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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Fusarium proliferatum: isolation, identification and evaluation of its association with the model insect *Galleria mellonella* (L.) (Lepidoptera: Pyralidae)

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Abstract: The genus *Fusarium* is widely known to include important plant pathogens and mycotoxin producers. However, many *Fusarium* species have also shown promising potential for insect control. These species are characterised by high mortality rates, rapid action, and prolific sporulation, making them attractive candidates for agricultural pest control. In this study, the micro and macromorphology, DNA sequencing, and effects of *Fusarium proliferatum* isolated from soil using the trap method on the model insect *Galleria mellonella* were investigated. Morphological analyses revealed detailed colony and spore structures on different agar media. The identification of the species was confirmed by DNA analysis using ITS and CaM gene sequences. *F. proliferatum* was observed to prolong the larval stage of the model insect *G. mellonella* while reducing pupal duration, weight, and total egg production. Our results suggest that the fungus exerts a dose-dependent effect on the development and reproduction of *G. mellonella*. Future studies should focus on identifying the secondary metabolites responsible for the observed biological effects, evaluating possible effects on non-target organisms, and conducting field trials under different environmental conditions.

Keywords: *Fusarium proliferatum*, *Galleria mellonella*, developmental biology, fungal pathogenicity, identification, isolation.

Fusarium proliferatum: izolasyonu, tanımlaması ve model böcek *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) ile ilişkisinin değerlendirilmesi

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Öz: *Fusarium* cinsi, önemli bitki patojenleri ve mikotoksin üreticileri içermesiyle dikkat çekmesine rağmen birçok *Fusarium* türünün böcekleri kontrol etmede etkili olduğu ve yüksek ölüm oranlarına neden olma, hızlı etki ve bol sporlanma gibi tarımsal zararlı kontrolü için umut verici özellikler sergilediği bilinmektedir. Bu çalışmada, topraktan tuzak metodu ile izole edilen *Fusarium proliferatum*'un mikro-makro morfolojisi, DNA dizilimi ve model böcek *Galleria mellonella* üzerindeki etkisi araştırılmıştır. Morfolojik analizler, farklı agarlarda ayrıntılı koloni ve spor yapıları ortaya koymuştur. DNA analizi ile ITS ve CaM gen dizileri kullanılarak tür tanımlamasını doğrulanmıştır. *F. proliferatum*'un model böcek *G. mellonella* 'nın larval evresi uzattığı ve pupa süresini, ağırlığını ve toplam yumurta sayısını azalttığı gözlenmiştir. Çalışmamız fungusun *G. mellonella* 'nın gelişme ve üremesi üzerinde doza bağlı bir etkiye sahip olduğunu göstermektedir. Gelecekteki çalışmalar, gözlenen biyolojik etkilerden sorumlu ikincil metabolitlerin tanımlanmasına, hedef dışı organizmalar üzerindeki olası etkilerin değerlendirilmesi ve farklı çevre koşulları altında saha denemelerinin yürütülmesine odaklanmalıdır.

Anahtar kelimeler: *Fusarium proliferatum*, fungal patojenite, *Galleria mellonella*, gelişim biyolojisi, izolasyon, tanımlama.

INTRODUCTION

Fusarium is a genus widely distributed in nature, frequently isolated from soil and various substrates, including decaying organic materials, and is known to engage in both pathogenic and non-pathogenic interactions with plants, animals, and other living organisms (Sharma & Marques, 2018). Given their importance as plant pathogens and mycotoxin producers, *Fusarium* species have been studied over the last two centuries mainly using isolates from crop plants (Summerell & Leslie, 2011). Nevertheless, alternative environments and hosts such as natural

ecosystems, diversified agricultural systems (Laurence et al., 2015), clinical and veterinary samples (O'Donnell et al., 2010) and insects (Aoki et al., 2019) have also proven to be noteworthy sources for the study of *Fusarium* diversity. *Fusarium* species are able to interact not only with plant hosts (De Silva et al., 2017), but also with a variety of animals and even other fungi (Van Diepeningen & De Hoog, 2016; Torbati et al., 2018).

