ISOLATION AND PATHOGENICITY OF FUNGI ASSOCIATED WITH *HALYOMORPHA HALYS* (STAL) (HEMIPTERA: PENTATOMIDAE) FOR MICROBIAL CONTROL

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Abstract. In this study, the fungal flora associated with *Halyomorpha halys* (Stal) (Hemiptera: Pentatomidae) was investigated for the first time in terms of its potential use in microbial control. Adult *H. halys* were collected from infested areas in Artvin and brought to the laboratory for further analysis. Thirteen fungal species from five genera were isolated from both live and dead insects. The colony morphologies of the isolates were determined on Sabouraud dextrose agar (SDA). These fungal isolates were identified using ITS rDNA analysis as *Fusarium solani* (Hf1, Hf2), *Penicillium citrinum* (Hf3), *Alternaria* sp. (Hf4, Hf9), *Fusarium trincinctum* (Hf5, Hf12), *Cladosporium cladosporioides* (Hf6), *Aspergillus niger* (Hf7, Hf11), *Fusarium proliferatum* (Hf8, Hf10), and *Alternaria alternata* (Hf13). Furthermore, the entomopathogenic fungus *Beauveria bassiana* (PaF09) was utilized against this pest. The isolate, previously obtained from *Pristiphora abietina*, demonstrated entomopathogenicity against pests from various insect orders. The pathogenicity of all fungal isolates was evaluated against *H. halys* at a concentration of 1×10^7 conidia/ml and 60 ± 1% relative humidity. Mortality was recorded daily over a 10-day period. The Hf1 isolate was found to be the most virulent, with a mortality rate of 60.8 ± 7.0. Future studies should focus on field trials to further evaluate the effectiveness of this isolate.
Keywords: *Halyomorpha halys, fungal flora, pest, Fusarium, entomopathogenic fungi*

Introduction

The brown marmorated stink bug, *Halyomorpha halys* (Stal, 1855) (Hemiptera: Pentatomidae), is an invasive pest species native to East Asia. It feeds on a wide range of plant species from approximately 49 families and causes significant damage to agricultural crops (Gapon, 2016). *H. halys* was first identified in Turkey in 2017 (Çerçi and Koçak, 2017). Typically, it completes one generation per year, but under favorable conditions, it may complete 4 to5 generations. Female individuals lay their eggs in groups of 20-30 on the undersides of plant leaves or on stems. Both adults and nymphs cause damage by feeding on the leaves and fruits of host plants. The pest is especially damaging to crops such as hazelnuts and is commonly found in agricultural areas near forests. The spread of *H. halys* throughout the entire Eastern Black Sea region will present a serious threat to hazelnut producers and lead to significant economic losses (Aksu, 2019).

Current management strategies for *H. halys* primarily involve the application of broad-spectrum insecticides to vulnerable crops within agricultural systems (Hamilton et al., 2018). However, only a limited number of pesticides have been classified for use against *H. halys*, and many conventional insecticides are not effective in controlling this pest. Additionally, the pest's wide host range and continuous movement complicate its control

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with insecticides. Unfortunately, the need for multiple applications per growing season further renders insecticide use both destructive and unsustainable (Blaauw et al., 2015).

Entomopathogenic fungi (EPF) are recognized as significant microbial control agents worldwide. They can exert either epizootic or enzootic effects on host populations. The mechanism of action of these fungi involves the attachment of a spore to the insect's cuticle, followed by germination, penetration of the cuticle, and subsequent spread within the insect's body (Mora et al., 2017). Notably, extensive research on EPF has been conducted for over a century (Feng and Johnson, 1990; Hu et al., 1996; Kılıç et al., 2009; Vega and Kaya, 2012; Örtücü and İskender, 2017; Ozdemir et al., 2020; Awan et al., 2021).

However, limited research has been conducted on the microbial control of *H. halys*. In a study, the virulence of several entomopathogenic fungi, including *Beauveria bassiana* and *Metarhizium anisopliae* isolates, against *H. halys* was investigated for the first time. The *B. bassiana* isolates, originating from BotaniGard and coded as GHA, were found to produce mortality rates of 85% and 100% after 9 and 12 days of application under 70-75% relative humidity, respectively. In addition, it was reported that the *M. anisopliae* isolates resulted in lower mortality rates compared to the *B. bassiana* isolates (Gouli et al., 2011). In another study, the biological control potential of 11 entomopathogenic bacterial strains and 1 fungal isolate was evaluated against *H. halys* nymphs, and their effects were tested at a humidity level of 65-70%. The results reported that the bacterial strains exhibited mortality rates ranging from 75% to 100%, with *Bacillus cereus* and *Pantoea agglomerans* as the isolates that achieved the highest efficacy, reaching 100% mortality. As for *B. bassiana*, it has been reported to result in a mortality rate of 76.19%. (Tozlu et al., 2019). Other studies have focused on evaluating the efficacy of certain local entomopathogenic bacteria and fungi against the pest (Burjanadze et al., 2020; Mantzoukas et al., 2024). To date, no studies have been conducted on the fungal flora of *H. halys*.

