in the torn part, and a small coremium in the center. The texture is velvety, with no exudate, and a light-yellow soluble pigment is present (Fig. 1a). The reverse of the colony is initially orange, later turning dark brown (Fig. 1e). Colonies in MEA incubated at 28°C for one week were light gray-green and velvety. Radial grooves and elevations that increase towards the edges are present on the colony surface. There is beige-light yellow basal mycelium at the edges and the colony edges are wrinkled (Fig. 1b). The reverse of the colony is dark orange brown (Fig. 1f). Colonies in PDA incubated at 28°C for one week were 2.5-3.5 cm in diameter with irregular cream-colored basal mycelium at the edges. Towards the centre, it is irregularly gray. No exudate is present. An orange-black pigment diffuses into the agar along the colony perimeter (Fig. 1c). The reverse side of the colony appeared dark orange to black (Fig. 1g). Colonies in SDA incubated at 28°C for one week were 3.5-4 cm in diameter, zones, with a thin light greenish basal mycelium at the edges. The centre is dark, with the middle part being light green. An orange pigment diffuses into the agar. No exudate is present (Fig. 1d). The reverse of the colony is similar to that of PDA (Fig. 1h).

Conidial heads were 30-150 µm in diameter, smooth-walled, globose or pyriform. Conidiaophores were smooth-walled, uncoloured, septate, 60-900 x 4-7-(12) µm (Fig. 1j & Fig. 1m). Vesicles were globose or pyriform (7.5) 25-30 µm in diameter, with some elongated. Phialides were uniseriate, (5) 6-8 x 3-4 µm, swollen in the middle, phialides cover three-quarters of the vesicle surface (Fig. 1k). Conidia subglobose or ovoid with some being elliptical, (2.5)-4.5-5 (6) µm (Fig. 1n). Ascoma (cleistothecium) is 50-100 (150) µm in diameter. The asci were globose or subgloboz 11-14 µm. Ascospores were

globose or lens-shaped, smooth-walled, and 4.5-5 x 3.4-3.8 µm (Fig. 1k & Fig. 1n).

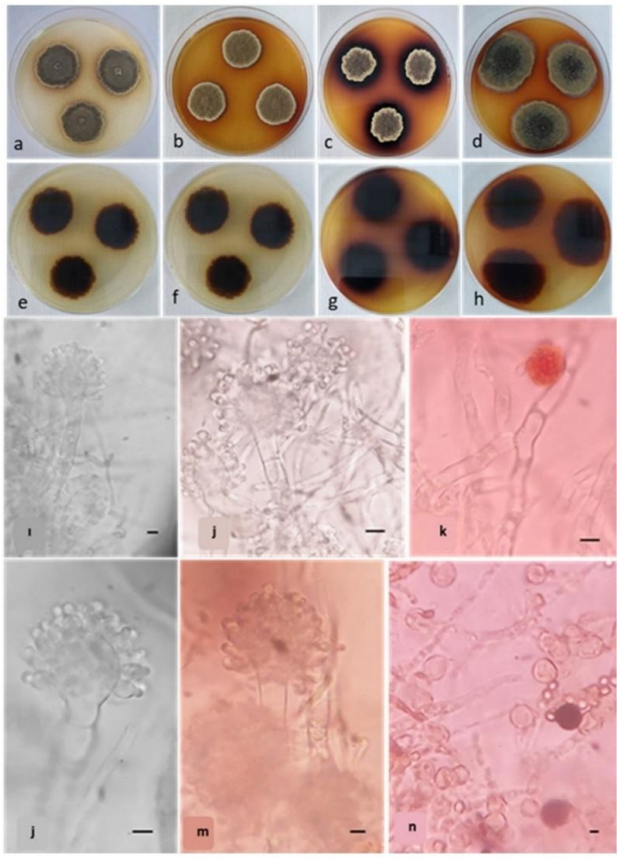

Figure 1. Overview of *A. chevalieri* on various agar media. a) CDA; b) MEA; c) PDA; d) SDA, Bottom view of colonies: e) CDA; f) MEA; g) PDA h) SDA. ı-j and m-n) Conidial heads, vesicle and phialide. Reproductive structures: k-n) Ascoma, ascus, and ascospores Bars: ı-j: 10 µm; k: 50 µm; m-n: 5 µm.

3.2. Moleculer Identification

The obtained DNA sequences were analyzed using the BioEdit biological sequence alignment editor to obtain contigs from bidirectional sequencing, resulting in a consensus sequence for each region. BLAST analysis of the sequences in the NCBI database revealed the following similarities: the ITS region showed 98.19% similarity with *A. chevalieri* (accession number NR_135340.1), the beta-tubulin benA gene showed 100% similarity with *A. chevalieri* (accession number OR241570.1), and the RPB2 gene showed 100% similarity with *A. chevalieri* (accession number LC494262.1). After alignment with MAFFT and trimming with Gblocks, the final base counts for each region were: 503 bases for the ITS region, 362 bases for the beta-tubulin region, and 927 bases for the RPB2 region. The concatenated dataset was 1793 bases long. A Maximum Likelihood (ML) tree was constructed to demonstrate the position of the obtained DNA sequence among the sequences of closely related taxa. Based on the ML tree analysis, the F3-2 strain was conclusively identified as *A. chevalieri* and the sequence was deposited in the NCBI GenBank with the accession numbers PQ014273, PV700104, PQ111849 for ITS, RPB2 and beta-tubulin loci respectively (Fig. 2). The phylogenetic analysis using the Maximum Likelihood method revealed a well-supported tree with the F3-2 strain clustering closely with *A. chevalieri* sequences. The tree was rooted with *Eurotium medium* as the outgroup, providing a clear distinction between the ingroup and outgroup taxa. The F3-2 strain shared a branch with high similarity to *A. chevalieri*, confirming its identification. Other species included in the analysis, such as *Aspergillus cristatus*, *Aspergillus costiformis*, *Aspergillus amstelodami*, *Aspergillus brunneus*, *Aspergillus neocarnoyi*, *Aspergillus cibarius*, *Aspergillus montevidensis*, *Aspergillus intermedius*, *Aspergillus niveoglaucus*, *Aspergillus ruber*, *Aspergillus glaucus*, *Aspergillus aurantiacoflavus*, and *Aspergillus endophyticus* formed distinct clades further validating the robustness of the phylogenetic reconstruction (Table 2). The final analysis demonstrated the precise placement of the F3-2 strain within the *Aspergillus* genus, specifically aligning it with *A. chevalieri*, supported by significant bootstrap values (Fig. 2). This alignment corroborates the molecular identification results obtained through BLAST analysis, solidifying the taxonomic classification of the F3-2 strain.

Table 2. List of sequences used for phylogenetic tree construction.