Among the various interactions between *Fusarium* species and insects, pathogenic relationships have received growing attention, with numerous studies revealing the potential of lesser-known *Fusarium* taxa as promising candidates for biological control (da Silva Santos et al., 2020). Nevertheless, the broader application of *Fusarium* in the control of agriculturally important insect pests and as a source of novel insecticidal metabolites remains limited. A key reason is the apprehension surrounding the unintentional introduction of phytopathogenic strains and their associated mycotoxins into ecosystems (O'Donnell et al., 2012). This concern is exacerbated by the taxonomic complexity of the genus, as accurate species identification necessitates a polyphasic methodology that synthesizes morphological traits, biological behaviour, and phylogenetic data (Summerell & Leslie, 2011). Although few studies have investigated the host range of entomopathogenic *Fusarium* species, the available research indicates that certain isolates the cause significant insect mortality also exhibit a degree of host specificity, and pose minimal risk to crop plants. This is attributed to the necessity for host-specific adaptations in successful parasitism (Fan et al., 2014). Furthermore, advances in molecular phylogenetics have improved the resolution of species relationships within the *Fusarium* genus and have become increasingly accessible for use in identification (Geiser et al., 2004). Several *Fusarium* species are important species that exhibit natural entomopathogenic properties, including *Fusarium acuminatum*, *Fusarium proliferatum*, *Fusarium avenaceum*, *Fusarium merismoides*, *Fusarium verticillioides*, *Fusarium oxysporum*, *Fusarium culmorum*, *Fusarium pseudograminearum*, *Fusarium solani*, and *Fusarium subglutinans* (Sharma & Marques, 2018).

In the current study, in addition to classical identification methods, molecular identification of *F. proliferatum* isolated from soil using the trap method was performed using calmodulin (CaM), a powerful DNA marker for rapid identification of fungi, and the Internal Transcribed Spacer (ITS) region, a non-coding DNA sequence separating the 18S, 5.8S, and 28S ribosomal RNA genes that provides good species-level resolution for *Fusarium* species. In addition, its activity on the model organism *G. mellonella* was evaluated, and a step was aimed for applications to deepen our understanding of *Fusarium*-insect association and to explore their potential in agricultural applications.

MATERIAL AND METHOD

Rearing of G. mellonella larvae: The adult moths were obtained from colonies kept at the Department of Biology, Faculty of Sciences and Arts, Balikesir University (BAUN), Turkey. The larvae were reared under controlled laboratory conditions at 30°C, 65±5% relative humidity, and a 12:12 hour light:dark cycle. They were fed with a synthetic diet consisting of 500 g wheat bran, 150 mL honey, 300 mL glycerol, 150 mL distilled water, and 200 g beeswax according to the method described by Sak and Uckan (2009). For this study, larvae weighing 250-300 mg were specifically selected.

Isolation and trap method: In 2020, a total of ten soil samples were collected from various locations in Balıkesir, Türkiye. The samples were collected from a depth of 20–40 cm, with approximately 1 kg of soil obtained from each site. The collected samples were stored in dark at +4°C in sealed and properly labelled plastic bags (Sanchez Pena et al., 2011). For the trap method, each soil sample was transferred to 200 ml culture containers and moistened with sterile distilled water. Five *G. mellonella* larvae were then placed in each container and kept in the dark at a temperature of 26±2°C for 10 days. Larvae exhibiting signs of fungal infection were retrieved and subjected to surface sterilization by immersion in 5% sodium hypochlorite for 5 seconds, followed by rinsing in sterile distilled water. The samples were then subjected to a 5-second treatment with 70% ethanol, followed by three rinses with sterile distilled water. Subsequently, the samples were placed on blotting paper to facilitate the removal of excess moisture. The sterilized larvae were then subjected to an incubation process at a temperature of 26 ± 2°C, within Petri dishes lined with moist filter paper, for a period of 7–10 days. Subsequent isolation of pure fungal cultures was undertaken from larvae exhibiting external fungal growth during the incubation period (Padmaja et al., 2001).