In this study, the fungal flora of *H. halys* was investigated for the first time to assess its potential as a microbial control agent, and the pathogenicity of these fungal isolates against the pest was determined under laboratory conditions. Moreover, in line with the stated objective, the *Beauveria bassiana* (Paf 09) isolate, which had previously demonstrated entomopathogenic activity against various insect species, was also utilized.

Materials and methods

Collection of insects

The adult *H. halys* were collected from Artvin-Borçka during field studies conducted between May and June of 2020 to 2023 and were carefully transported to the laboratory under aseptic conditions.

Isolation and purification of fungi

The identification of the internal fungal flora of the adults was conducted using healthy specimens, while dead individuals were selected for the isolation of entomopathogenic fungi. Two distinct culture media, Potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA), were utilized for fungal isolation. Initially, both healthy and dead adult insects underwent surface sterilization using 70% ethanol for 5 min. Subsequently, the samples were rinsed with sterile distilled water to remove residual ethanol, and this

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procedure was repeated three times. After surface sterilization, the insects were placed in 20 mL test tubes containing 10 mL of sterile physiological saline. The insects were then aseptically macerated, homogenized, and filtered through three layers of sterile gauze. From the prepared homogenate, 0.1 mL was plated onto PDA and SDA agar plates. The inoculated plates were then incubated at 25°C for two weeks. Following the incubation period, the fungal colonies that developed were isolated and purified. The purified cultures were subsequently transferred to slanted SDA agar tubes and incubated at 25°C for one week before being stored at +4°C in a refrigerator.

Molecular characterization of fungi and phylogenetic tree construction

Molecular characterization of the fungi was performed using ITS rDNA analysis. For DNA isolation, approximately 100 mg of fresh mycelium was collected from newly cultured fungi. The sample was homogenized in a homogenizer using liquid nitrogen. Genomic DNA was then isolated according to the manufacturer’s protocol using a commercial Fungal DNA Isolation Kit (Norgen, Biotek Corp., Canada). DNA amplification was carried out using the ITS1 (TCC GTA GGT GAA CCT GCG G) and ITS4 (TCC TCC GCT TAT TGA TAT GC) primers.

The PCR mixture was prepared in a final volume of 20 µL according to the PCR Master Mix (A.B.T.TM) protocol. For each sample, the total 20 µL PCR mixture consisted of 10 µL of Hs-Taq master mix, 1 µL of ITS1 primer, 1 µL of ITS4 primer, 4 µL of template DNA, and 4 µL of dH2O. The prepared samples were placed in a PCR thermocycler, programmed with the following conditions: 5 min of denaturation at 94°C, followed by 35 cycles of 30 s of denaturation at 94°C, 45 s of annealing at 57°C, and 45 s of extension at 72°C, with a final extension step of 7 min at 72°C. The amplified DNA fragments were then run on a 1% agarose gel in 1 × TAE buffer to confirm the presence of the desired region. The ITS rDNA region was sequenced in both directions by a commercial service provider. The obtained ITS1 and ITS4 sequences were compared using BLAST (Basic Local Alignment Search Tool). Sequence alignment was performed using CLUSTAL W (Hall, 1999). Phylogenetic analysis was conducted using MEGA software version 11.0 (Tamura et al., 2021).

Conidial suspension

The fungal isolates were inoculated onto 9 cm diameter SDA agar plates for pathogenicity testing/assays and incubated at 25°C for two weeks. After incubation, 5 ml of sterile water containing 0.01% Tween 80 was added to the Petri dishes, and the conidia were carefully harvested. The resulting conidial suspension was then filtered through a sterile four-layer gauze to remove any hyphal fragments. Finally, the suspension was adjusted to a concentration of 1×10^7 conidia/mL using a Neubauer hemocytometer under a microscope (Zeiss, Germany) and used in the experiments.

Testing of fungi under laboratory conditions

Fungal isolates obtained from the internal flora of *H. halys* (Hf1, Hf2, Hf3, Hf5, Hf6, Hf7, and Hf12) were tested against the pest. The applications were carried out in 100 mL experimental containers with screw caps. Adult insects were individually immersed in a conidial suspension (1×10^7 conidia/mL) for 30 s, then allowed to dry at room temperature before being placed into the experimental containers. Each experimental container contained 10 adult insects, and fresh apple pieces were provided for feeding.

