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Identification of main pathogenic *Fusarium* species for biological control of poppy plants *in* Afghanistan

H. Bahlolzada
H. Saremi ✉
M. Fallahi
M. Javan-Nikkhah
Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

Abstract: Afghanistan is one of the major countries which contributes to illegal poppy (*Papaver somniferum* L.) cultivation globally. The objectives of the present study were to report the *Fusarium* species associated with *P. somniferum* and to investigate a potential biocontrol agent against *P. somniferum*. A total number of 155 *Fusarium* isolates were obtained from four provinces in Afghanistan, including Helmand, Kandahar, Daikundi and Bamyan. The pathogenic *Fusarium* spp. isolates were characterized by morphological and molecular identification, using the translation elongation factor1-α (*TEF1-α*) gene. The results of isolation revealed the most predominant species as *F. proliferatum* (27%), followed by *F. equiseti* (26%), *F. solani* (13%), *F. incarnatum* (12%), *F. acuminatum* (7%), *F. oxysporum* (5%), *F. verticillioides* (5%), *F. pseudograminearum* (2%) and *F. coeruleum* (2%). With the exception of *F. oxysporum* and *F. solani*, other identified species were the first reported *Fusarium* species associated with poppy plants in Afghanistan. Furthermore, pathogenicity testing of *F. oxysporum*, *F. proliferatum*, *F. acuminatum* and *F. verticillioides* isolates were performed. Although disease symptoms were observed in all species, *F. oxysporum* infected poppy samples showed an early development of wilt symptoms and their disease severity was significantly more than all other isolates. The results suggest that *F. oxysporum* isolates have the potential to be used as biological control agents against poppy plants in Afghanistan.

Keywords: Pathogenicity, morphological characters, biocontrol agent

INTRODUCTION

Opium poppy (*Papaver somniferum* L.) is an important medicinal plant known for its beneficial effects and immediate pain relief. More than 2500 different compounds have been identified in opium poppy that are used in pharmaceutical industries (Mishra et al. 2013). However, the major drawback of this species is its potential for abuse and addiction, posing serious threats to public health, governance and security. According to World Drug Report (2019), about 271 million people aged between 15 and 64 years old have used drugs in the previous year and among them an estimated of 54 million people used opium. With 263,000 ha under illicit opium poppy cultivation in 2018, Afghanistan remained the largest global contributor (UNODC 2018). Afghanistan's largest area of opium poppy cultivation in Helmand province, the country's major producer, was estimated at 136,798 hectares (UNODC 2018). Despite the efforts deployed by international organizations to suppress or reduce illegal opium poppy cultivation, it still remains a major challenge in Afghanistan.

Today, acquisition and application of novel natural enemies have become a relevant and efficient strategy for weed control worldwide (McEvoy & Coombs 2000; Aneja 2009). Biological control is a well-established tool for invasive species control. It involves releasing predators, parasitoids, pathogens or antagonist populations into the field (Heimpel & Mills 2017). The literature is rich with studies illustrating the efficiency of biocontrol agents against plant pathogens, but very few studies have taken this approach to control narcotics supply in Afghanistan.

Fusarium species are one of the most important genera of plant pathogenic fungi on earth. Members of this genus are usually terrestrial and cause various symptoms and diseases such as dry rot, sudden or prolonged wilting, head blight, chlorosis and eventual death of plants (Saremi & Saremi 2013; Fallahi et al. 2019). *Fusarium oxysporum* formae speciales has specific host ranges and can remain in soil as chlamydospores for years, or even decades (Nelson 1981).

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✉ Corresponding Author E-mail: hsn.saremi@ut.ac.ir
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Strains of pathogenic *F. oxysporum* are one of the most studied mycoherbicides for parasitic weeds due to high levels of specificity (Cohen et al. 2002; Altinok 2013). Moreover, this species can tolerate environmental stresses such as drought and heat (Nazer Kakhaki et al. 2017). Many studies have illustrated *F. oxysporum* ability to control and manage various agricultural parasitic weeds, including broomrape species (*Phelipanche* spp. and *Orobanche* spp.) (Nazer Kakhaki et al. 2017; Nosratti et al. 2017) and witchweed (*Striga hermonthica*) (Ciotola et al. 2000).

Fusarium oxysporum is not only used against plant diseases and invasive weeds but also has the potential use of fungal strains as mycoherbicides against illicit drug crops. McPartland & Hillig (2004) reported the use of *F. oxysporum* f.sp. *cannabis* and *F. oxysporum* f. sp. *vasinfectum* to control plants in the genus cannabis, including *Cannabis sativa* (fiber hemp and cannabis). They caused complete wilt in different regions of the world, including eastern and western Europe, central Asia and North America, over 50 years ago. In a study by Bailey (1998), seven isolates of *F. oxysporum* f. sp. *erythroxyli* were tested for pathogenicity on coca plants. Although the mortality of coca plants was variable among the strains, ranging from 35% to 85%; the research demonstrated *F. oxysporum* as a potential biocontrol agent against coca. Many other studies have also reported *F. oxysporum* strains as an alternative to control narcotic plants, including hemp (*Cannabis sativa* L.) (e.g. Hildebrand & McCain 1978), poppy (e.g. Saremi et al. 2018) and coca (e.g. Sands et al. 1997).