Macro and micromorphology of fungi: The media used in this study included Czapek-Dox Agar (CDA, Millipore), Sabouraud Dextrose Agar (SDA, Millipore), Malt Extract Agar (MEA, Oxoid), and Potato Dextrose Agar (PDA, Millipore). These were used to determine the microscopic and macroscopic characteristics of the fungus and to obtain a conidial suspension. Inoculation was carried out using the three-point method, and the plates were incubated at 28°C for 14 days. After the incubation period, both macro-morphological and micro-morphological examinations of the fungus were conducted. The main macro-morphological features recorded included colony diameter, upper and lower surface color and surface characteristics. For the microscopic examination, various

techniques were utilized, including the Scotch tape method (Larone, 1995), the lamellar culture technique (Fujita, 2013), and the application of Lactophenol Cotton Blue solution (Sigma, Aldrich).

DNA isolation, DNA barcode sequence and phylogenetic analysis: Genomic DNA was extracted from the sample following the manufacturer's instructions using the Plant DNeasy Kit (Qiagen Inc.). Polymerase Chain Reaction (PCR) was conducted in a total reaction volume of 50 µL. The universal primers ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) were employed to amplify the ITS region, whereas cmdD3 (5′-GACTCCCTGACCGAA GAGCA-3′) and cmdA2 (5′-GCCTCGCGGATCATCTCATC-3′) were used for the CaM gene, following the protocols outlined by White et al. (1990) and Sang et al. (1995). For DNA sequencing, PCR products were submitted to a commercial sequencing service for bidirectional sequencing using both forward (5′) and reverse (3′) primers. The obtained sequences were verified and aligned using BioEdit (Hall, 1999), a sequence alignment editor for Windows. Further confirmation was performed through BLAST analysis in the NCBI database. Phylogenetic analysis was carried out using PAUP 4.0 and BioEdit (Hall, 1999), incorporating closely related sequences from the database.

Preparation and application of conidial suspensions: Conidia were collected after 10 days of incubation on SDA at 28 ºC in darkness. To harvest the spores, 10 mL of sterile water containing 0.01% Tween 20 was added to the Petri dishes, and the surface was gently scraped using a glass rod. During the preparation of the spore suspension, mycelial and agar fragments were removed by filtering the mixture through four layers of gauze into sterile 50 mL Falcon tubes. The concentration of conidia was then determined using a Thoma counting chamber under a microscope. Finally, the suspension was adjusted to the desired concentration of 1×10^9 conidia per mL (Fancelli et al., 2013).

Pathogenicity and re-isolating of fungi from G. mellonella larvae: Healthy larvae were used for both the control and experimental groups. The control group was inoculated with sterile physiological serum, while larvae in the experimental group were inoculated with *F. proliferatum* at a specified concentration of 10^7 conidia/mL. The efficacy of the treatment was evaluated by observing the infected insects under controlled laboratory conditions. The ten larvae that died due to the application were placed in a trap adapted from White Trap before the fungus began to develop. To make the trap, a small Petri dish (6 cm diameter) was placed in a larger Petri dishes (9 cm diameter), on which a 5 cm diameter filter paper was placed. After 15 mL of distilled water had been added to the Petri dishes, the larvae were placed on the filter paper in the smaller Petri dishes. The larvae were incubated in Petri dishes with moist filter papers in an incubator set at 26±2°C for 7-14 days. The sporulating dead larvae were photographed. The microorganisms isolated from these larvae were cultured on PDA plates to examine micro- and macromorphological characteristics, followed by DNA extraction (Gradmann, 2008).