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For the control treatment, the same procedure was followed, but sterilized distilled water with 0.01% Tween 80 was used in place of the conidial suspensions. The experiments were conducted at 25°C, with a relative humidity of 60 ± 1% and a 16:8-h light/dark photoperiod. Mortality was assessed daily for 10 days. Dead larvae were removed every 24 h, and the mortality rate of the pests over time was recorded to evaluate the efficacy of the pathogens under laboratory conditions. The experiment was replicated three times. The deceased adult insects were surface sterilized with 70% ethanol, dried, and subsequently placed in Petri dishes containing moist filter paper for a 10-day observation period to evaluate the development of mycosis. Fungal-induced mortality was confirmed through microscopic examination of spores on the larvae's surface (Cherry et al., 2005).

Statistical analysis

The percentage mortality rates were calculated using Abbott's formula (Abbott, 1925). The experiments were performed with three replications, each replicate involving 10 adult insects. The data were analyzed using a one-way ANOVA at a significance level of 0.05, utilizing SPSS 15.0 software, and mean comparisons were conducted using Duncan's multiple range test.

Results

Isolation and identification of fungi from H. halys

In this study, a total of thirteen fungal isolates from five genera were obtained from both live and dead adult insects. The fungal isolates were identified using molecular methods and classified as *Fusarium solani* (Hf1, Hf2), *Penicillium citrinum* (Hf3), *Alternaria* sp. (Hf4, Hf9), *Fusarium trincinctum* (Hf5, Hf12), *Cladosporium cladosporioides* (Hf6), *Aspergillus niger* (Hf7, Hf11), *Fusarium proliferatum* (Hf8, Hf10), and *Alternaria alternata* (Hf13). The species *F. solani*, *F. trincinctum*, and *A. alternata* were isolated only from dead insects. *P. citrinum*, *Alternaria* sp., and *C. cladosporioides* were isolated exclusively from live insects. No fungi were isolated from the SDA medium, except for *Alternaria* sp. and *C. cladosporioides*. The fungal isolates, grouped according to isolation method and the media used, were presented in *Table 1*. Furthermore, the colony morphologies of the isolates on SDA medium were determined and are shown in *Figure 1*.

Table 1. *Fungi isolated based on the isolation methods and the culture media used*

Isolates	Homogenate			
	Dead adult (60)		Live adult (35)	
	PDA	SDA	PDA	SDA
Fusarium solani	+	-	-	-
Penicillium citrinum	-	-	+	-
Alternaria sp.	-	-	-	+
Fusarium trincinctum	+	-	-	-
Cladosporium cladosporioides	-	-	-	+
Aspergillus niger	+	-	+	-
Fusarium proliferatum	+	-	+	-
Alternaria alternata	+	-	-	-