Taxon	ITS	RPB2	Beta-tubulin
	Accession No.	Accession No.	Accession No.
A. chevalieri F3-2	PQ014273	PV700104	PQ111849
A. chevalieri	MT316337.1	LC494262.1	OR241570.1
A. cristatus	MN968351.1	KF923734.1	KF923739.1
A. costiformis	OL711815.1	LT671054.1	FR837970.1
A. amstelodami	MK503961.1	KX696417.1	KX421532.1
A. proliferans	MG650614.1	KX696407.1	OQ446689.1
A. brunneus	PP702148.1	LT671007.1	MW729303.1
A.neocarnoyi	LT670955.1	LT671111.1	OQ446669.1
A. cibarius	MT028084.1	LT671048.1	MZ826405.1
A.montevidensis	MT487826.1	LC494264.1	MZ826409.1
A. intermedius	MH855971.1	LT671084.1	EF651892.1
A. niveoglaucus	MH806377.1	LT671126.1	OR935008.1
A. ruber	KY629639.1	OK500095.1	OR394833.1
A. glaucus	KM232504.1	OR231525.1	LT671070.1
A. aurantiacoflavus	OP479992.1	LT670995.1	LT670993.1
A. endophyticus	OL711847.1	LT671069.1	LT671067.1
E. medium	EF652056.1	EF651938.1	EF651904.1

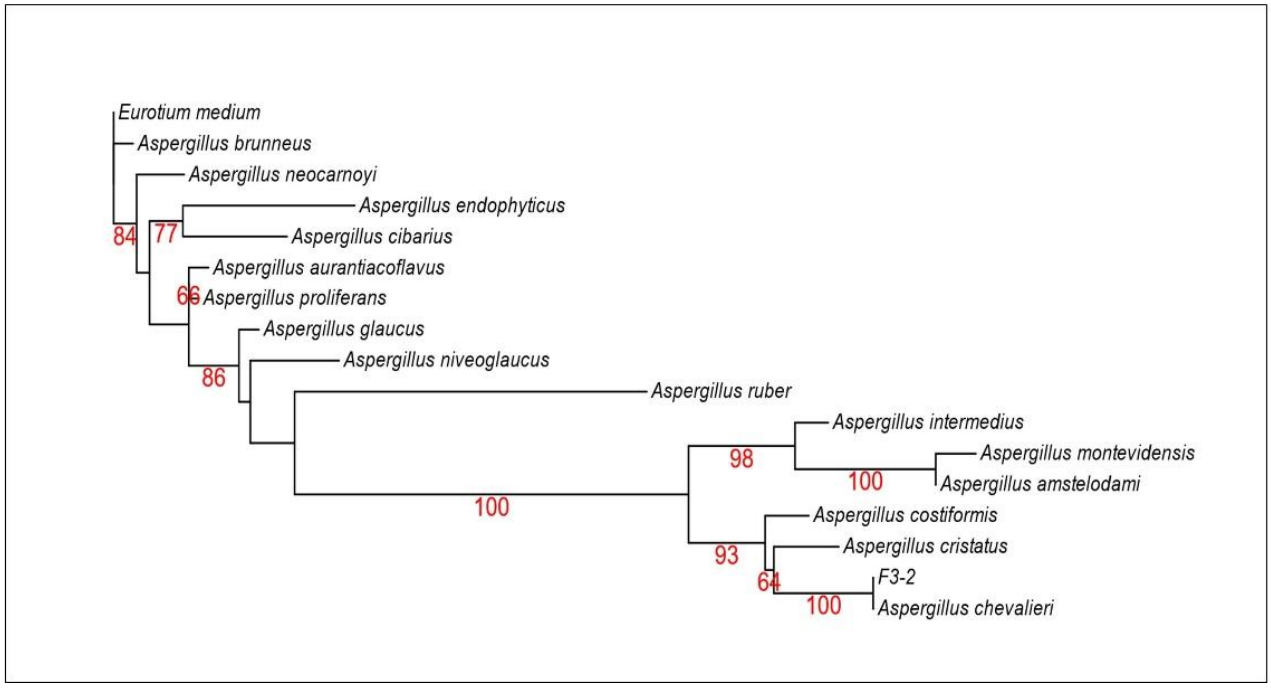

Figure 2. A Maximum Likelihood (ML) phylogenetic tree was constructed using combined data of beta-tubiline, ITS and RPB2 sequences from the included species. Sequences of other fungi were obtained from the NCBI database and their accession numbers were shown in Table 2. The reliability of branches was tested by performing 1000 bootstrap replicates and those with values exceeding 60% were depicted at the nodes. *E. medium* EF652056.1 was employed as the outgroup in the analysis.

3.3. Pathogenicity of *A. chevalieri* aganist *E. kuehniella*

At the end of the incubation period, the fungus developed and sporulated on the head, thorax, and abdomen of the larval cadavers, with its color changing from white to yellow or green (Fig. 3). The sporulated cadavers were then used to re-inoculate healthy larvae, leading to fungal colonization; the mycelium enveloped the host and ultimately caused its death. The identity of the reisolated fungal species was confirmed to match the original strain through both classical and molecular identification methods.