Effect of F. proliferatum on the developmental biology of G. mellonella: The experiments were performed in triplicate, utilizing a total of 15 larvae. The larvae were divided into groups of five and inoculated with 2 µL of conidial suspensions at concentrations of 1×10^5, 1×10^6, 1×10^7, 1×10^8, and 1×10^9 conidia/mL in Phosphate Buffered Saline (PBS). Control groups consisted of untreated larvae and larvae treated with 2 µL of PBS. All groups were maintained under standardized laboratory conditions at 26°C, 60% relative humidity, and a 12:12 light:dark cycle. To investigate the effects of fungal exposure on developmental biology, various parameters were assessed, including the duration of larval and pupal stages, time to adult hatching, adult weight, adult lifespan and fecundity. The eggs laid by the adult females hatching during the experiment were collected daily. The total number of eggs produced by each female was recorded until death and counted using an Olympus SZ51 stereomicroscope (Olympus, Japan).

Statistical analysis: In the context of statistical analysis, the Levene test was employed to ascertain the normality of the data distribution, while one-way analysis of variance (ANOVA) was utilized to evaluate the significance of observed differences. The differences were separated using Tukey's honestly significant post hoc tests (HSD). Using SPSS software (SPSS 18.0 for Windows) for data analysis revealed that all tests' differences were statistically significant when $P < 0.05$.

RESULTS

***Morphological identification*:** Colonies incubated on CYA agar at 28 °C for one week exhibit a diameter of 6–7 cm, with a woolly texture, elevated growth, and arachnoid colony edges. The sunken mycelium is well-developed and widely distributed. The colony's reverse is yellowish at the edges, red-veined in the middle, and orange at the center. On CDA, colonies reach 4.5–5 cm in diameter within one week. The colony edges are gelatinous, 1.5–2 cm wide, while the center appears cottony and slightly elevated. The colony is pinkish-white, with a reverse that is white at the edges and pinkish-white to yellow toward the center. Colonies grown on MEA at 28 °C for a week measure 4–4.5 cm in diameter. They display a woolly texture, light pink coloration at the edges and center, and a slightly elevated structure. The middle parts of the colony exhibit a cherry-purple hue. The

colony's reverse is tan in the outer regions, cherry-colored at the center, with darker tones forming a smooth surface. On PDA, colonies reach 5–6 cm in diameter in one week. They are woolly, high, and produce dusty rose-colored aerial mycelia. The colony appears vivid, transitioning from pink-dusty rose to cherry-colored at the center, with a smooth texture. Colonies incubated on SDA at 28 °C for a week measure 6–7 cm in diameter. They are woolly, whitish-mauve in color, with high aerial mycelia that darken toward the center. The colony's reverse features truncated yellow edges, a truncated brown middle section, and darker tones concentrated in the center (Figure 1). Micromorphology: Conidiophores long septate, regular 1.5-3 µm wide. Branch 12-30 x 2.5-3.5 µm, 2-3 phialides arising from branches, phialides 25-32 x 2-3.5 µm. Macroconidia borne in short chains, curved two or more compartmented. Macroconidia are the following sizes: 2-compartment 18-20 x 4-4.3 µm, 3-compartment 15-25 x 4-5 µm, 4-compartment 35-40 x 4-5 µm and 5-compartment 55-80 x 5-6 µm. Microconidia elongate ovoid, centrally swollen, posterior end rounded, 7.5-12 (15) x 3.5-6 µm. False heads (sporogonium) are globose, subglobose or clavate; diameters are 12-55 µm up to 75 µm and broader (Figure 1).