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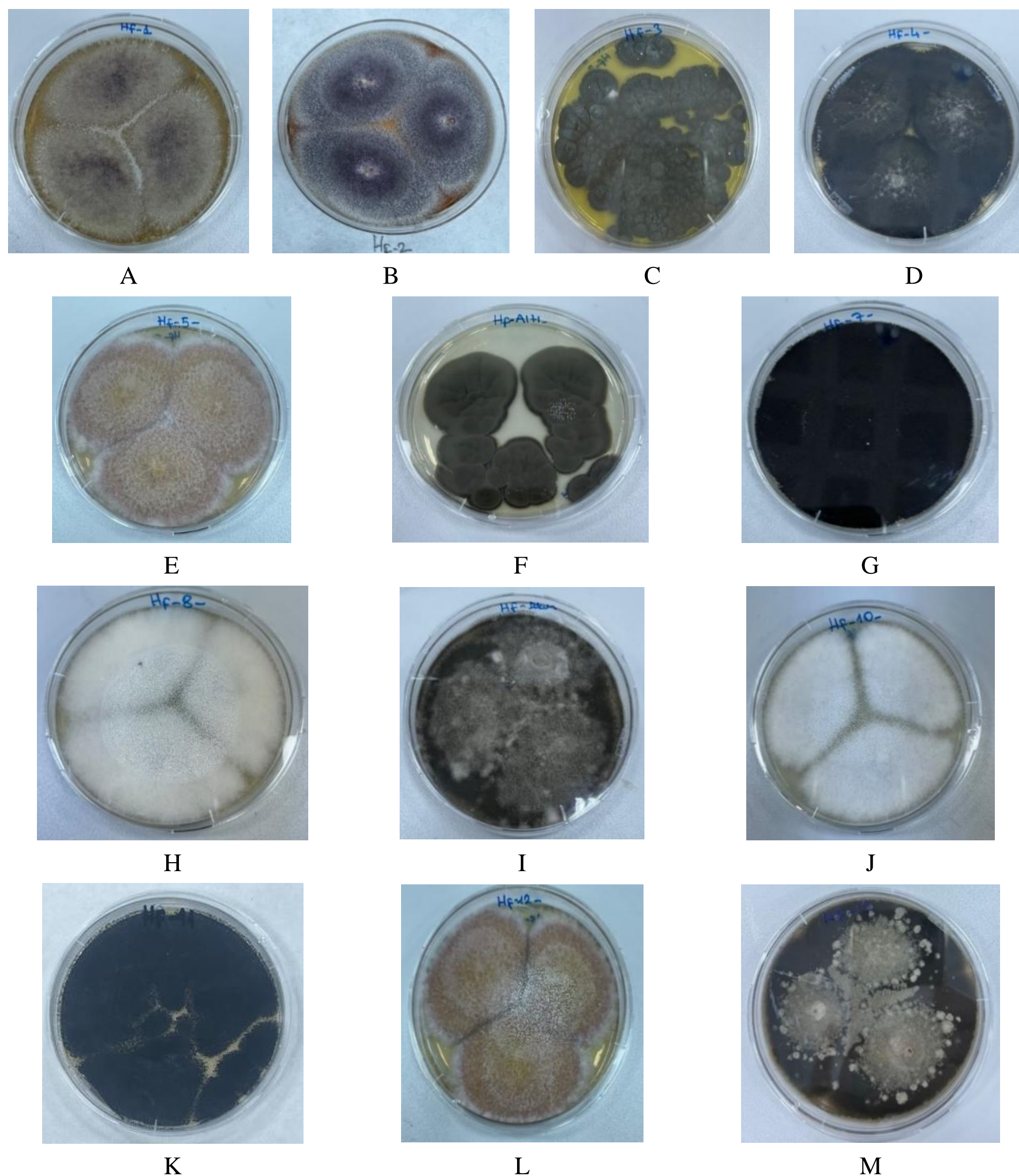

Figure 1. *Colony morphologies of fungi isolated from H. halys on SDA medium. A: Hf1, B: Hf2, C: Hf3, D: Hf4, E: Hf5, F: Hf6, G: Hf7, H: Hf8, I: Hf9, J: Hf10, K: Hf11, L: Hf12, M: Hf13*

The sequences data were deposited in the GenBank database under accession numbers PP999445-PP999457, respectively. The phylogenetic tree was constructed using the Neighbor-Joining (NJ) method with bootstrap analysis (1000 replicates), as shown in *Figure 2*.

Pathogenicity test results of fungal isolates

All fungal isolates and a *Beauveria bassiana* (Paf 09) isolate were tested against adult pests at a concentration of 1×10^7 conidia/ml. In previous studies, the *B. bassiana* PaF09 isolate, obtained from *Pristiphora abietina*, was selected due to its entomopathogenic activity against Coleoptera, Lepidoptera, and Hymenoptera orders,

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specifically *Dendroctonus micans*, *Hyphantria cunea*, and *P. abietina* (Albayrak İskender et al., 2012, 2013, 2017). The Hf1 isolate resulted in the highest mortality, with 60.8 ± 7.0% mortality observed after 10 days of application ($F = 13.967$, $df = 8$, $p < 0.05$). The Paf 09 isolate followed with 56.5 ± 6.6% mortality. The Hf6 isolate was identified as the one causing the lowest mortality with 8.69 ± 3.0. Other isolates led to mortalities ranging from 17.3 ± 3.6% to 43.4 ± 5.6%. According to the pathogenicity test results, a significant difference in mortality was found between the tested fungal isolates and the control group ($F = 13.967$, $df = 8$, $p < 0.05$). The mortality rates recorded on the 10th day post-application are presented in *Figure 3* and *Table 2*. Due to a reduction in sporulation, an insufficient spore concentration was obtained, and consequently, the Hf4, Hf8, Hf9, Hf10, and Hf13 isolates were excluded from the pathogenicity assays. Among the Hf7 and Hf11 isolates, only the Hf7 isolate, which was obtained from dead adults, was considered to have a higher potential for pathogenicity and, therefore, was preferred in the experiments.