Despite various attempts to control *P. somniferum* cultivation in Afghanistan, not many advances in scientific research on biocontrol have been undertaken in this region and opium poppy continues to be cultivated by local populations. The main objectives of this study were to isolate and identify the most pathogenic *Fusarium* species associated with *P. somniferum* and to explore whether these isolates can be used as biocontrol agents for opium poppy in Afghanistan.

MATERIALS AND METHODS

Field survey

The field survey was carried out in the 2018 and 2019 cropping seasons, from opium poppy fields in four provinces in Afghanistan, including Helmand, Kandahar, Daikundi and Bamyan[1] (Fig. 1). A total number of 80 samples were collected.

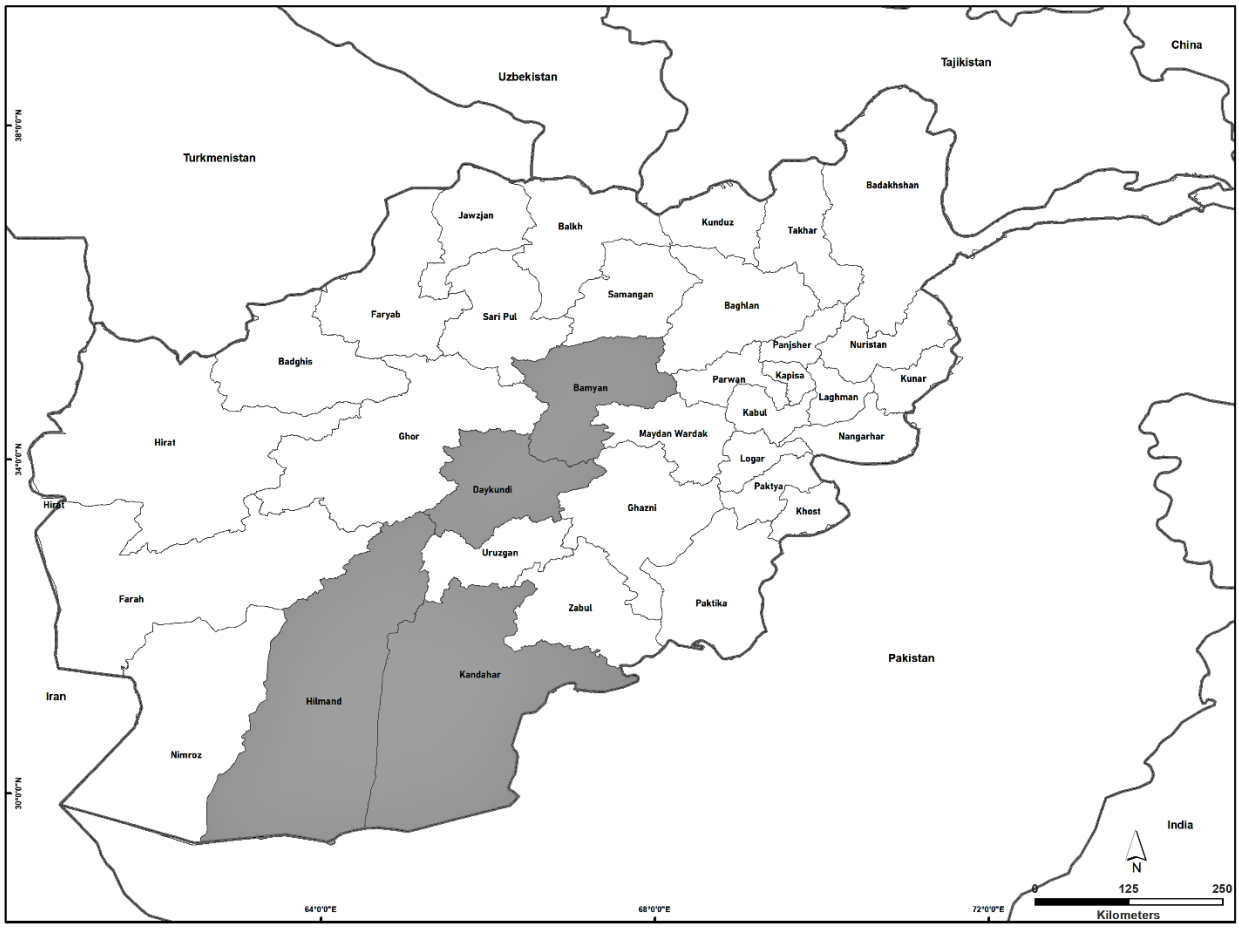

Fig. 1. Map showing the sampling regions in grey.

