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Study on *Fusarium oxysporum* as a biocontrol agent *on Papaver somniferum*

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Abstract

Opium poppy (*Papaver somniferum*) is one of the most important medicinal plants which can be cultured for use of the alkaloid morphine too. Plant poppy have been also associated with the most pests and diseases all over the world. One of the major disease is related to *Fusarium oxyspoprumt* which can also control plant cultivation in biggest producer of opium. However¸ the objectives of this study were to evaluate the effect of *Fusarium oxysporum* isolates against opium poppy and verify their potential as biocontrol agent. For this, a 2-year experiment was carried out under glasshouse (2 trials) and field conditions (2 trials). From infected poppy plants, a total of 16 pathogenic fungal strains were identified as *F. oxysporum* and used for the experiments. The isolates Ghr18, Ghr5-2, Mr28 and Ghr5-4 caused the highest wilting symptoms on sample plants ($P < 0.001$). In addition, no significant differences were observed between field and glasshouse conditions ($P > 0.5$). Moreover, the results showed a clear host specificity of the selected pathogenic isolates. These results suggest that *Fusarium* isolates have the potential to be used as biological control agents against poppy plants where legal policy surrounding the growing of this plant.

Keywords: Medicinal plant, Papaver**,** *Fusarium oxysporum*, poppy

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Introduction

Opium poppy (*Papaver somniferum*) is one of the most important medicinal plants which are the widely used in medicine all over the world (Blamey et all. 2003, Bailey, et all. 2010).. It is grown as an agricultural crop on a large scale, for one of three primary purposes. Firstly to produce seeds that are eaten by humans, known usually as poppy seed (Krist, et. All. 2005). The second is to produce opium for use mainly by the pharmaceutical industry and the third is to produce alkaloids, that are processed as the drugs. A comparatively small amount of *Papaver somniferum* is also produced commercially for ornamental purposes. Some species of the poppies are ornamental plants and the seeds of other species can be used in the food industry. t is increasingly a mistakenness to call *Papaver somniferum* the opium poppy and continue to be produced, that do not yield a significant quantity of opium (Gaevskii, 1999. Schulz, et. al, 2004). The variety known as Sujata produces no latex at all and breadseed poppy is more accurate as a common name today because all varieties of *Papaver somniferum* produce edible seeds. This differentiation has strong implications for legal policy surrounding the growing of this plant. (Gaevskii, A.V. 1999).

Despite many distinct political and governance tensions related to the attempts to control opium (*Papaver somniferum)* cultivation in the world, the production of opium poppy has increased in the last couple of years.

Afghanistan remains the biggest producer of opium, with an estimated area of 224,000 hectares under cultivation in 2014, a 7% increase from the previous year (UNODC, 2014). Not only Afghanistan suffers ravages of terror and insecurity as results of drug production, countries around its borders face security concerns with regards to drug trafficking issues. A sizable proportion of opium in Afghanistan is trafficked and cultivated illegally in Iran, as Iran shares 1,923 km-long Eastern border with this country, and consequently sent on to consumer markets in Europe. Although Iran efforts to combat drug trafficking has been praised by United Nations and Interpol, as the country typically accounting for 74% of the world's opium seizures and 25% of the world's heroin and morphine seizures (Paoli et al., 2009; UNODC, 2014), the Afghanistan opium cultivation remains a major challenge for Iran. One of the major consequences, is the illegal poppy field cultivations and small-scale heroin productions in rugged hillsides of western

boarders of Iran which are influenced by the cultivation patterns in Afghanistan.

Biocontrol procedures emphasize host-specificity testing to select highly specific candidate agents to reduce the risks associated to non-target species (Bajpai et.al; 1999. Lutwick and Lutwick, 2009). Around the globe, most of the biocontrol programs schemes have mainly attacked plant pathogens, the main threat on crops; however, very limited studies have used plant pathogens as bioherbicides to control narcotics supply in the Middle East. Researchers tried to use of the biological control agents against poppy plants where legal policy surrounding the growing of this plant. (Askitopoulou, et. al, 2002; Yadav, et.al, 2006).