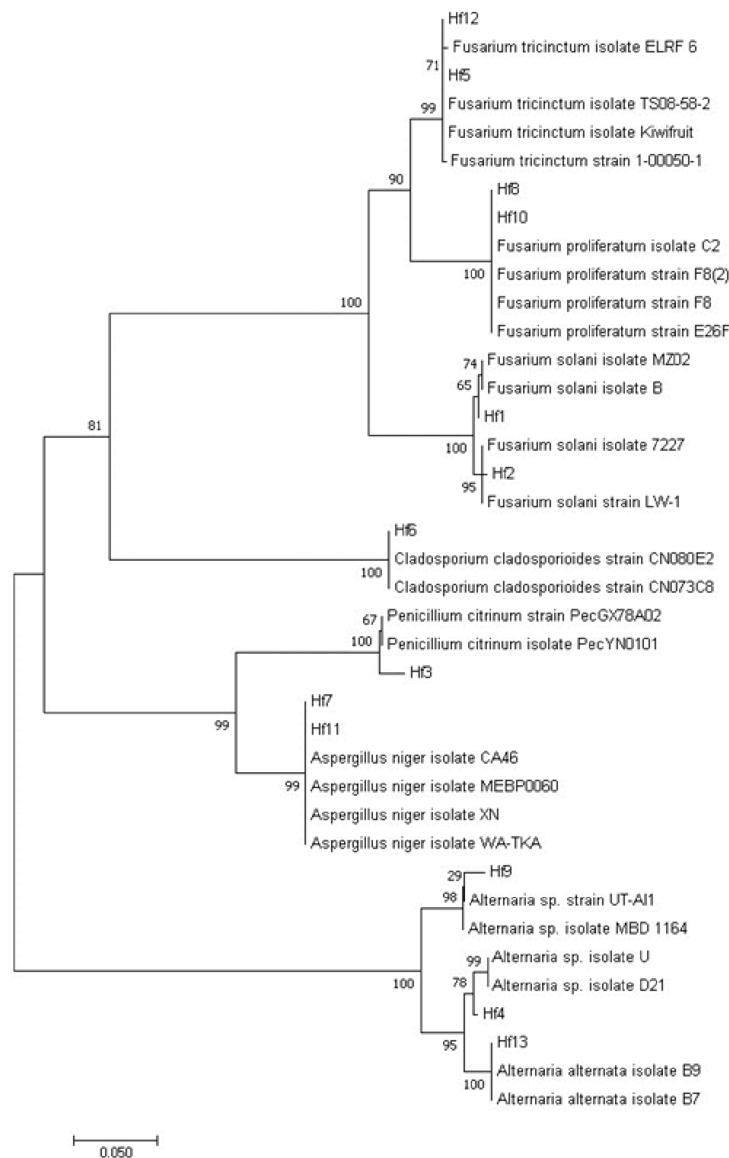

Figure 2. *Phylogenetic tree based on ITS rDNA analysis of fungal strains and closely related species. The scale on the bottom of the dendrogram shows the degree of dissimilarity*

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Table 2. *Percentage mortality rates of fungal isolates on the 10th day post-application ($p < 0.05$; ± SE)**

Isolate	Mortality (%)
Hf1	60.8 ± 7.0^{a}
Hf2	34.7 ± 5.0^{bc}
Hf3	17.3 ± 3.6^{cde}
Hf5	43.4 ± 5.6^{ab}
Hf6	8.69 ± 3.0^{de}
Hf7	43.4 ± 5.6^{ab}
Hf12	26.0 ± 4.3^{bcd}
Paf09	56.5 ± 6.6a
Control	2.3 ± 2.3^{f}

*The differences between the values represented by the same letters in the same column are not statistically significant ($p \leq 0.05$; Duncan's test)

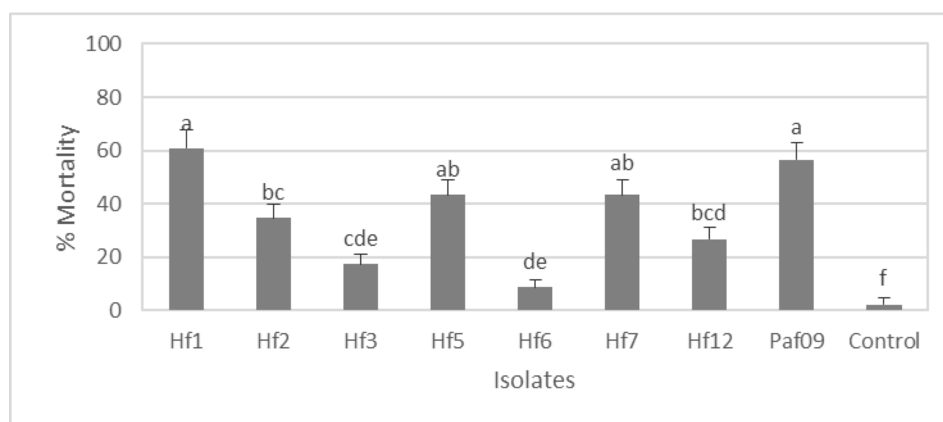

Figure 3. *Mortality of the fungal isolates from the pests and B. bassiana (Paf 09) isolate on the 10th day of the application ($p < 0.05$; ± SE; Duncan's test)**