[1] Due to security restrictions, the exact locations of the study areas are not exposed.

Isolation of *Fusarium* species

The sampling method was based on a hierarchical method (McDonald et al. 1999). Opium plants with symptoms (traces of *F. oxysporum*) were placed in paper bags, transferred to the laboratory and air-dried at room temperature for 6-7 days. The prepared samples were surface sterilized for 1 min in 3% sodium hypochlorite solution, rinsed twice in sterile distilled water, dried on filter paper and placed on Nash and Snyder medium (Nash & Snyder 1962), including 15 g peptone, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 20 g agar, 1 g pentachloronitrobenzene (PCNB; Terraclor 75% WP) in 1 liter of distilled water. All the plates were incubated at 25 °C for 5-7 days. Mycelial tips of the fungal cultures grown on the medium were transferred to new potato dextrose agar (PDA) plates using a single spore technique (Leslie & Summerell 2006).

Morphological identification

Colony characteristics of the fungal isolates were observed after the cultures were grown on both carnation leaf agar (CLA) and synthetic nutrient agar (SNA; 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCL, 0.2 g glucose, 0.2 g sucrose and 20 g agar per liter) medium for 14 days. The *Fusarium* species were identified on the basis of macroscopic traits such as colony appearance, growth rate of the colony, pigmentation, absence or presence of microconidia, characteristic of macro- and microconidia and conidial measurement using Leslie & Summerell (2006) key of identification.

Molecular identification

Total genomic DNA of *Fusarium* species was isolated using the method described by Zhong & Steffenson (2001). Approximately 100 mg mycelium of each isolate were finely grounded and suspended in 400 µL of extraction buffer (50 mM Tris-HCl (pH 8.0), 150 mM EDTA, 1% sarkosyl, and 300 µg proteinase K per mL). The contents were transferred into a 1.5 mL microtube, mixed by vortex for 10 s and incubated for 30 min at 65°C. After incubation, 400 µL of chloroform: isoamyl alcohol (24:1) was added, mixed for 10 min, and then centrifuged at 10,000 rpm for 10 min at 4°C. The aqueous clear phase was mixed with an equal amount of isopropanol and stored overnight for precipitation of DNA at -20°C. DNA was recovered by centrifugation at 10,000 rpm for 3 min and precipitated with 70% ethanol. The DNA pellet was then rinsed twice with 1 ml of 10 mM Tris-HCl and 0.1 mM EDTA or sterile distilled water buffer. The purity and quantity of extracted DNA were determined using NanoDrop 2000c Spectrophotometer (Thermo Scientific).

Polymerase chain reaction

A standard polymerase chain reaction (PCR) assay was performed using the elongation factor (*TEF*-1*α*) gene sequencing. The *TEF*1-*α* gene fragment was amplified with EF-1 (5´-ATGGGTAAGGAGGACA AGAC-3´) and EF-2 (5´-GGAAGTACCAGTGATCA TGTT-3´) primers.

The reaction mixture of PCR was prepared in a final volume of 25 µL containing 3 µL Taq DNA Polymerase 2X Master Mix RED (Ampliqon, Denmark), 1 µL of each primer, and 10 µL ddH_2O. Amplifications were conducted with PTC-200 Peltier thermal cycler (MJ research inc., Watertown, MS, USA) using the following conditions: initial denaturation at 95°C for 5 min; 35 cycles of denaturation for 50 s at 94 °C, annealing for 40 s at 58 °C, initial extension for 50 s at 72°C, and final extension of 5 min at 72°C. PCR products were detected through gel electrophoresis using 1% agarose gel.

Sequencing and phylogenetic analysis

DNA sequencing of *Fusarium* isolates were aligned with reference sequences obtained from GenBank, using the multiple alignment program, EditSeqTM 6.1 (DNASTAR Inc., Madison, WI), and the resulting alignment was manually edited in BioEdit sequence editor version 7.2.5 (Hall 1999). The EF-1α sequence was downloaded from National Centre for Biotechnology Information (NCBI) using the Basic Local Alignment Tool (BLAST) and used as the reference genome. It was annotated by aligning genomic DNA sequences using the program MEGA 6.0 (Tamura et al., 2013). In addition, to validate the phylogenetic position of *Fusarium* sp., the sequences of *Microdochium nivale* were used as the out-group for phylogenetic analysis. The phylogenetic tree was constructed using the Maximum Likelihood (ML) method based on the Kimura two-parameter model. To determine the position of each strain in a phylogeny, several sequences representative of *Fusarium* species were selected from the database to construct the phylogenetic trees. To ensure consistency of data and group support in the phylogenetic tree, bootstrap values were calculated with 1000 replicates.