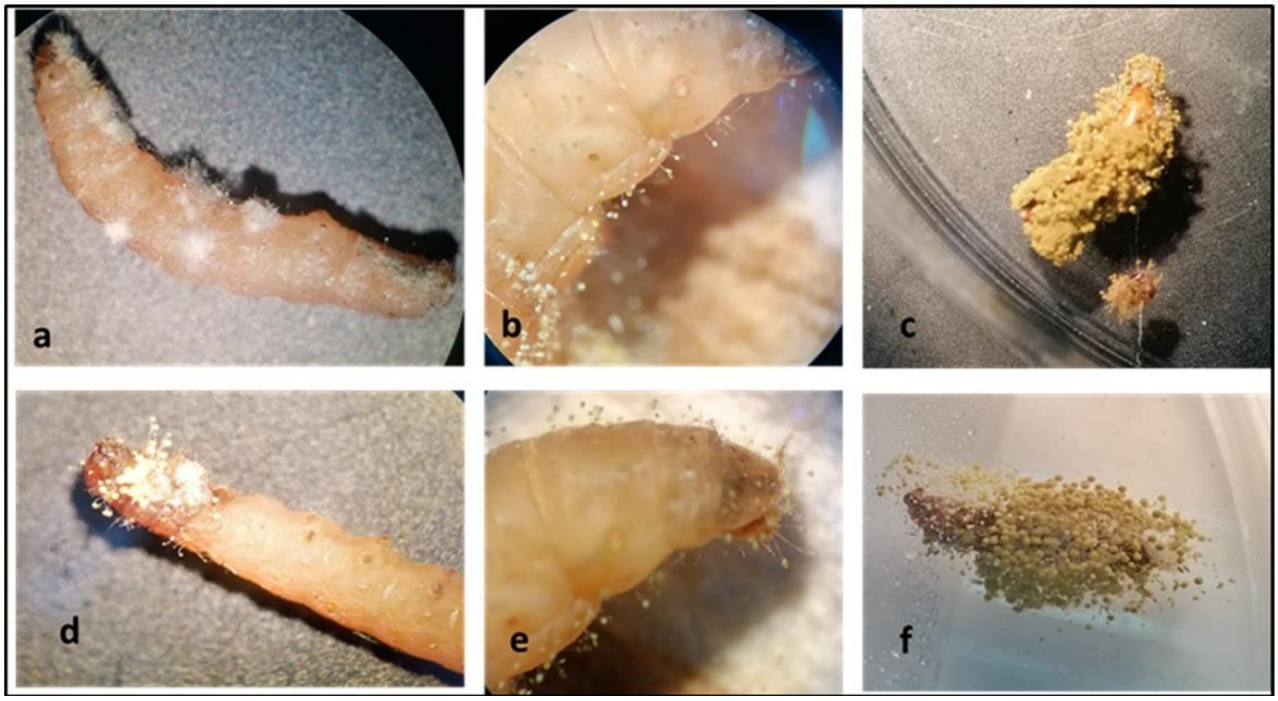

Figure 3. Pathogenicity of *A. chevalieri* aganist *E. kuehniella*. a, b, d and e: The hyphal growth and sporulation of the fungus and changes in fungal colouration in different larvae on day 5 after death. c and f: Dead larvae for 10 days after death showing the sporulation of fungus.

3.4. Developmental Biology of *E. kuehniella*

The results indicated that varying concentrations of conidial suspensions and ethanol extract doses elicited differential responses in *E. kuehniella*. The changes in larval and pupal periods between the experimental and control groups were presented in Table 3. In the conidial

suspension applications, only the highest concentration (10^8 conidia/mL) resulted in a statistically significant reduction compared to the control group [$F(4,70) = 6, 213$, $p < .001$]. In contrast, during the pupal stage, all doses, except the lowest one, caused a statistically significant decrease relative to the control [$F(4,70) = 17, 158$, $p < .001$]. When the effects of different concentrations of ethanol extract on the larval period were examined, no statistically significant difference was observed between the control and the other doses, [$F(5, 84) = 0.424$, $p = .831$]. In the pupal period, although increases and decreases were observed between the control and experimental groups, a statistically significant difference was found between the doses compared to the control. A significant increase in pupal period was observed only at 10 mg/mL compared to 0.5 mg/mL [$F(5, 84) = 2.675$, $p = .027$].

Table 3. Changes in larval and pupal period of *E. kuehniella.*

	Dose	Larval stage(day)		Pupal stage (day)	
		Min.-Max.	$\bar{X}$ ±SH*	Min.-Max.	$\bar{X}$ ±SH*
Conidial Suspansion	Control	4-10	7.53±0.49a	8-17	11.86±0.51a
	10^5 conidia/mL	4-10	7.13±0.54a	10-17	13.00±0.56a
	10^6 conidia/mL	5-17	8.73±1.08a	8-12	9.53±0.32b
	10^7 conidia/mL	2-12	6.73±0.77a	4-12	8.06±0.52b
	10^8 conidia/mL	2-9	3.80±0.61b	6-12	9.20±0.49b
Ethanol Extract	Control	2-11	4.60±0.66a	5-10	7.93±0.37ab
	0.5 mg/mL	2-6	4.13±0.29a	5-12	7.27±0.48a
	1 mg/mL	2-11	4.73±0.56a	4-12	7.80±0.62ab
	2 mg/mL	3-8	3.80±0.41a	7-10	8.53±0.26ab
	5 mg/mL	3-8	4.40±0.55a	7-10	8.60±0.32ab
	10 mg/mL	2-10	4.60±0.68a	7-11	9.13±0.29b

*The means in each column followed by the same letter are not significantly different (P>.05; Tukey's HSD test)

The changes in adult emergence time and longevity between the experimental and control groups were evaluated based on the conidial suspension doses applied to *E. kuehniella* (Table 4). No statistically significant differences were observed between any treatment dose and the control in terms of adult emergence time and longevity. However, a significant reduction in adult emergence time was found between 10^7 and 10^5 conidia/mL treatments [$F(4, 70) = 3.696$, $p = .009$]. Similarly, adult longevity significantly decreased at higher doses compared to 10^5 conidia/mL [$F(4, 70) = 3.686$, $p = .009$]. In the evaluation of the effect of different concentrations of ethanol extract on adult emergence time in *E. kuehniella*, a significant decrease was observed at the lowest doses (0.5 mg/mL and 1 mg/mL) compared to the control [$F(5, 84) = 3.789$, $p = .004$]. However, no significant difference was observed among the doses in longevity compared to the control [$F(5, 84) = 1.570$, $p = .178$].

Table 4. Changes in adult emergence time and longevity of *E. kuehniella.*

	Dose	Adult emergence time(day)		Adult longevity (day)	
		Min.-Max.	$\bar{X}$ ±SH*	Min.-Max.	$\bar{X}$ ±SH*
Conidial Suspansion	Control	17-26	21.46±0.65ab	5-17	10.73±0.92ab
	10^5 conidia/mL	17-28	21.66±0.83b	2-16	10.86±0.93b
	10^6 conidia/mL	16-31	20.80±1.18ab	2-12	7.86±0.84a
	10^7 conidia/mL	11-26	18.00±0.92a	1-14	7.06±1.08a
	10^8 conidia/mL	13-27	18.26±0.92ab	2-17	7.20±1.12a
Ethanol Extract	Control	10-16	12.20±0.47a	7-14	10.33±0.55a
	0.5 mg/mL	7-18	10.27±0.69b	5-14	9.07±0.85a
	1 mg/mL	7-14	9.87±0.59b	2-17	8.20±1.01a
	2 mg/mL	10-12	11.27±0.23ab	9-12	10.33±0.37a
	5 mg/mL	10-14	11.53±0.29ab	7-14	9.87±0.56a
	10 mg/mL	10-14	11.67±0.27ab	5-14	8.87±0.62a

*The means in each column followed by the same letter are not significantly different (P>.05; Tukey's HSD test)

The changes in adult body weight and total number of eggs between the experimental and control groups are presented in Table 5. No significant difference in weight was observed across the conidial suspension-treated groups compared to the control ($F(4, 70) = 1.003$, $p = .412$). The total number of eggs exhibited a significant reduction in all treatment groups except the lowest dose (10^5 conidia/mL) when compared to the control ($F(4, 70) = 8.692$, $p < .001$). In *E. kuehniella*, no significant difference in adult weight was observed across ethanol extract

application doses compared to the control. However, a statistically significant increase was detected at 2 and 5 mg/mL compared to 0.5 and 1 mg/mL ($F(5, 84) = 5.578$, $p<.001$). Ethanol extract applications also resulted in a statistically significant reduction in the total number of eggs across all doses compared to the control ($F(5, 56) = 15.444$, $p<.001$).

Table 5. Changes in weight and total number of eggs of *E. kuehniella*.