Discussion

In this study, we aimed to investigate the fungal flora of *H. halys* for the first time and identify potential microbial control agents against this pest. In addition, to contribute to these microbial control studies, the *B. bassiana* (Paf 09) isolate, which was previously identified as highly entomopathogenic in earlier studies, was also utilized.

Total of thirteen fungal isolates were obtained from both live and dead adult insects. The fungal isolates were identified using molecular methods and classified as *Fusarium solani* (Hf1, Hf2), *Penicillium citrinum* (Hf3), *Alternaria* sp. (Hf4, Hf9), *Fusarium trincinctum* (Hf5, Hf12), *Cladosporium cladosporioides* (Hf6), *Aspergillus niger* (Hf7, Hf11), *Fusarium proliferatum* (Hf8, Hf10), and *Alternaria alternata* (Hf13).

The isolates of Hf3, Hf4, Hf6 and Hf9 obtained exclusively from healthy insects suggest the ability of these fungi to colonize healthy insect hosts, which may indicate latent infections or their role in the normal microbiota. In contrast, the isolates of Hf1, Hf2, Hf5, and Hf12 obtained solely from dead insects reflect the pathogenic potential of these fungi. The isolation of Hf7, Hf11, Hf8, Hf10 and Hf13 from both healthy and dead individuals of the same insect species provides valuable insights into the

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pathogenic characteristics of these fungi and the immune responses of the insects. While these fungi may establish a balance with healthy hosts, environmental stressors or a compromised immune system may lead to lethal outcomes.

The species belonging to the genera *Fusarium*, *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* that were isolated have previously been reported from insects of the orders *Orthoptera*, *Isoptera*, *Homoptera*, *Coleoptera*, *Hemiptera*, *Diptera*, and *Lepidoptera* (Carrion and Bonet, 2004; Geib et al., 2012; Kim et al., 2014; Marquez-Fernandez et al., 2020; Singh et al., 2020).

Literature reviews have identified that genera such as *Fusarium* (Teetor-Barsch and Roberts, 1983; Santos et al., 2020), *Alternaria* (Machowicz-Stefaniak and Miczulski, 1985; Christias et al., 2001; Lebody et al., 2021), *Aspergillus* (Essien, 2004; Kaur et al., 2016), *Cladosporium* (Pan et al., 1989; Jeyarani et al., 2011) and *Beauveria* (Alves et al., 2002; Bugeme et al., 2008) host entomopathogenic fungal species.

A total of thirteen fungal isolates were obtained from *H. halys* six of which belonged to the genus *Fusarium*. Among the isolates, *Fusarium* species exhibited the highest prevalence, comprising 46.1% of the total. With the exception of *F. trincinctum* (Hf5, Hf12), species such as *F. solani* (Hf1, Hf2) and *F. proliferatum* (Hf8, Hf10) have previously been isolated from various insect species, with their pathogenicity documented in prior studies (Panyasiri et al., 2007; Qi et al., 2011; Tosi et al., 2015; Sharma et al., 2018; Al-Ani et al., 2018; Abrar et al., 2023). However, this study represents the first evaluation of the pathogenicity and insect isolation of *F. trincinctum* (Hf5, Hf12).

For the first time, *Fusarium solani* has been documented as a native entomopathogen in the pupal stage of the sugar beet root maggot, *Tetanops myopaeformis*. Field surveys conducted on the prevalence of *F. solani*, along with its identification and isolation from *T. myopaeformis* pupae, revealed that the fungus infected 44% of the larvae. Moreover, the pathogenicity of the fungus was confirmed under controlled laboratory conditions, with an average LC50 value of 1.8×10^6 conidia/ml. Symptoms of *F. solani* infection included rapid atrophy of pupal tissue and the failure of adult emergence (Majumdar et al., 2008).