Pathogenicity test

Glasshouse experiments were performed based on the previous detection results on several selective isolates. Accordingly, poppy plants at 4-5 leaf stage were selected and inoculated using a root-dip inoculation method. The spore suspension was prepared according to Garibaldi et al. (2004) method. Ten-day-old cultures grown on PDAincubated at 25 ºC under 12 h of fluorescent light per day were used as the primary inoculum sources. Fungal spores were harvested by scraping the surface of the cultures maintained in PDA with a sterile loop, and spore suspensions were made with sterilized distilled water. Spore concentrations were determined using a hemocytometer and diluted to 1×10^5 microconidia mL^{-1} water. Thirty healthy poppy plants were randomly chosen, their roots were dipped for 1 min in the spore suspension of each isolate and later transplanted to 15-cm-diameter pots. Pots were filled with mixtures of sand, soil and animal manure in the

proportion of 4:2:1 and were maintained in the glasshouse at a temperature ranging between 25ºC to 28 ºC for 50 days. Control plants were prepared similarly but soaked in plain deionized water. The assay was performed with 3 replicates per isolate, and the experiment was repeated twice. The observations of wilt incidence and symptoms were recorded every 10 days and evaluated by wilting percentage. The *Fusarium*-infected plants were rated visually on a scale of 0 to 100 as described by Bertetti et al. (2015), where 0 represented a healthy plant; 25 showed a growth reduction of 25% (vascular discoloration and slight chlorosis) in comparison with healthy control; 50 corresponded to strong growth reduction (vascular discoloration and chlorosis); 75 represented wilting with initial collapse symptoms (dark streaks in the vascular system and unilateral yellowing of leaves) and 100 indicated a dead plant.

Statistical analysis

Statistical analyses were conducted using R Studio version 1.1.463 (R Studio Team, 2018). A Generalized Linear Model (GLM) with a normal error structure and an identity link function were applied to determine the effects of isolates within the sample plants.

RESULTS

***Fusarium* species**

Based on morphological characteristics, a total number of 155 isolates were recovered from the infested poppy plants and 9 different *Fusarium* species were identified and grouped. These were, namely *Fusarium proliferatum* (27%), *F. equiseti* (26%), *F. solani* (13%), *F. incarnatum* (12%), *F. acuminatum* (7%), *F. oxysporum* (5%), *F. verticillioides* (5%), *F. pseudograminearum* (2%), *F. coeruleum* (2%). *F. proliferatum* was found as the most prevalent *Fusarium* species in poppy fields in the region.

The distribution of *Fusarium* species isolates varied within the four locations. As can be seen in Fig. 2, Helmand province recorded the most isolates (38%), followed by Bamyan (25%). All of the 9 *Fusarium* species were observed in Helmand and Kandahar; however only 8 species were found in Bamyan and *F. pseudograminearum* was not identified in this area. The region with the least diversity of *Fusarium* species was Daikundi, since *F. verticillioides*, *F. pseudograminearum* and *F. coeruleum* species were not identified in this region.

Sequence analysis

Besides the morphological identification, the identity of 12 pathogenic *Fusarium* isolates was confirmed using the universal primers EF-1 and EF-2 (Table 1). These *EF1-α* gene sequences were subjected to produce a dendrogram using the ML method with bootstrap analysis of 1000 replications. In the phylogenetic analysis (Fig. 3), the isolates fall into 8 distinctive clades, namely, clades I to VIII. Most of the support values for these clades were very high (more than 90% bootstrap value; BP), with the exceptions of clade IV (64% BP).

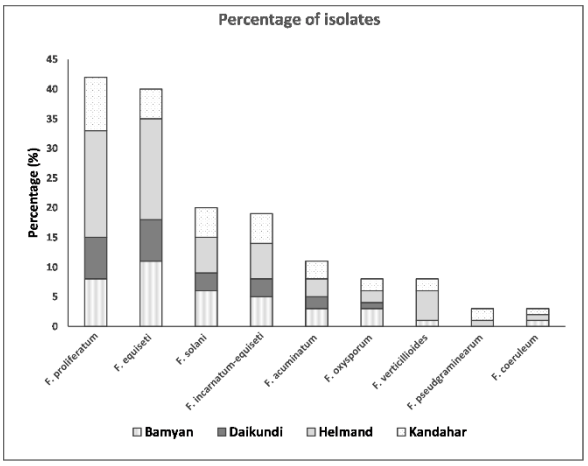

Fig. 2. Distribution of *Fusarium* isolates collected from different sampling sites in Afghanistan.

Table 1. List of *Fusarium* species isolated from poppy plants that were used to perform phylogenetic analysis.