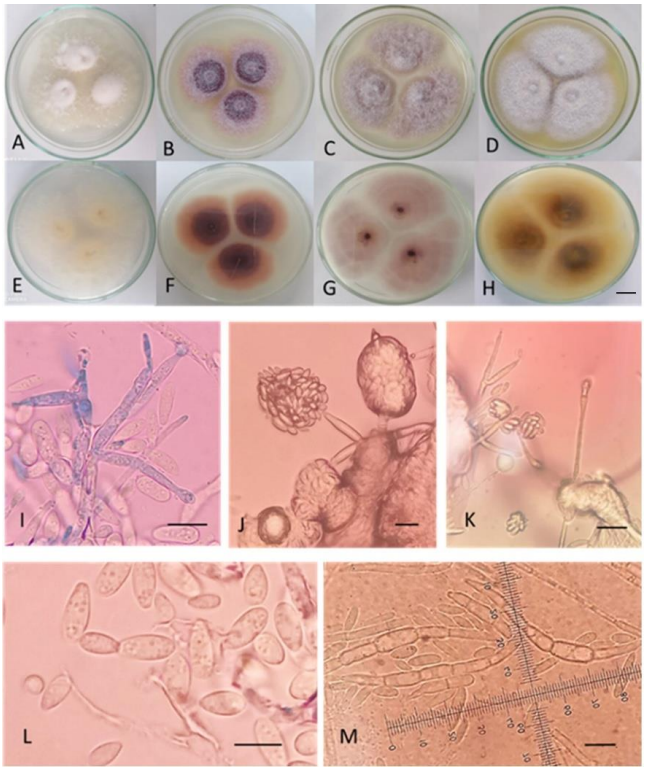

Figure 1. View of the upper surface of colonies A) CDA, B) MEA C) PDA, D) SDA; View of the bottom surface of colonies E) CDA, F) MEA G) PDA, H) SDA I) Detailed observation of conidiophores polyphialids and conidia J) False heads (sporogonium), K) An erect, Acremonium like conidiophore and false heads, L) Microconidia M) Micro and macroconidia. Bars: A-H 1 cm, I 20 µm, J-M 10 µm.

DNA isolation, management of DNA barcode sequence and phylogenetic analysis: The ITS sequence showed 100% similarity to the *F. proliferatum* ITS sequence with the accession number PP702342 in the NCBI database. The BLAST analysis of the CaM gene also showed 100% similarity to the *F. proliferatum* AJ560773. To further support this finding and to show the position of the P4-EP19 strain, an NJ tree was constructed by using closely related sequences like *F. proliferatum, F. verticillioides, F. sacchari, F. annulatum, F. solani, F. subglutinans, F. temperatum, F. oxysporum, F. fujikuroi, F. circinatum, F. anthophilum, F. pseudocircinatum* and *Aspergillus clavatus* as outgroup. The tree clearly showed that the P4-EP19 strain was identified as *F. proliferatum*. The obtained sequences were registered in the NCBI GenBank with the accession numbers PQ164440 for the ITS sequence and PQ514132 for the CaM gene sequence (Table 1; Figure 2).

Table 1. List of sequences used to create the phylogenetic tree.

Taxon	ITS	Calmodulin
	Accession No.	Accession No.
F. proliferatum	PQ164440	PQ514132
F.proliferatum	PP702342.1	AJ560773.1
F. annulatum	OM837286.1	MN534220.1
F. fujikuroi	KJ000442.1	OP913274.1
F. oxysporum	FJ867936.1	OM282988.1
F. temperatum	ON209623.1	ON601574.1
F. subglutinans	PP336543.1	MN534166.1
F. anthophilum	LS422780.1	MW402368.1
F. solani	KY679576.1	KC964172.1
F.sacchari	OP740478.1	MN534226.1
F. pseudocircinatum	OR538655.1	GU737372.1
F. verticillioides	OM956055.1	LS423396.1
F. circinatum	PQ008590.1	MW402364.1
A. clavatus	KP749943.1	LC820498.1

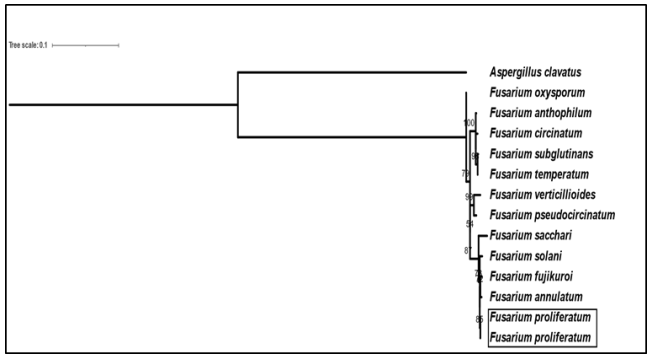

Figure 2. A phylogenetic tree (Neighbor-Joining (NJ)) was constructed using the combined CaM-ITS sequence data of the included species. The sequences of other taxa, together with their accession numbers retrieved from the NCBI database, are listed in Table 1. Branch reliability was assessed using 1,000 bootstrap repeats, and bootstrap values greater than 50% are indicated at the appropriate nodes. *Aspergillus clavatus* was used as an outgroup for the analysis.