	Dose	Weight(mg)		Total number of eggs	
		Min.-Mak.	$\bar{X}$ ±SH*	Min.-Max.	$\bar{X}$ ±SH*
Conidial Suspansion	Control	5.80-15.90	11.79±0.74a	0-166	91.46±17.33a
	10^5 conidia/mL	4.50-20.00	11.15±0.97a	0-163	80.73±18.00a
	10^6 conidia/mL	8.60-76.00	14.63±4.39a	0-111	29.26±10.09b
	10^7 conidia/mL	6.80-10.80	9.80±0.28a	0-62	20.00±6.38b
	10^8 conidia/mL	4.40-10.90	9.47±0.44a	0-41	11.26±3.76b
Ethanol Extract	Control	8.1-18.5	11.95±0.82ab	93-252	158.10±17.26a
	0.5 mg/mL	8.1-12.6	10.47±0.40a	18-128	82.78±12.38b
	1 mg/mL	8.7-12.8	10.37±0.30a	11-182	69.38±21.03bc
	2 mg/mL	10.6-17.7	13.53±0.52b	7-102	56.40±8.74bc
	5 mg/mL	9.2-18.7	13.43±0.72b	3-81	39.08±7.73bc
	10 mg/mL	6.1-15.3	11.06±0.67ab	1-75	24.42±7.22c

*The means in each column followed by the same letter are not significantly different (P>.05; Tukey's HSD test)

3.5. Cytotoxicity Test and Mycotoxin Analyses

As a result of the analyses carried out by TUBITAK MRC in cytotoxicity tests on L929 mouse cells, it was established that the ethanol extract of *A. chevalieri*, when applied to cell lines for a period of 24 hours, demonstrated a cytotoxic effect at concentrations exceeding 0.78 mg/mL. The IC_{50} value was determined to be 40.87 mg/mL (Fig. 4). The results of the mycotoxin analysis demonstrated that *A. chevalieri* did not produce aflatoxin or ochratoxin (Table 6).

Table 6. Analysis of Mycotoxins by HPLC.

Matrix	Mycotoxin	Result	Method
Ethanol extract	Aflatoxin (G1-G2)	Not detected (µg/kg)	D.05.G116
	Aflatoxin B1	Not detected (µg/kg)	AOAC 999.07
	Aflatoxin B2	Not detected (µg/kg)	AOAC 999.31
	Ochratoxin A	Not detected (µg/kg)	R Hone-Diagnostics OTA

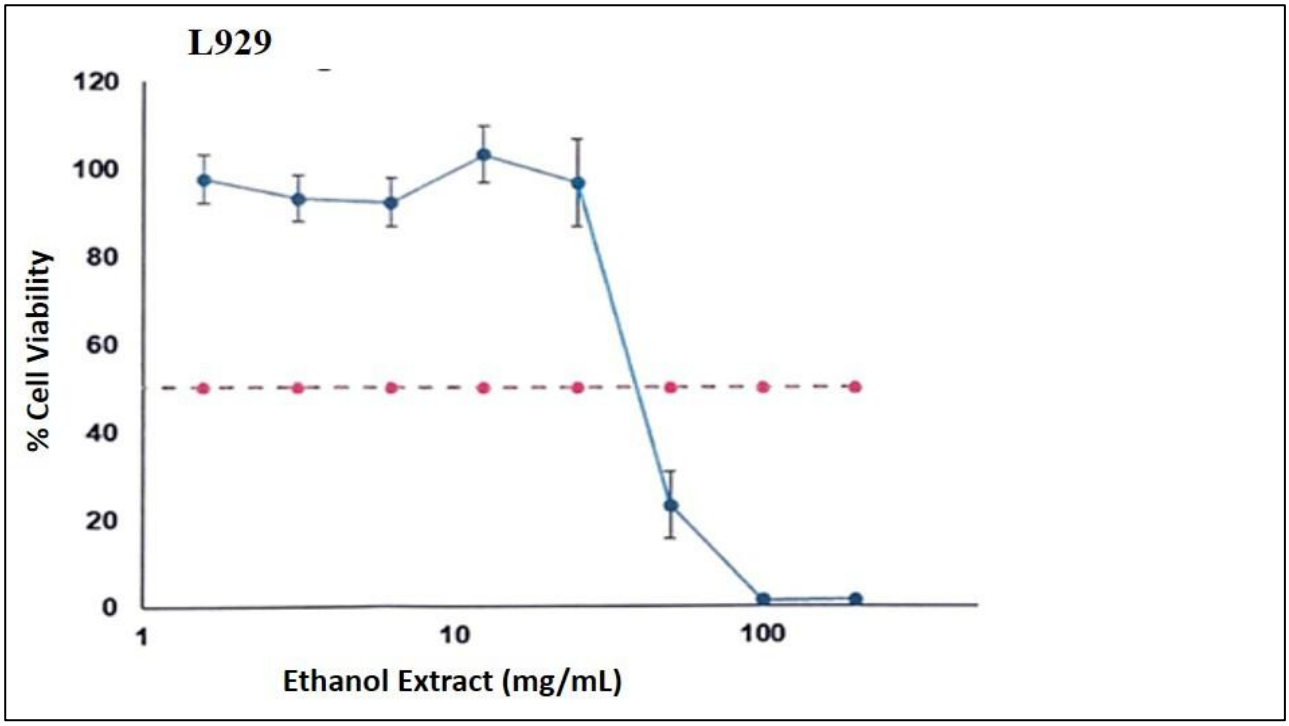

Figure 4. Dose-response relationship of ethanol extract on L929 cells after 24 hours of exposure, expressed as percent cell viability normalized to the negative control.

4. Discussion

Fungi are genetically and morphologically distinct from other organisms, such as plants and animals, which makes their identification and classification challenging. Alternative molecular diagnostic tools like PCR techniques have been developed to overcome these difficulties. These techniques employ species-specific primers as an efficacious method for distinguishing fungal taxa at the species level. In this context, DNA barcoding is a modern molecular biology technique designed to overcome these challenges (Schoch et al., 2012). In this study, a morphological review, a detailed examination of the physical characteristics of *A. chevalieri*, showed that the testing results for *A. chevalieri* using ITS sequences, benA, and RPB2 loci agreed with the results obtained when morphological characteristics were used to identify the species.

In the current study, during the assessment of the pathogenicity of *A. chevalieri*, the effectiveness of the conidial suspension applied to *E. kuehniella* larvae was monitored between the 5th and 10th days post-application. After the 3rd day, larvae infected with the fungus became lethargic and immobile. During the insecticidal process of EPFs, the hyphal mass within the insect hemocoel increases, leading to the disruption of vital physiological functions due to either nutrient depletion or the action of fungal toxins. These toxins have been reported to cause host mortality within 3–5 days (Zhang et al., 2015). Furthermore, under suitable environmental conditions, fungal hyphae were observed to emerge from between the host's body segments within two days after death (Gökçe & Er, 2003).