Species	Isolate	Sampling site
F. pseudograminearum	AFHMG1-8	Gerishk - Helmand
F. acuminatum	AFBYW8	Waras - Bamyan
F. oxysporum	AFBYW24	Waras - Bamyan
F. verticillioides	AFHMN79	Nad-e Ali - Helmand
F. proliferatum	AFDKN10۳	Daikundi - Nili
F. equiseti	AFDHM71	Garmsir - Helmand
F. solani	AFHMD49	Garmsir - Helmand
F. incarnatum-equiseti	AFHMD45	Garmsir - Helmand
F. incarnatum-equiseti	AFHMD32	Garmsir - Helmand
F. acuminatum	AFJHMAF9	Nahar-e-Seraj - Helmand
F. equiseti	AFBYW22	Waras - Bamyan
F. coeruleum	AFHMN70	Nad-e Ali - Helmand

AFHMN79 isolate in clade I was shown to be highly homologous to *F. verticillioides*. This isolate, among other isolates such as *F. proliferatum* (AFDKN103-clad II) and *F. sacchari* (NRRL 13999), were associated with *Fusarium fujikuroi* species complex (FFSC). The isolate AFBYW24, with 97% homology, was closely related to *F. oxysporum.* Based on the morphological characteristics, AFHMN70 isolate was very similar to *F. oxysporum.* However, in a phylogenetic tree with low support (64% BP) it was identified as *F. coeruleum*. With high support, AFJHMAF9 and AFBYW8 isolates (clade V) were identified as *F. acuminatum* and AFHMD49 as *F. solani* (clade VI). The AFHMG1-8 isolate in group VII was located next to *F. verticillioides* reference sequence and separated from *F. graminearum*.

Four isolates were identified as *F. equiseti* and *F. incarnatum*, which belong to the *F. incarnatum-equiseti* species complex (FIESC) based on morphological characteristics (Fig. 3).

The FIESC members, the *Equiseti* and *Incarnatum* clades were identified to the phylogenetic species level, as illustrated in Fig. 4. AFBYW22, AFHMD71, AFHMD45 and AFHMD32 isolates were clustered separately with high bootstrap support from all other *Fusarium* species and were confirmed to be a member species of the FIESC 14, FIESC 5, FIESC 23 and FIESC 24, respectively.

Pathogenicity test

Following the identification of the isolates, four isolates with higher bootstrap values than 90% were selected for the glasshouse trial. These were AFBYW24 (*F. oxysporum*), AFBYW8 (*F. acuminatum*), AFDKN103 (*F. proliferatum*) and AFHMN79 (*F. verticillioides*). The plants inoculated with these isolates showed external symptoms of wilting and leaf yellowing, however, with different wilting severity. The first symptoms of disease observed 10-15 days after inoculation. Among these pathogenic isolates, AFBYW24 was highly virulent and showed severe wilting symptoms in comparison with the control sample ($P < 0.001$). The presence of the *F. oxysporum* isolate in the plants caused an average of 83% growth loss. Other isolates also caused wilting symptoms; however, symptoms varied depending on the *Fusarium* species inoculated. AFBYW8 and AFHMN79 showed an average growth reduction of 50% ($P < 0.01$) and AFDKN103 reduced growth by 42% ($P < 0.05$). While all isolates showed significant differences with control, *F. oxysporum* led to the early development of wilt symptoms and significantly increased disease severity compared to all other isolates. Symptoms caused by *F. oxysporum* consisted of root and crown rot, yellowing and wilting in aerial parts of the plants (Fig. 5). The non-inoculated seedlings exhibited no symptoms.