Pathogenicity of F. proliferatum against G. mellonella: A total of five *F. proliferatum*-treated *G. mellonella* cadavers were obtained and incubated in moist petri dishes for a period of two weeks. During this time, hyphal growth and sporulation of the fungus were observed on the head, thorax and abdomen of the cadavers. Additionally, the fungus exhibited colour from bright white (Figure 3).

Re-isolating of F. proliferatum from G. mellonella larvae: The sporulating dead larvae were photographed and the fungi growing on these larvae were isolated, purified and cultured on PDA, and the micro and macromorphological

features were determined. When the purified culture was re-inoculated into healthy larvae, mycelium enveloped the larvae, resulting in death. It was confirmed that the fungal species isolated matched the initial microorganism. The results were supported by the DNA extraction method.

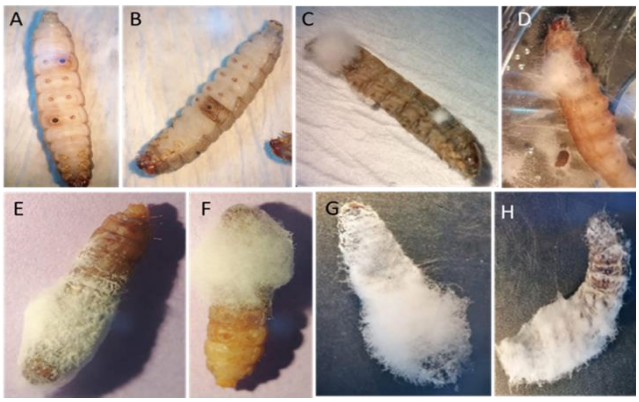

Figure 3. Pathogenicity of *F. proliferatum* aganist *G. mellonella*. A and B: Before fungal growth begins dead larvae (day 5), C and D: The hyphal growth and sporulation of the fungus in different larvae on day 7 after death, E and F: Other dead larvae for 10 days after death showing the sporulation of fungus, G and H: dead larvae for 14 days after death showing the sporulation of fungus.

Effect of F. proliferatum on the developmental biology of G. mellonella:

The effects of conidial suspensions at varying concentrations on the larval and pupal periods of *G. mellonella* larvae are presented in Table 2. The data reveals significant increases in the larval period at conidial doses of 10^6, 10^7, 10^8, and 10^9 conidia/mL compared to the control ($F = 8.934$; $df = 6.\ 121$; $p = 0.000$). While there was no significant difference between the two control groups and 10^5 conidia/mL during the larval period, the increase observed between 10^5 and the higher doses (10^7, 10^8, and 10^9 conidia/mL) was statistically significant. In terms of the pupal period, a highly significant decrease was observed at these doses (10^6, 10^7, 10^8, and 10^9 conidia/mL) compared to the control groups ($F = 6.024$; $df = 6.\ 121$; $p = 0.000$). No statistically significant differences were observed in the adult emergence time ($F = 3.109$; $df = 6.\ 121$; $p = 0.067$) and longevity ($F = 30.273$; $df = 6.\ 121$; $p = 0.317$) at any of the conidial doses compared to the control (Table 3). Significant increases in weight were observed in all treatment groups, including the PBS-control and conidial doses ranging from 10^5 to 10^9 conidia/mL, compared to the control group ($F = 4.625$; $df = 6.\ 121$; $p = 0.000$). In the number of eggs data, significant reductions were observed in the PBS-control group and at all other doses compared to the control group; however, the reduction at the 10^6 conidia/mL dose was not statistically significant ($F = 13.146$; $df = 6.\ 121$; $p = 0.000$). Additionally, the reduction in number of eggs between the lowest doses (10^5 conidia/mL) and the highest doses (10^9 conidia/mL) was statistically significant (Table 4).