Findings from this study indicated that *A. chevalieri* exhibited traits consistent with known EPF mechanisms. The emergence of adult individuals—excluding those that died during pathogenicity testing—following treatment with *A. chevalieri* conidial suspension and ethanol extract enabled the evaluation of biological parameters from larval to adult stages. Fungal spores and secondary metabolites can impact insects physiologically, resulting in effects such as prolonged development time, decreased longevity, and morphological abnormalities (Kaur et al., 2014). In the current study, the application of different conidial suspension concentrations to the third instar larvae did not cause changes in the adult emergence time, longevity, and weight compared to the control. Decreases were observed in the pupal period and egg number of *E. kuehniella* treated with different conidial suspension concentrations at all doses. A significant shortening of the larval period was detected only at the highest dose (10^8 conidia/mL), possibly due to accelerated physiological exhaustion caused by higher fungal load or earlier toxin expression (Chandler, 2017). It was determined that ethanol extract applications did not affect larval and pupal development or adult longevity compared to the control group; however, they did cause changes in body weight, total number of eggs, and adult emergence time. These findings suggest that fungal secondary metabolites, independent of conidial infection, can influence host physiology through stress-related pathways. Such compounds may interfere with endocrine regulation, disrupt metabolic balance, or impose energetic costs on detoxification and immune responses resulting in altered developmental trajectories and reproductive investment (Los & Strachecka, 2018). In biological control, it is important to study how insect pre-adult stages change. This effect shows how they interact with natural enemies. (Alikhani et al., 2019). In a study conducted by Senthilkumar et al. (2014), extracts derived from *A. flavus* and *Nigrospora sphaerica* were observed to possess insecticidal properties against *Hyblaea purea*, *Atteva fabriciella*, and *Eligma narcissus*. Similarly, another study reported that the EtOAc extract of the fermentation broth from *A. fumigatus*, isolated from the leaves of *Acrostichum speciosum* endemic to Hainan Island, demonstrated insecticidal effects against *S. litura* (Guo Zhikai et al., 2017).

Although several studies have investigated the insecticidal properties of various *Aspergillus* species, this is the first to explore the impact of *A. chevalieri* on the developmental biology of *E. kuehniella*. Both the conidial suspension and ethanol extract of *A. chevalieri* were found to influence developmental parameters and reduce total egg production. This decline in fecundity may result from either the invasive nature of fungal mycelia or the toxic effects of fungal metabolites. Furthermore, higher concentrations of conidia may divert host energy resources toward combating infection, thereby enhancing fungal pathogenicity while suppressing reproduction (Roy et al., 2006). The reduction in egg production observed in both conidial and extract treatments may stem from the energetic trade-offs associated with immune activation and survival under fungal stress. Host resources may be reallocated from reproduction to defense and repair as previously observed in other insect-fungus systems (Rumbos & Athanassiou, 2017). Supporting this, Güner et al. (2025) found that high concentrations of *Penicillium mallochii* conidia and ethanol extract decreased egg-laying in *Cadra cautella*. Similarly, Castillo et al. (2000) reported a 65% reduction in the fertility of the Mediterranean fruit fly (*Ceratitis capitata*) following infection by *Paecilomyces fumosoroseus*. Treatments with *Metarhizium anisopliae* and *Aspergillus ochraceus* also led to a 40–50% decrease in fertility in the same pest species. These findings support the view that EPF affects host populations not only by inducing mortality but also by disrupting reproductive capacity through physiological stress or direct toxin action.

Mycotoxins play an important role in fungal infection and are small molecules that are toxic to different organisms (Erzurum, 2001). EPFs produce multiple mycotoxins to improve fungal pathogenesis and virulence. Ochratoxin and Aflatoxin are produced by many different fungi including *Aspergillus* and *Penicillium*. Ochratoxin and Aflatoxin are known to be toxic and carcinogenic in animals (Guyonnet et al., 2002). Secondary metabolites such as aflatoxins (AFs) and OTA produced by *Aspergillus* species can be used for biological control against pests (Escriva et al., 2021). Among the important mycotoxins, aflatoxin B is effective on *Periplaneta americana*, while patulin, citrinin, and ochratoxin are toxic to *Drosophila melanogaster* (Srivastava et al., 2009). In studies on *Aspergillus* species, it was observed that *Aspergillus clavatus* produces cytochalasin E and patulin, while certain strains of *A. flavus* were identified as aflatoxin producers. Additionally, these compounds were identified as having adverse effects on humans and animals (Pitt, 2000). Despite the concerns about *Aspergillus* species producing toxins, studies on using them in biological control have

gained attention. Mohanty & Prakash (2010) found that using *Aspergillus* to control mosquito larvae in unsuitable water sources is acceptable. Extracts prepared from *A. chevalieri* cultures did not contain any of the commonly known *Aspergillus* mycotoxins including aflatoxins, sterigmatocystin, OTA, rubratoxin B, or patulin. However, later studies indicated that *A. chevalieri* can produce aflatoxin, citrinin, flavoglaucin, gliotoxin, and sterigmatocystin but does not produce OTA (Chen et al., 2021). In addition to these studies, current study revealed that *A. chevalieri* does not produce Ochratoxin A or Aflatoxin (Aflatoxin B1, B2, G1, and G2). The increase in fungal contamination rates and mycotoxin production that occurs before and after harvest is influenced by a number of environmental agents including humidity, water activity, and temperature (Mannaa & Kim, 2017; Singh et al., 2023).

The effects of radiation on genetic material for pest control purposes have previously been investigated in species such as *Plodia interpunctella* (Imamura et al., 2004), *Curculio sikkimensis* (Todoriki et al., 2006), *Plutella xylostella* (Koo et al., 2011), *Liriomyza trifolii* (Koo et al., 2012), and *Spodoptera litura* (Yun et al., 2014). However, studies addressing the cellular and genetic-level effects of microbial biopesticides remain limited. In this study, we examined the cytotoxic effect of the ethanol extract obtained from *A. chevalieri* which has potential as a biological control agent. The findings indicate that the extract exhibited a cytotoxic effect at doses above 0.78 mg/mL in the L929 mouse cell line. In our study, ethanol extract was applied at concentrations both above and below the established cytotoxic thresholds. Taken together, these findings indicate that the ethanol extract of *A. chevalieri* exhibits a mild cytotoxic effect only at relatively high concentrations and is devoid of major mycotoxins such as aflatoxins and ochratoxins. This profile supports its potential for safe use in pest control.

5. Conclusion

This study demonstrated that *A. chevalieri* exhibits entomopathogenic potential against *E. kuehniella* as evidenced by a significant reduction in egg production and alterations in developmental parameters. Importantly, the conidial suspensions and ethanol extract of *A. chevalieri* showed biological activity without producing major mycotoxins such as aflatoxins (B1, B2, G1, G2) or ochratoxin A. The ethanol extract exhibited low cytotoxicity, with an IC_{50} value of 40.87 mg/mL in the L929 cell line, suggesting a favorable safety profile. These findings are highly relevant for the development of environmentally friendly pest management strategies. The absence of key mycotoxins and the limited cytotoxic effect indicate that *A. chevalieri* may serve as a safe biocontrol agent with minimal risk to non-target organisms. Furthermore, its ability to negatively impact insect reproduction without direct lethality supports its potential as a population-suppressing agent. While these initial results are promising, further studies, including field trials, detailed metabolite profiling, and comprehensive ecotoxicological assessments, are essential to validate the efficacy and biosafety of *A. chevalieri* under agricultural conditions. With proper evaluation, *A. chevalieri* may contribute significantly to integrated pest management as a sustainable and effective fungal biocontrol agent.