Fusarium species are one of the largest genera of fungi that cause various diseases such as crown rot, head blight, and scab on crops (Saremi and Saremi, 2013). Many studies have demonstrated the potential of non-pathogenic *F. oxysporum* in controlling various *Fusarium* diseases, including *Fusarium* wilt, based on actions of competition, mycoparasitism, antibiosis and induction of plant defense reactions (Cachinero et al., 2002; Larkin and Fravel, 1998; Lecomte et al., 2016; Mandeel and Baker, 1991; Minuto et al., 1997; Shishido et al., 2005). Strains of pathogenic *F. oxysporum* have also been selected as potential biological control agents and mycoherbicides to control and manage various parasitic weeds by destroying the tissues (Ndambi et al., 2011; Saremi and Okhovvat, 2008; Zarafi et al., 2014).

Genrally, the numerous studies have demonstrated the potential use of fungal strains as biological control agents for various narcotic crops. O’Neill et al. (2000) reported *Dendryphion penicillatum* and *Pleospora papaveracea* as destructive seedborne pathogens to *P. somniferum* which caused complete poppy blight; however, *P. papaveracea* was more virulent and produced ascospores in addition to conidia, therefore has more potential for use as a mycoherbicide. Isolates of *Fusarium oxysporum* f. sp. *erythroxyli* showed significant effect on coca plants (*Erythroxylum coca* var. *coca*) death and high disease rates, when applied to soil in both greenhouse and field experiments (Bailey et al., 1996). Other studies have also used host specific *F. oxysporum* strains as an alternative to control coca (e.g. Bailey et al., 1997; Sands et al., 1997), hemp (*Cannabis sativa* L.) (e.g. Hildebrand and McCain, 1978; McCain and Noviello, 1985) and *P.*

somniferum (Connick et al., 1998; McCarthy et al., 1995).

The use of mycoherbicides on other countries (agricultural bioterrorism) and the threat of simulation to cause direct damage in agricultural sector have resulted in efforts to reduce the biocontrol programs especially towards the narcotic plants (e.g. Suffert et al., 2009). Nevertheless, the benefits of no detrimental effect on human and animal health which present a low risk for environmental damage over man-made chemical solutions as well as the host specificity of *F. oxysporum* (Buxton, 2006), can suggest biocontrol approach as one of the strategies to reduce narcotics supply in particular at local and regional scales. Present study was initiated to isolate and identify strains of *F. oxysporum* from three different regions and to find out whether the isolates were efficient biocontrol agents for opium poppy. To the best of our knowledge, this is the first report on *P. somniferum* biocontrol that was carried out under both greenhouse (laboratory scale) and field conditions, in the Middle East.

Materials and methods

Fielded study

Surey was carried out within three different locations[2] (hereafter referred as Ghr, Mr and Z) in Iran and Afghanistan. Normally, Opium plants (*Papaver somniferum* var. album) showing symptoms of chlorosis, foliar wilting and necrosis (traces of *F. oxysporum)*, and rhizosphere soil samples were collected from the three regions. The three locations were almost aligned along a straight line and the calculated distance between Ghr and Mr was 180 km and Mr to Z was ٦0 kilometers. A total of 40 samples were collected from each site, overall 120 samples.

Generally, Opium poppy largely tends to be winter or spring crop (sown from October to February) and harvested between 120 to 250 days later depending on the variety and environmental conditions (Chouvy, 2011). The mean elevation, average temperature, average relative humidity and the annual rainfall of the study areas are approximately 800 m asl, 20 °C, 18% and 100 mm, respectively.

F. oxysporum isolation

plant samples were soaked in ethanol and 1% NaOCl and rinsed with sterile distilled water and later cultured in Peptone PCNB Agar (PPA) (Burgess et al., 2008). In order

[2] Due to security restrictions, the names of the study areas are not exposed.

to isolate *Fusarium* species from soil samples the serial dilution technique was used, serial dilutions (10^{-2} to 10^{-4}) were prepared and plated onto PPA medium. After 5-7 days of incubation, the colonies were purified by hyphal tipping (Burgess et al., 2008; Saremi and Saremi, 2013). For the species to be identified at genus level, they were sub-cultured on water agar and after couple of days were mounted on microscope slides for further investigation.Identification was based on the morphological characteristics of single-spored isolates as described by Leslie and Summerell (2006).