Table 2. Changes in the larval and pupal period of *G. mellonella*.

	LARVAL STAGE (DAY)		PUPAL STAGE(DAY)	
	Min.-Max.	$\bar{X}$ ±SH*	Min.-Max.	$\bar{X}$ ±SE*
Control	1-2	1.86±0.09a	14-17	15.53±0.27a
PBS-Control	2-3	2.06±0.06ab	10-26	14.33±0.99a
10^5 conidia/mL	2-16	3.57±0.61abc	2-51	11.50±1.75ab
10^6 conidia/mL	2-28	7.47±1.81bcd	2-14	8.26±0.61b
10^7 conidia/mL	3-28	10.05±1.75d	3-14	9.11±0.99b
10^8 conidia/mL	2-15	8.87±1.19cd	5-14	9.06±0.55b
10^9 conidia/mL	7-28	11.86±1.45d	4-12	8.60±0.55b

The means ($\bar{X}$) in each column that share the same letter are not significantly different (P > 0.05; Tukey's HSD test); SE: Standard error.

Table 3. Changes in the adult emergence time and adult longevity of *G. mellonella*.

	ADULT EMERGENCE TIME (DAY)		ADULT LONGEVITY (DAY)	
	Min.-Max.	$\bar{X}$ ±SH*	Min.-Max.	$\bar{X}$ ±SE*
Control	19-57	27.06±2.53a	5-20	12.66±1.28a
PBS-Control	15-57	27.73±3.27a	10-26	15.73±1.25a
10^5 conidia/mL	9-65	23.84±2.91a	2-21	13.15±1.03a
10^6 conidia/mL	9-78	19.13±3.04a	1-21	11.82±1.03a
10^7 conidia/mL	8-25	16.94±1.16a	2-21	11.83±1.21a
10^8 conidia/mL	12-21	17.31±0.59a	8-21	13.56±0.90a
10^9 conidia/mL	12-31	16.93±1.49a	2-23	12.33±1.57a

The means ($\bar{X}$) in each column that share the same letter are not significantly different (P > 0.05; Tukey's HSD test); SE: Standard error.

Table 4. The variation in the weight and the number of eggs of *G. mellonella*.

	WEIGHT (mg)		NUMBER OF EGGS	
	Min.-Max.	$\bar{X}$ ±SH*	Min.-Max.	$\bar{X}$ ±SE*
Control	15.80-88.40	37.01±4.62a	262-366	321.87±14.03a
PBS-Control	24.30-96.60	65.68±5.80b	6-444	185.30±42.29bc
10^5 conidia/mL	36.30-98.30	59.43±3.67b	5-325	205.05±22.29bc
10^6 conidia/mL	17.40-114.30	61.83±5.58b	102-365	259.61±21.55ab
10^7 conidia/mL	31.00-101.90	65.32±4.29b	1-306	108.50±26.14cd
10^8 conidia/mL	49.30-137.80	76.69±6.20b	5-146	64.08±14.68d
10^9 conidia/mL	27.10-103.10	60.88±5.79b	2-108	70.44±11.49d

The means ($\bar{X}$) in each column that share the same letter are not significantly different (P > 0.05; Tukey's HSD test); SE: Standard error