A study conducted in Western Georgia reported the isolation of three *Beauveria bassiana* sensu lato isolates and one *Isaria cf. fumosorosea* isolate from infected adult *H. halys* displaying symptoms of fungal diseases, although no study on the pathogenicity of these isolates was conducted. Furthermore, a laboratory study testing the locally produced, registered Georgian mycoinsecticide (Bover-Ge), derived from soil-origin *B. bassiana-024* strain, on adult stink bugs was conducted. The mycoinsecticide was tested at two different concentrations (1×10^7 and 1×10^8 conidia/ml), and the mortality rate was reported to range from 72.0% to 90.5% on the 12th day. The same researchers found that local nematode isolates (*Heterorhabditis bacteriophora* and *Steinernema borjomiensis*) exhibited low potential for pest control, based on the results of pathogenicity tests, at a humidity level of 80%, with mortality rates of 33-47% and 13-33%, respectively (Burjanedze ve ark., 2020). In a study by Mantzoukas et al. (2024), the impacts of 15 native entomopathogenic fungal isolates on the egg and nymphal stages of *H. halys* were thoroughly examined. The egg hatch duration ranged from 4.5 to 7.0 days, while the survival times of the nymphs varied, with second instar nymphs surviving between 2.1 and 6.6 days, and fourth instar nymphs surviving between 3.7 and 6.8 days. Among all the isolates tested, *Beauveria varroae* demonstrated the highest level of toxicity at all developmental stages,

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positioning it as a promising candidate for the biological control of *H. Halys*. In this study, the *F. solani* Hf1 isolate was first isolated from *H. halys* and exhibited the highest virulence, with a mortality rate of 60.8 ± 7.0% at a concentration of 1 × 10^7 conidia/ml. Compared to similar studies in the literature, the Hf1 isolate demonstrated notable success, with the mortality rate at a low humidity level of 60 ± 1%. pest (Gouli et al., 2011; Tozlu et al., 2019). This can be attributed to the fact that increased humidity enhances pathogenicity, whereas decreased humidity results in a decline in pathogenicity (Sivasankaran et al., 1998). Fungi require high humidity levels for sporulation, germination, and insect infections (Charnley and Collins, 2007). Indeed, the lower the environmental humidity required for optimum pathogenicity, the higher the biological control efficacy of the isolate.

Other *Fusarium* isolates, including *F. solani* Hf2, *F. trincinctum* Hf5, and *F. trincinctum* Hf12, resulted in mortalities of 34.7 ± 5.0%, 43.4 ± 5.6%, and 26.0 ± 4.3%, respectively. This study also demonstrates that fungi of the same genus exhibited varying levels of pathogenicity against the same insect species. Additionally, the fact that the isolates Hf1, Hf2, Hf5, and Hf12 were obtained solely from dead insects suggests that these *Fusarium* strains may be pathogenic to *H. halys* adults.

Beauveria bassiana (Bals.) Vull. is recognized as an effective entomopathogenic fungus against pest insects. It was found that PaF09 caused 100% mortality in *D. micans* larvae at a concentration of 1 × 10^9 conidia/ml and a humidity level of 65 ± 5%. Additionally, at the same humidity level and a concentration of 1 × 10^6 conidia/ml, mortality rates of 80 ± 6.4% and 90 ± 5.7% were recorded in *P. abietina* and *H. cunea* larvae, respectively (Albayrak İskender et al., 2012, 2013, 2017). In the current study, the PaF09 isolate induced 56.5 ± 6.6% pathogenicity in *H. halys* (Hemiptera: Pentatomidae) adults at a concentration of 1 × 10^7 conidia/ml. When compared to the results reported in the literature, the lower humidity level (60 ± 1%) at which the PaF09 isolate was tested is thought to have led to a reduction in its pathogenicity (Sivasankaran et al., 1998).

The *Penicillium citrinum* Hf3 isolate, obtained from the body homogenate of healthy *H. halys* adults, resulted in an observed mortality rate of 17.3 ± 3.6%. However, *P. citrinum* is particularly notable for its high mortality rates in insect larvae (Nguyen et al., 2023). For instance, Maketon et al. (2014) reported that the *P. citrinum* CM-010 isolate, obtained from the fourth-instar larvae of *Culex quinquefasciatus*, induced 100% mortality in third-instar larvae within 2 h at a concentration of 1 × 10^6 conidia/ml under laboratory conditions. Similarly, *P. citrinum* isolated from soil in South Sumatra, Indonesia (*BKbTp*) caused 98.67% mortality in *Spodoptera litura* larvae when applied both as a mycoinsecticide culture filtrate and at a concentration of 1 × 10^6 conidia/ml (Herlinda et al., 2020). These findings highlight the potential of *P. citrinum* as a biocontrol agent, particularly for larvae. Contrary to the existing literature, the low mortality of the Hf3 isolate may be attributed to the more developed defense and resistance mechanisms in adults compared to larvae. Indeed, it is well-established that immune systems in insects begin to develop from the early larval stages (Li et al., 2005), and that the flora defending the insect and competing with its antagonists is less developed in larvae, gradually maturing over time (Behar et al., 2008). Moreover, a fungus that exhibits pathogenic properties in one insect may not show the same effects in other insect species.