Identification of *F. oxysporum*

The *Fusarium* species were identified on the basis of macroscopic characteristics such as growth rate of the colony, pigmentation, absence or presence of microconidia, characteristic of macro- and microconidia and conidial dimension. Genomic DNA of *F. oxysporum* was isolated using the method described by Raeder and Broda (1985). To generate molecular markers, the polymerase chain reaction (PCR) approach was used. For the molecular identification of *F. oxysporum* isolates, two primers designed specifically to the internal transcribed spacer (ITS) region of the rDNA operon of *F. oxysporum* were used (Mishra et al., 2003). All isolates were identified using *F. oxysporum* specific primers FOF1 (5′-ACA TAC CAC TTG TTG CCT CG-3′) and FOR1 (5′-CGC CAA TCA ATT TGA GGA ACG-3′). PCR reactions were carried out in 50 µl reaction mixture containing 5 µl of 10 x PCR buffer, 0.6 µl of MgCl2 (50 mM),1 µl of each dNTPs (10 mM), 2 U *Taq* DNA polymerase, 1.5 µl of each primers, 6 µl of DNA (10 ng) and 31.4 µl ddH2o. Amplification was performed with an Corbett DNA thermocycler (Corbett Research, Mortlake, Australia) and based on the method suggested by Mishra et al. (2003), in a program comprising of 34 cycles of the initial denaturation at 94 °C for 60 s, annealing at 53 °C for 60 s, and extension at 72 °C for 1.5 min with an initial denaturation of 5 min at 94 °C before cycling and final extension of 5 min at 72 °C after cycling.

Glasshouse examination

The pathogenicity test results of selective isolates were performed in Glasshouse experiments (2 trials). In order to investigate the pathogenicity of *F. oxysporum* and identify the *forma specialis,* isolates were maintained in potato dextrose agar (PDA) slants and incubated for seven days at 29-30 °C. The mycelial plugs (5 mm diameter) of the isolates were then positioned in sterile sand: maize meal medium (50 g + 1.5 g

maize meal + 10 ml water) and incubated for 15 days at 28±2 °C (for preservation, isolates were stored on silica gel at 4°C (Windels et al., 1988)). Pots of 25 cm diameter were filled with mixtures of sand, soil and animal manure in the proportion of 4:2:1. For inoculation, before seeds were sown in the pots, the top 5 cm of soil was removed and mixed with 15 g of inoculum and distributed through the pots. The sterile seeds were then planted immediately in 1 cm depth and compost were added to the surface, and irrigated afterwards.

Furthermore, single-spored isolates of *F. oxysporum* were sub-cultured onto PDA media and grown for 10 days at 20 °C. Spore suspensions were produced by adding sterile distilled water to the Petri dishes, gently removing spores using a glass spreader. The spore suspension concentration was adjusted to a concentration of 1×10^6 spore/ml using a hemocytometer. At the beginning of pod development (R3 stage), root surface and the adjacent tissues of the lower stem of the plants were inoculated with a drop (1 ml) of spore suspension. The observation on wilt incidence and symptoms were recorded at harvest approximately 110 days after sowing.

The glasshouse temperature was approximately 20 °C, and daylight was supplemented with light from fluorescent tubes to provide 14 h of continuous light. The experiment was set up in a completely randomized design with three replicates (pots) per isolate and three replicates for negative control pots (equivalent weight of maize meal media without the inoculated *Fusarium*).

Field assessments

In order to verify results from the glasshouse trials, field trials were carried out. Based on the pathogenicity results from the first-year glasshouse experiment, the high virulent isolates were selected to carry out the first field trial. Subsequently, the most virulent isolates from the first-year field trial along with several new isolates were selected and used for the second-year glasshouse experiment.

Field plots consisted of three rows, 1.5 m long in 1 m centers (with three plot replicates for each treatment). Soil at the plots was classified as clay-loam. The mean elevation, average temperature, average relative humidity, and the annual rainfall of the field plots were recorded as approximately 1260 m asl, 14 °C, 40% and 250 mm, respectively.