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References

Alikhani, M., Safavi, S.A., & Iranipou, S. (2019). Effect of the entomopathogenic fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin, on demographic fitness of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Egyptian Journal of Biological Pest Control,* 29, 1-7. https://doi.org/10.1186/s41938-019-0121

Bovio, E., Garzoli, L., Poli, A., Luganini, A., Villa, P., Musumeci, R., McCormack, G.P., Cocuzza, C.E., Gribaudo, G., Mehiri, M., & Varese, G.C. (2019). Marine fungi from the sponge Grantia compressa: biodiversity, chemodiversity, and biotechnological potential. *Marine drugs,* 17(4), 220. https://doi.org/10.3390/md17040220

Castillo, M.A., Moya, P., Hernández, E., & Primo-Yufera, E. (2000). Susceptibility of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) to entomopathogenic fungi and their extracts. *Biological control*, 19(3), 274-282. https://doi.org/10.1006/bcon.2000.0867

Chandler, D. (2017). Basic and applied research on entomopathogenic fungi. In Microbial control of insect and mite pests (pp. 69-89). Academic Press.

Chen, W., Xie, W., Cai, W., Thaochan, N., & Hu, Q. (2021). Entomopathogenic Fungi Biodiversity in the Soil of Three Provinces Located in Southwest China and First Approach to Evaluate Their Biocontrol Potential. *Journal of Fungi,* 7, 984. https://doi.org/10.3390/jof7110984

Chevrette, M.G., Carlson, C.M., Ortega, H.E., Thomas, C., Ananiev, G.E., Barns, K.J., & Currie, C.R. (2019). The antimicrobial potential of Streptomyces from insect microbiomes. *Nature Communications*, 10(1), 516. https://doi.org/10.1038/s41467-019-08438-0

de Oliveira, G.P., Barreto, D.L.C., Ramalho Silva, M., Augusti, R., Evódio Marriel, I., Gomes de Paula Lana, U., & Takahashi, J.A. (2022). Biotic stress caused by in vitro co-inoculation enhances the expression of acetylcholinesterase inhibitors by fungi. *Natural Product Research,* 36(16), 4266-4270. https://doi.org/10.1080/14786419.2021.1975701

Deka, B., Baruah, C., & Babu, A. (2021). Entomopathogenic microorganisms: their role in insect pest management. *Egyptian Journal of Biological Pest Control*, 31, 1-8. https://doi.org/10.1186/s41938-021-00466-7

Diao, H., Xing, P., Tian, J., Han, Z., Wang, D., Xiang, H., ... & Ma, R. (2022). Toxicity of crude toxin protein produced by *Cordyceps fumosorosea* IF-1106 against *Myzus persicae* (Sulze). *Journal of Invertebrate Pathology*, 194, 107825. https://doi.org/10.1016/j.jip.2022.107825

Erzurum, K. (2001). Gıdalarda mikotoksin oluşumunu etkileyen faktörler. *Gıda*, 26 (4), 289-293. Retrieved from: https://dergipark.org.tr/tr/pub/gida/issue/6931/92553

Escriva, L., Agahi, F., Vila-Donat, P., Mañes, J., Meca, G., & Manyes, L. (2021). Bioaccessibility study of aflatoxin B1 and ochratoxin A in bread enriched with fermented milk whey and/or pumpkin. *Toxins*, 14(1), 6. https://doi.org/10.3390/toxins14010006

Fancelli, M., Dias, A.B., Delalibera, I.J., Cerqueira de Jesus, S., Souza do Nascimento, A., & Oliveira e Silva, S. (2013). *Beauveria bassiana* Strains for Biological Control of *Cosmopolites sordidus* (Germ.) (Coleoptera: Curculionidae) in Plantain. *BioMed Research International,* 2013(1), 184756. https://doi.org/10.1155/2013/184756

Fujita, S. (2013). Simple modified method for fungal slide preparation. *Journal of Medical Mycology*, 54(2), 141-146. https://doi.org/10.3314/mmj.54.141

Glass, N.L., & Donaldson, G.C. (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology, 61(4), 1323-30. https://10.1128/aem.61.4.1323-1330, PMID: 7747954; PMCID: PMC167388.

Gökçe, A., & Er, M.K. (2003). First description of the disease by *Conidiobolus osmodes* on *Tipula paludosa* larvae with the report of a natural epizootic. *Journal of Invertebrate Pathology*, 84(2), 83-89. https://doi.org/10.1016/j.jip.2003.09.004

Gradmann, C. (2008). A matter of methods: the historicity of Koch's postulates. *Medical Journal*, 43(2), 121–148. https://www.jstor.org/stable/25805450

Güner, P., Aşkun, T., & Er, A. (2023). Entomopathogenic Fungi and Their Potential Role in the Sustainable Biological Control of Storage Pests. *Commagene Journal of Biology*, 7(1), 90-97. https://doi.org/10.31594/commagene.1284354

Güner, P., Aşkun, T., Er, A., & Sönmez, D.G. (2025). Assessment of the potential pathogenicity of *Penicillium mallochii* conidial suspensions and ethanol extract against almond moth *Cadra cautella* (Walker) (Lepidoptera: Pyralidae). *Journal of Plant Diseases and Protection*, 132, 3. https://doi.org/10.1007/s41348-024-01039-0

Guo ZhiKai, G.Z., Gai CuiJuan, G.C., Cai CaiHong, C.C., Chen LiangLiang, C.L., Liu ShouBai, L.S., Zeng YanBo, Z.Y., Mei, W., & Dai HaoFu, D.H. (2017). Metabolites with insecticidal activity from Aspergillus fumigatus JRJ111048 isolated from mangrove plant Acrostichum specioum endemic to Hainan Island. *Marine Drugs*, 15(12), 381. https://doi.org/10.3390/md15120381

Guyonnet, D., Belloir, C., Suschetet, M., Siess, M.H., & Le Bon, A.M. (2002). Mechanisms of Protection Against Aflatoxin B(1) Genotoxicity in Rats Treated by Organosulfur Compounds from Garlic. *Carcinogenesis*, 23(8), 1335-1341. https://doi.org/10.1093/carcin/23.8.1335

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *In Nucleic Acids Symposium Series*, 41, 95–98.