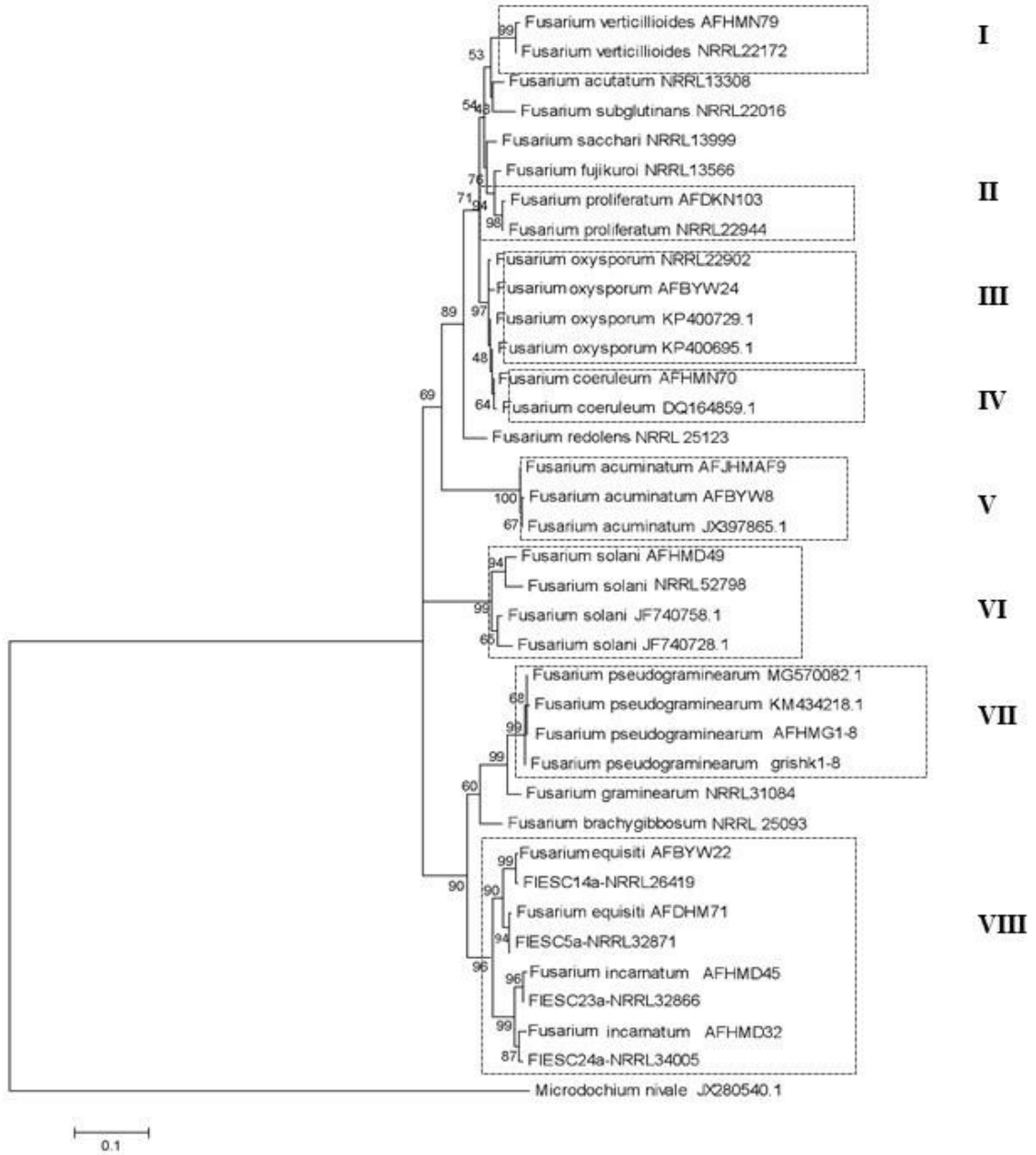

Fig. 3. Maximum likelihood phylogenetic tree showing the diversity of *Fusarium* isolates associated with poppy plants from Afghanistan. The *Michrodochium nivale* was used as the out-group. Bootstrap values are shown next to the branches.