The inoculum (using maize meal media) was prepared as mentioned above and was mixed with the soil surface area before planting the seeds. After the seeds were planted, they were covered with a thin layer of animal manure and were irrigated. The plots were irrigated every 4 days for the entire season since careful irrigation management schedule is crucial to the success of poppy crop. Similar to glasshouse pots, at the beginning of pod development (R3 stage), 1 ml of spore suspension was inoculated to the root surface and the adjacent tissues of the lower stem of each plant. The three row plots were established in a randomized complete block design with three replicates (plots) per isolate and three replicates for negative control plots.

Statically evaluates

In order to examine the host specificity of the isolates, some of the highly virulent isolates were selected and inoculated (similar techniques and conditions used in poppy plants) on several non-host species, including saffron, zucchini, beans and lambsquarters. Three pots per treatment (isolates) arranged in a completely randomized design were used in this experiment. This experiment was conducted twice.

Healthy and *Fusarium*-infected plants were evaluated by wilting percentage to assess the effect of disease symptoms on plants,. For this purpose, the infected samples were compared to the unwilted control samples and each individual plant, based on the wilting severity, was further classified into two groups; Mild Wilting-MW (with less than 50% wilting) and Severe Wilting-SW (with more than 50% wilting). Consequently, the percentage of leaves, stems and shoots of each sample with minor and less than 50% wilting symptoms was calculated under "MW" group and the percentage of plants with more than 50% wilting symptoms was calculated under "SW" group. The relationships between the variables (2 wilting groups) in each treatment were investigated by Pearson correlation tests. Statistical analyses were conducted with R Studio version 1.0.143 (R Studio Team, 2015).

The Generalized Linear Model (GLM) with a normal error structure and an identity link function was applied separately in each greenhouse and field trial to determine the effects of isolates within the sample plants. An additional GLM was also performed to test the effect of environments (i.e. glasshouse and field experiments in each year).

Results

Isolated *Fusarium* spp.

Due to morphologic characterization and macro- and microconidia characteristics, 10 different *Fusarium* species (overall 50 isolates) were recovered from the infested poppy plant material along with their root tissue. These were: *Fusarium solani*, *F. oxysporum*, *F. equiseti*, *F. longipes*, *F. lateritium*, *F. crookwellense*, *F. sambucinum*, *F. subglutinans*, *F. armeniacum* and *F. compactum.*

F. oxysporum identification

Overall, 16 isolates were identified as *F. oxysporum* on the basis of colony morphology and characteristics of macroconidia and microconidia. In addition, PCR with species-specific primers amplified a single 340 bp DNA fragment specific to *F. oxysporum*, therefore confirming the species-specific identification. Figure 1a illustrates the 12 isolates used in the first-year glasshouse trial.

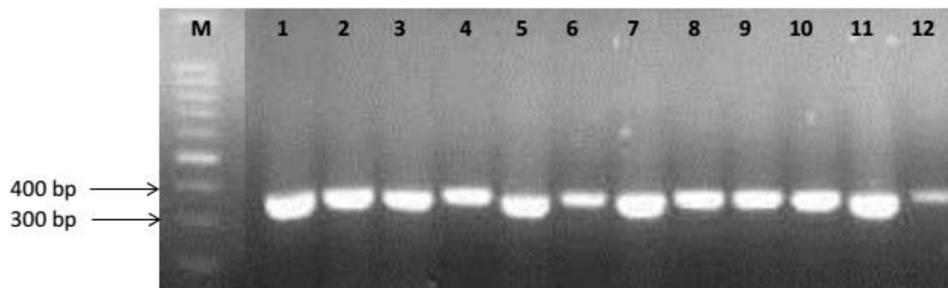

Fig. 1. (a) Agarose gel electrophoresis of the internal transcribed spacer region base pair (bp) products of the *F. oxysporum*. Lane M - 100 bp DNA ladder; Lanes 1 to 9, *F. oxysporum* isolates..

A total of 12 *F. oxysporum* isolates were selected for the first glasshouse trials, including Ghr1, Ghr4, Ghr7, Ghr18 and Ghr28, from the first location, Mr28 from the second location and Z11, Z12 and Z13 from the last location. The plants inoculated with these 12 isolates expressed external symptoms of wilting and leaf yellowing, however with different wilting severity. Depending on the severity of the wilted parts, the percentage were calculated for each MW and SW wilting group. A significant correlation between both MW and SW groups were found (Pearson test: *t-value* = 2.52; *df* = 28; r^2 = 0.43; *p* = 0.01), therefore a strong relationship exists between percentage of the mild wilting parts and percentage of severe wilting parts of each plant.