Imamura, T., Todoriki, S., Sota, N., & Nakakita, H. (2004). Efect of sof-electron (low-energy electron) treatment on three stored-product insect pests. *Journal of Stored Products Research*, 40(2), 169–177. https://doi.org/10.1016/S0022-474X(02)00095-4

Jacob, T.A., & Cox, P.D. (1977). The influence of temperature and humidity on the life-cycle of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). *Journal of Stored Products Research*, 13(3), 107-118. https://doi.org/10.1016/0022-474X(77)90009-1

Karthi, S., Vaideki, K., Shivakumar, M.S., Ponsankar, A., Thanigaivel, A., Chellappandian, M., Hunter, W.B., & Senthil-Nathan, S. (2018). Effect of *Aspergillus flavus* on the mortality and activity of antioxidant enzymes of *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) larvae. *Pesticide Biochemistry and Physiology*, 149, 54-60. https://doi.org/10.1016/j.pestbp.2018.05.009

Kaur, S., Thakur, A., & Rajput, M. (2014). A laboratory assessment of the potential of *Beauveria bassiana* (Balsamo) Vuillemin as a biocontrol agent of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae). *Journal of Stored Products Research*, 59, 185–189. https://doi.org/10.1016/j.jspr.2014.08.004

Kaur, T., Kaur, J., Kaur, A., & Kaur, S. (2016). Larvicidal and growth inhibitory effects of endophytic *Aspergillus niger* on a polyphagous pest, Spodoptera litura. *Phytoparasitica*, 44, 465-476. https://doi.org/10.1007/s12600-016-0541-2

Khan, H.A.A., & Khan, T. (2023). Efficacy of entomopathogenic fungi against three major stored insect pests, *Rhyzopertha dominica*, *Sitophilus zeamais* and *Trogoderma granarium*. *Journal of Stored Products Research*, 104, 102188. https://doi.org/10.1016/j.jspr.2023.102188

Klich, M.A. (2002). Identification of common Aspergillus species. Centraalbureau voor schimmelcultures.

Koo, H.N., Yoon, S.H., Shin, Y.H., & Yoon, C. (2011). Efect of electron beam irradiation on developmental stages of *Plutella xylostella* (Lepidoptera: Plutellidae). *Journal of Asia-Pacific Entomology*, 14(3), 243–247. https://doi.org/10.1016/j.aspen.2011.03.001

Koo, H.N., Yun, S.H., Yoon, C., & Kim, G.H. (2012). Electron beam irradiation induces abnormal development and the stabilization of p53 protein of American serpentine leafminer, *Liriomyza trifolii* (Burgess). *Radiation Physics and Chemistry*, 81(1), 86–92. https://doi.org/10.1016/j.radphyschem.2011.09.008

Letunic, I., & Bork, P. (2024). Interactive Tree of Life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. *Nucleic Acids Research*, 52(W1), W78-W82. https://doi.org/10.1093/nar/gkae268

Lin, W.J., Chiu, M.C., Lin, C.C., Chung, Y.K., & Chou, J.Y. (2021). Efficacy of Entomopathogenic fungus *Aspergillus nomius* against *Dolichoderus thoracicus*. *BioControl*, 66(4), 463-473. https://doi.org/10.1007/s10526-021-10086-7

Liu, S., Li, J., Feng, Q., Chu, L., Tan, Z., Ji, X., & Jin, P. (2023). Insecticidal Effect of the Entomopathogenic Fungus *Lecanicillium araneicola* HK-1 in *Aphis craccivora* (Hemiptera: Aphididae). *Insects*, 14(11), 860. https://doi.org/10.3390/insects14110860

Liu, Y.J., Whelen, S., & Hall, B.D. (1999). Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. *Molecular Biology and Evolution*, 16(12), 1799-1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092

Los, A., & Strachecka, A. (2018). Fast and Cost-Effective Biochemical Spectrophotometric Analysis of Solution of Insect Blood and Body Surface Elution. *Sensors*, 18(5), 1494. https://doi.org/10.3390/s18051494

Mannaa, M., & Kim, K.D. (2017). Influence of temperature and water activity on deleterious fungi and mycotoxin production during grain storage. *Mycobiology*, 45(4), 240-254. https://doi.org/10.5941/MYCO.2017.45.4.240

Marcinkevicius, K., Salvatore, S.A., Bardon, A.D.V., Cartagena, E., Arena, M.E., & Vera, N.R. (2017). Insecticidal activities of diketopiperazines of *Nomuraea rileyi* entomopathogenic fungus. *International Journal of Environment, Agriculture and Biotechnology*, 2(4), 1586-1596. https://doi.org/10.22161/ijeab/2.4.18

Mensah, R.K., & Young, A. (2017). Microbial control of cotton pests: use of the naturally occurring entomopathogenic fungus *Aspergillus* sp.(BC 639) in the management of *Bemisia tabaci* (Genn.)(Hemiptera: Aleyrodidae) and beneficial insects on transgenic cotton crops. *Biocontrol Science and Technology*, 27(7), 844-866. https://doi.org/10.1080/09583157.2017.1349875

Mohanty, S.S., & Prakash, S. (2010). Comparative efficacy and pathogenicity of keratinophilic soil fungi against *Culex quinquefasciatus* larvae. *Indian Journal of Microbiology*, 50, 299-302. https://doi.org/10.1007/s12088-010-0051-8

Nguyen, B.T., Shuval, K., Bertmann, F., & Yaroch, A.L. (2015). The Supplemental Nutrition Assistance Program, food insecurity, dietary quality, and obesity among US adults. *American Journal of Public Health*, 105(7), 1453-1459. https://doi.org/10.2105/AJPH.2015.302580

Nguyen, H.C., Lin, K.H., Nguyen, T.P., Le, H. S., Ngo, K.N., Pham, D.C., Su, C.H., & Barrow, C.J. (2023). Isolation and Cultivation of *Penicillium citrinum* for Biological Control of *Spodoptera litura* and *Plutella xylostella*. *Fermentation*, 9(5), 438. https://doi.org/10.3390/fermentation9050438

Ningsih, B.N.S., Rukachaisirikul, V., Phongpaichit, S., Preedanon, S., Sakayaroj, J., & Muanprasat, C. (2023). A nonadride derivative from the marine-derived fungus *Aspergillus chevalieri* PSU-AMF79. *Natural Product Research*, 37(14), 2311-2318. https://doi.org/10.1080/14786419.2022.2039651

Orozco, R.A., Lee, M.M., & Stock, S.P. (2014). Soil sampling and isolation of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae). *Journal of Visualized Experiments*, 89, 52083. https://doi.org/10.3791/52083

Pitt, J.I. (2000). Toxigenic fungi and mycotoxins. *British Medical Bulletin*, 56(1), 184-192. https://doi.org/10.1258/0007142001902888

Roy, H., Steinkraus, D.C., Eilinberg, J., Hajek, A.E., & Pell, J.K. (2006). Bizarre interactions and endgames: entomopathogenic fungi and their arthropod hosts. *Annual Review of Entomology*, 51, 331–357. https://doi.org/10.1146/annurev.ento.51.110104.150941

Rumbos, C.I., & Athanassiou, C.G. (2017). Use of entomopathogenic fungi for the control of stored-product insects: can fungi protect durable commodities. *Journal of Pest Science*, 90, 839–854. https://doi.org/10.1007/s10340-017-0849-9