Some species within the entomopathogenic fungi, particularly those belonging to the genus *Cladosporium*, have been less studied. Although *Cladosporium* species are not traditionally considered part of the entomopathogenic fungal community, increasing

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evidence suggests that these fungi can infect insects. Specifically, *C. cladosporioides* s.c. has been shown to exhibit virulence against various arthropod taxa, leading to mortality rates (Abdelaziz et al., 2018; Singh et al., 2020; Nicoletti et al., 2024). Bahar et al. (2011) reported that the *Cladosporium* RM16 isolate, obtained from the eggs of *Helicoverpa armigera* (Lepidoptera: Noctuidae), caused 54% mortality in the early instars of *H. armigera*, while it was not pathogenic to later larval stages. This observation suggests that the entomopathogenic potential of *Cladosporium* species may vary across different insect developmental stages, with certain strains demonstrating pathogenicity only at specific life stages. In this study, the Hf6 isolate exhibited the lowest virulence, causing a mortality rate of 8.69 ± 3.0% in H. halys adults.

Aspergillus niger has been previously isolated from various insect species, including *Pectocera fortune* (Coleoptera: Elateridae), *Spodoptera littoralis* (Lepidoptera: Noctuidae), and *Musca domestica* (Diptera: Muscidae) (Sales et al., 2002; Kim et al., 2014; Elkhateeb et al., 2021). In the present study, *A. niger* (Hf7, Hf11) isolates were obtained from insects belonging to the Hemiptera order. During the pathogenicity assays against *H. halys*, the Hf7 isolate, obtained from dead insects, was utilized. Mazid et al. (2015) isolated *A. niger* and *A. flavus* from *Oligonychus coffeae* and tested their pathogenicity at three concentrations (1×10^6, 1×10^7, 1×10^8 conidia/ml). They reported that *A. niger* was more pathogenic than *A. flavus* at all three concentrations. Pasaru et al. (2014) identified local entomopathogenic fungi, including *Aspergillus* sp., *A. flavus*, and *Verticillium lecanii*, isolated from *Helopeltis spp.* (Hemiptera: Miridae), a potential pest of cocoa plants. These fungal isolates exhibited pathogenicity against *Helopeltis spp.* under laboratory conditions, with reported mortality rates of 90%, 80%, and 77%, respectively. The Hf7 isolate resulted in a mortality rate of 43.4 ± 5.6% in the pest.

Numerous studies have documented the pathogenic interactions between *Fusarium* species and insects. The entomopathogenic potential of *Fusarium* species presents a promising and effective strategy for the biological control of agricultural pests. However, the use of these fungi in insect control requires a thorough evaluation of both their beneficial properties and potential adverse effects. Some studies investigating the effects of entomopathogenic *Fusarium* species on plants report harmless effects (Kuruvilla and Jacob, 1979), while others indicate that these species are infective (Kilpatrick, 1961). It is crucial to ensure the safe and efficient use of *Fusarium* species, while minimizing their environmental impact. In this context, the development of reliable markers capable of distinguishing *Fusarium* strains with entomopathogenic properties is essential. Additionally, the necessity of molecular studies aimed at distinguishing the genetic loci responsible for entomopathogenic traits from those causing phytopathogenicity is coming to the forefront. Such research will enable the more effective use of *Fusarium* strains in the biological control of pest insects (Sharma and Marques, 2018). Indeed, the phytopathogenicity of *Fusarium* strains can be controlled through specific mutations, and these can be adjusted for biological control of insects (Navarro-Velasco et al., 2011). Therefore, all these considerations must be taken into account before field trials are conducted on pathogenic species.

Conclusions

Currently, the use of microbial flora isolated directly from the pest itself yields more effective results in pest control. In this study, the internal fungal flora of *Halyomorpha halys* was investigated for the first time. Thirteen fungal species belonging to five

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genera were isolated from both live and dead insects. The pathogenicity of the fungal isolates and *B. bassiana* (PaF09) was evaluated on *H. halys*. The Hf1 isolate was identified as the most virulent, with a mortality rate of 60.8 ± 7.0%. Consequently, this isolate was found to possess potential as a microbial control agent against the pest. Future studies should focus on field trials to evaluate the effectiveness of this isolate.

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