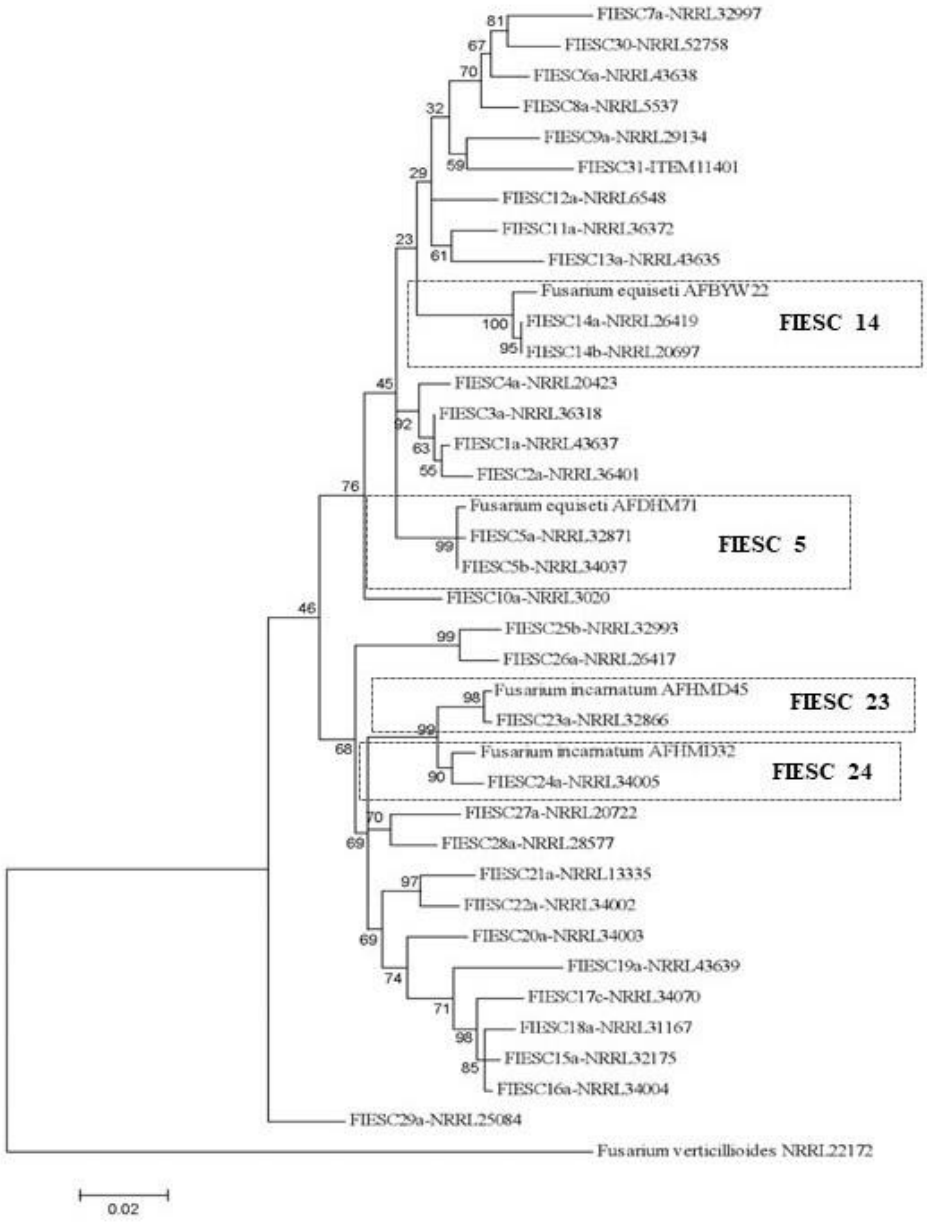

Fig. 4. Phylogenetic analysis of FIESC sequences of four AFBYW22, AFHMD, AFHMD45, AFHMD32 isolates based on of EF-1α gene. *Fusarium verticillioides* is used as the out-group. The phylogenetic relationships were inferred by using the Maximum Likelihood method, with 1000 bootstrapped replicates.

Fig. 5. Pathogenicity of *Fusarium oxysporum* AFBYW24 on the poppy plant. a. Symptoms after 15 days of inoculation; b. Symptoms of crown and root rot after 15 days of inoculation, c. Control plant after 15 days of inoculation.

DISCUSSION

Based on morphological and molecular identification, *F. proliferatum* and *F. incarnatum-equiseti* species complex (FIESC), respectively, were found to be the dominant species in the study area. Helmand and Kandahar (Southern Plateau), with the mean annual temperature of 23.3 ºC and annual precipitation of 116 mm, showed more variety than Bamyan and Daikundi (Central Highlands) with an average temperature of 5.2 ºC and 332 mm of annual rainfall. *Fusarium pseudograminearum* has been reported to be more prevalent in relatively warm and dry environmental conditions (Chakraborty et al. 2006; Poole et al. 2013). The absence of the *F. pseudograminearum* in central highlands could probably be a result of cooler temperatures and high rainfall of the regions. Geographical and climatic factors are significant in determining the occurrence and pattern of infection of different *Fusarium* species; however, very limited studies have been conducted about the distribution of *Fusarium* species in Afghanistan. Further investigations and measurements are needed to assess the contributions of geographical and climatic effects on this genus in the study area.

Results of this study revealed that several *Fusarium* species could infect poppy plants. *Fusarium solani, F. oxysporum* and *F. equiseti* were also reported by Saremi et al. (2018) as species recovered from infected poppy plants. However, to the best of our knowledge, this is the first study to associate *F. coeruleum, F. proliferatum, F. pseudograminearum,* and *F. verticillioides* species with poppy plants. Furthermore, *F. acuminatum, F. incarnatum* and *F. equiseti* are reported for the first time on poppy plants in Afghanistan.

To date, considerable effort has been put into examining the taxonomy and correct detection and identification of *Fusarium* species. The results showed the *TEF*-1*α* gene is a useful phylogenetic marker for distinguishing *Fusarium* spp. and members of the species complex. Partial sequences of *EF*-1*α* have proven to be a useful gene for resolving species boundaries in *Fusarium* spp. and provide phylogenetic information at deeper levels for this species (Geiser et al. 2004; Villafana & Rampersad 2020). Within the FIESC, 33 (with the designations of FIESC 1 – FIESC 33), phylogenetically distinct species have been recognized, where FIESC 1-14 were molecular siblings of *F. equiseti* and the remaining were grouped as *F. incarnatum*. The species-level phylogenetic analysis of the four isolates in our study showed two groups including *Incarnatum* (FIESC 23 and FIESC 24) and *Equiseti* (FIESC 5, FIESC 14) species based on the *TEF*-1*α* gene region. Although many researchers have applied molecular phylogenetic analysis approaches for clarification of taxonomic ambiguities in *Fusarium* species, more comprehensive phylogenetic analyses with suitable nucleotides and amino acid substitution rates are required.