Şahin Taylan, Z., & Er, M.K. (2024). Mortality Effects of *Beauveria bassiana* and *Purpureocillium lilacinum* Isolates and Efficacy of a Wettable Formulation on *Palemona prasina* (Hemiptera: Pentatomidae) Nymphs. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 27(6), 1392-1400. https://doi.org/10.18016/ksutarimdoga.vi.1425131

Sani, I., Ismail, S. I., Abdullah, S., Jalinas, J., Jamian, S., & Saad, N. (2020). A review of the biology and control of whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae), with special reference to biological control using entomopathogenic fungi. *Insects*, 11(9), 619. https://doi.org/10.3390/insects11090619

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., & White, M.M. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences*, 109(16), 6241-6246. https://doi.org/10.1073/pnas.1117018109

Senthilkumar, N., Murugesan, S., & Babu, D.S. (2014). Metabolite profiling of the extracts of endophytic fungi of entomopathogenic significance, *Aspergillus flavus* and *Nigrospora sphaerica* isolated from tropical tree species of India, *Tectona grandis* L. *Journal of Agriculture and Life Sciences*, 1(1), 108-114.

Shahrajabian, M.H., Kuang, Y., Cui, H., Fu, L., & Sun, W. (2023). Metabolic changes of active components of important medicinal plants on the basis of traditional Chinese medicine under different environmental stresses. *Current Organic Chemistry*, 27(9), 782-806. https://doi.org/10.2174/1385272827666230807150910

Shakarami, J., Eftekharifar, R., Latifian, M., & Jafari, S. (2015). Insecticidal activity and synergistic effect of *Beauvaria bassiana* (Bals.) Vuill. and three botanical compounds against third instar larvae of *Ephestia kuehniella* Zeller. *Research on Crops*, 16(2), 296-303. https://doi.org/10.5958/2348-7542.2015.00044.3

Singh, B.K., Delgado-Baquerizo, M., Egidi, E., Guirado, E., Leach, J.E., Liu, H., & Trivedi, P. (2023). Climate change impacts on plant pathogens, food security and paths forward. *Nature Reviews Microbiology*, 21(10), 640-656. https://doi.org/10.1038/s41579-023-00900-7

Song, Y., Liu, X., Feng, S., Zhao, K., Qi, Z., Wu, W., ... & Qin, B. (2023). Discovery, Identification, and Insecticidal Activity of an *Aspergillus flavus* Strain Isolated from a Saline–Alkali Soil Sample. *Microorganisms*, 11(11), 2788. https://doi.org/10.3390/microorganisms11112788

Spescha, A., Weibel, J., Wyser, L., Brunner, M., Hermida, M.H., Moix, A., & Maurhofer, M. (2023). Combining entomopathogenic Pseudomonas bacteria, nematodes and fungi for biological control of a below-ground insect pest. *Agriculture, Ecosystems & Environment*, 348, 108414. https://doi.org/10.1016/j.agee.2023.108414

Srivastava, C.N., Maurya, P., Sharma, P., & Mohan, L. (2009). Prospective role of insecticides of fungal origin. *Entomological Research*, 39(6), 341-355. https://doi.org/10.1111/j.1748-5967.2009.00244.x

Talavera, G., & Castresana, J. (2007). Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic biology*, 56(4), 564-577. https://doi.org/10.1080/10635150701472164

Todoriki, S., Mahbub Hasan, M.D., Miyanoshita, A., & Imamura, T. (2006). Assessment of electron beam-induced DNA damage in larvae of chestnut weevil, *Curculio sikkimensis* (Heller) (Coleoptera: Curculionidae) using comet assay. *Radiation Physics and Chemistry*, 75(2), 292–296. https://doi.org/10.1016/j.radphyschem.2005.08.001

Wang, Z., Jin, Q., Li, Q., Ou, X., Li, S., Liu, Z., & Huang, J.A. (2022). Multiplex PCR identification of *Aspergillus cristatus* and *Aspergillus chevalieri* in Liupao tea based on orphan genes. *Foods*, 11(15), 2217. https://doi.org/10.3390/foods11152217

Warcup, J.H. (1955). On the origin of colonies of fungi developing on soil dilution plates. *Transactions of the British Mycological Society*, 38(3), 298-301. https://doi.org/10.1016/S0007-1536(55)80075-7

White, T.J., Bruns, T., Lee, S., & Taylor, J.W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds.) PCR Proto-cols: A Guide to Methods and Applications. Academic Press, Inc., New York, 315–322.

Yan, J., Liu, H., Idrees, A., Chen, F., Lu, H., Ouyang, G., & Meng, X. (2022). First record of *Aspergillus fijiensis* as an entomopathogenic fungus against Asian *Citrus psyllid*, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae). *Journal of Fungi*, 8(11), 1222. https://doi.org/10.3390/jof8111222

Yun, S.H., Lee, S.W., Koo, H.N., & Kim, G.H. (2014). Assessment of electron beam-induced abnormal development and DNA damage in Spodoptera litura (F.) (Lepidoptera: Noctuidae). *Radiation Physics and Chemistry*, 96, 44–49. https://doi.org/10.1016/j.radphyschem.2013.08.008

Zhang, S., Huang, Z., Xu, H., Liu, Q., Jiang, Z., Yin, C., Han, G., Zhang, W., & Zhang, Y. (2024a). Biological control of wheat powdery mildew disease by the termite-associated fungus *Aspergillus chevalieri* BYST01 and potential role of secondary metabolites. *Pest Management Science*, 80(4), 2011-2020. https://doi.org/10.1002/ps.7938

Zhang, P.Z.P., You YinWei, Y.Y., Song Yuan, S.Y., Wang YouZhi, W.Y., & Zhang Long, Z.L. (2015). First record of *Aspergillus oryzae* (Eurotiales: Trichocomaceae) as an entomopathogenic fungus of the locust, *Locusta migratoria* (Orthoptera: Acrididae). *Biocontrol Science and Technolgy*, 25, 1285–1298. https://doi.org/10.1080/09583157.2015.1049977

Zhang, Z., Tian, Y., Sui, L., Lu, Y., Cheng, K., Zhao, Y., Li, Q., & Shi, W. (2024b). First record of *Aspergillus nomiae* as a broad-spectrum entomopathogenic fungus that provides resistance against phytopathogens and insect pests by colonization of plants. *Frontiers in Microbiology*, 14, 1284276. https://doi.org/10.3389/fmicb.2023.1284276

Zin, W.W.M., Buttachon, S., Dethoup, T., Pereira, J.A., Gales, L., Inácio, Â., Costa, P.M., Lee, M., Şekeroğlu, N., Pinto, M.M.M., & Kijjoa, A. (2017). Antibacterial and antibiofilm activities of the metabolites isolated from the culture of the mangrove-derived endophytic fungus *Eurotium chevalieri* KUFA 0006. *Phytochemistry*, 141, 86-97. https://doi.org/10.1016/j.phytochem.2017.05.015