DISCUSSION

Fungi are genetically and morphologically distinct from other organisms, such as plants and animals, which make their identification and classification a complex task. To overcome these challenges, alternative molecular diagnostic methods, such as polymerase chain reaction (PCR), have been developed. These methods use species-specific primers to effectively differentiate fungal taxa to species level. DNA barcoding, a modern molecular biology technique, has been introduced to overcome these difficulties (Geiser et al., 2004; Nilsson et al., 2019). In our study, genomic DNA of the fungus isolated from the soil by the trap method was extracted, PCR was performed, and sequences were validated by BLAST analysis from the NCBI database, followed by phylogenetic analysis. *F. proliferatum* is assigned to the *F. fujikuroi* species complex, alongside *F. verticillioides*, *F. subglutinans*, and *F. anthophilum*. *F. proliferatum* shares many characteristics with *F. verticillioides*, with the exception that its microconidia are formed in short chains and have false heads on both monophialides and polyphialides. Several studies have emphasised the usefullness of the EF-1α gene (O'Donnell et al., 1998) for species identification. In addition, the internal transcribed spacer (ITS) region of ribosomal DNA (Gurjar et al., 2009) and the β-tubulin gene (Azor et al., 2009) have been used for identifying various *Fusarium* species. In this study, morphological characterization, combined with the analysis of ITS sequences and the CaM gene, confirmed the identification of *F. proliferatum* in agreement with morphological features.

Fusarium species, which are pathogenic to insects, can infect a wide range of hosts, including those from the orders Hymenoptera, Coleoptera, Hemiptera, Diptera, and Lepidoptera (da Silva Santos et al., 2020). The isolation of *Fusarium* species from different developmental stages of insects is important to understand their entomopathogenic potential. For this purpose, samples are taken from different life stages (larvae, pupae, and adults) and specific culture techniques are applied to identify the fungal species (Vannini et al., 2017). In the current study, conidial suspensions were applied to the larvae of the model insect *G. mellonella* to evaluate the pathogenicity of *F. proliferatum*. The efficacy of the treatment was monitored between the seventh and fourteenth day after application. No mortality was observed before the fifth day, and the lethargy and immobility observed in the infected larvae began after this period. Our research also assessed how *F. proliferatum* affects the normal development and reproductive processes of *G. mellonella*. The application of different conidial suspension concentrations to late-stage larvae caused changes in larval and pupal periods, weight, and egg counts compared to the control. Kuruvilla and Jacob (1979; 1980) had previously observed that certain *Fusarium* strains exhibit high pathogenicity in insects, noting that a 100% mortality rate was achieved within three days of applying *F. oxysporum* to the rice brown planthopper (*Nilaparvata lugens*, Delphacidae: Homoptera). Thangam et al. (2014) identified *F. proliferatum* as an entomopathogenic agent associated with mango hopper species, using sequencing of the Translation Elongation Factor-1α (EF-1α) gene. Similarly, Tosi et al. (2015) isolated *F. proliferatum* from a gall wasp and suggested its potential as a biological control agent against the Asian chestnut gall wasp (*Dryocosmus kuriphilus*, Hymenoptera: Cynipidae). In a separate study, Ganassi et al. (2001) observed that *Schizaphis graminum* (Hemiptera: Aphididae) nymphs exhibited mortality within 18 to 24 hours following exposure to *F. proliferatum*.

One of the main problems with the use of *Fusarium* in biological control is the potential unintentional release of phytopathogens into the environment (O'Donnell et al., 2012), as many species of this genus are known to cause plant diseases (Summerell & Leslie, 2011). In order to utilise *Fusarium* strains for insect control, it is important to evaluate the species in terms of their host specificity. The specificity of entomopathogenic fungi varies significantly not only between genera and species, but also among strains of the same species. For biocontrol applications, the isolate is more crucial than the species entity itself (Goettel et al., 2010), therefore the potential for strain-level interactions needs to be investigated (Leger et al., 2011). In our study, an isolate of *F. proliferatum* identified and isolated by the trap method was tested on a model organism to serve as a preliminary step for more targeted research.

CONCLUSION

In conclusion, the study of *Fusarium* species associated with insects provides new insights into their ecology and advances knowledge of *Fusarium* diversity and taxonomy. Despite the mounting evidence from numerous studies that these fungi have considerable potential for use in insect control, there is a paucity of research investigating several key areas. These include the specificity of these fungi for host insects, the production of undesirable secondary metabolites, and the side effects and safety tests with non-target organisms. Consequently, these aspects need to be carefully evaluated for all *Fusarium* species that are candidates for biological control.

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