Numerous studies have considered members of the FIESC as moderately aggressive phytopathogens or secondary invaders of plants (e.g., Summerell et al. 2003; Leslie & Summerell 2011). However, *F. oxysporum* has been reported as causing symptoms of chlorosis, foliar wilting and necrosis in poppy plants (Saremi et al. 2018). In this study, the pathogenicity and the capacity of *F. oxysporum* as a potential biocontrol agent against opium poppy in Afghanistan have been demonstrated. Based on the pathogenicity test, AFBYW24 was very virulent and caused the highest pathogenicity among the isolates ($P < 0.001$) with more than 80% growth reduction in poppy plants. The plants infected with this isolate showed rapid symptoms of wilting and necrotic spots on surfaces, while plants infected with other isolates showed mild disease symptoms. Our results were in agreement with Garibaldi et al. (2012), who observed symptoms of early leaf chlorosis, leaf and stem wilting, bending and eventual rotting of Iceland poppy when affected with *F. oxysporum*.

Although our study showed pathogenic strains of *F. oxysporum* could potentially be used against *P. somniferum* in controlled-environments, further studies are required to determine the virulence under highly diverse natural environments including field conditions. Saremi et al. (2018) reported that differences in soil types, temperatures and relative humidity under various field and glasshouse conditions had no significant effect on the disease severity of *F. oxysporum*; suggesting it would be an effective biocontrol agent in the field. However, further measurements and validations are needed to concisely determine the effect of *F. oxysporum* against opium poppy across different soil types and microclimates.

Although several reports have shown the advantage of biological control programs in agricultural industries, the implementation of bioherbicides for the control of illicit crops has not been widely undertaken, especially in Afghanistan. In recent years, the threat of simulation to cause direct damage in the agricultural sector and agricultural bioterrorism have raised widespread concerns about biocontrol programs on narcotic plants (Anderson et al. 2004). Nevertheless, the host specificity of *F. oxysporum* (Saremi et al. 2018) and its low risk for environmental damage compared to man-made chemical solutions suggest that this species could be effectively used as a biological control agent on poppy plants.

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شناسایی اصلی ترین گونه های بیماری زای فوزاریوم برای کنترل بیولوژیک گیاه خشخاش در افغانستان

حبیب اله بهلول زاده، حسین صارمی✉، مریم فلاحی، محمد جوان نیکخواه
گروه گیاهپزشکی، پردیس کشاورزی و منابع طبیعی، دانشگاه تهران، کرج

چکیده: افغانستان یکی از اصلی‌ترین کشورهایی است که در سطح جهان با کشت غیرقانونی گیاه خشخاش (*Papaver somniferum* L.) مواجه است. هدف ازاین تحقیق بررسی ارتباط گونه های حاضر فوزاریوم با گیاه خشخاش و پیدا کردن یک گونه بالقوه برای کنترل بیولوژیک این گیاه بر اساس آزمون بیماری زایی می باشد. در مجموع تعداد ۱۵۵ ایزوله فوزاریوم از چهار محل نمونه‌گیری مختلف در افغانستان جداسازی گردید. خصوصیات مورفولوژیک و مولکولی ایزوله‌های بیمارگر با استفاده از فاکتور طویل‌سازی ترجمه ۱ آلفا (*TEF-1α*) بررسی شدند. نتایج حاصل از ایزوله‌سازی نشان می‌دهد که گونه‌های غالب بدین شرح بوده‌اند: *F. proliferatum* (۲۷ درصد)، *F. equiseti* (۲۶ درصد)، *F. solani* (۱۳ درصد)، *F. incarnatum* (۱۲ درصد)، *F. acuminatum* (۷ درصد)، *F. oxysporum* (۵ درصد)، *F. verticillioides* (۵ درصد)، *F. pseudograminearum* (۲ درصد) و *F. coeruleum* (۲ درصد). به استثنا *F. oxysporum* و *F. solani* دیگرگونه‌های شناسایی شده برای اولین بار از این گیاه درافغانستان گزارش می گردند. علاوه بر این، آزمون بیماری‌زایی جدایه‌های *F. oxysporum*، *F. proliferatum* و *F. acuminatum* نیز انجام گردید. با وجود آنکه علائم بیماری در تمامی گونه‌ها مشاهده شد، نمونه‌های بیماری‌زایی‌شده گونه *F. oxysporum* علائم پژمردگی زودهنگام‌تری را نشان داد و شدت بیماری آن‌ها به‌طور قابل ملاحظه‌ای بیش از سایر ایزوله‌ها بوده است. نتایج نشان می‌دهد که *F. oxysporum* ظرفیت آن را دارد که به‌عنوان گونه بیمارگر در کنترل گیاه خشخاش درافغانستان مورد استفاده قرار بگیرد.

کلمات کلیدی: بیماری زایی، ویژگی های ریخت شناختی، کنترل بیولوژیک

مکاتبه کننده: حسین صارمی Email: hsn.saremi@ut.ac.ir
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