Glasshouse examinations

Among the 12 pathogenic isolates, 4 were highly virulent and showed severe wilting symptoms in comparison with the control (Mr28 ($P < 0.001$), Ghr18 ($P < 0.001$), Ghr7 ($P < 0.001$), Z13 ($P < 0.001$)). An average of 62% of *P. somniferum* in Ghr18, 52% of plants in Mr28, 27% of plants in Ghr7 and 30% of plants in Z13 treatments showed severe wilting symptoms (SW group) (Fig. 2); whereas an average of 20%, 17%, 33% and 30% of plants in Mr28, Ghr18, Ghr7 and Z13 treatments, respectively, showed mild symptoms (MW group). Similarly, no symptoms of wilt were observed on control samples. These four isolates showed to be the most effective with highest disease severity compared to the other treatments, therefore were used in the field trial.

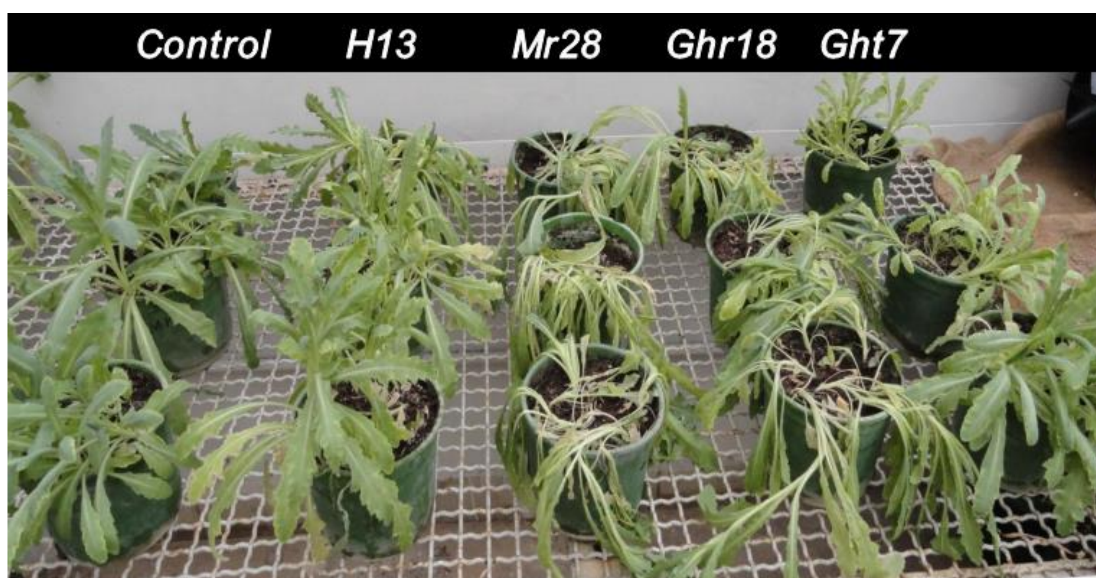

Fig. 2. The wilting percentage of poppy plants within each treatment in the first-year trials in the glasshouse.

Despite the ecological and biological differences in glasshouse and field environments, under natural condition the 4 selected isolates showed similar results as the glasshouse results, indicating significant wilting symptoms (Mr28 ($P < 0.001$), Ghr18 ($P < 0.001$), Ghr7 ($P < 0.001$), Z13 ($P < 0.001$)). In the plots treated with fungus, poppy plants showed symptoms of leaf chlorosis, wilting, stunting, leaf drop as well as smaller size seed capsules (pods); whereas in control plots (uninfested soil) no signs of infection were observed and plants sustained high growth rates.

Fusarium wilt (disease intensity) varied within the 4 treatments, where an average of

54% and 43% of plants infected by Ghr18 and Mr28 isolates showed severe wilting symptoms (SW) and only an average of 17% of plants within these treatments showed minor wilting symptoms (MW). The Ghr7 and Z13 substantially showed less virulence than Mr28 and Ghr18. Furthermore, the comparison of results across field and glasshouse environments did not indicate significant differences among them (GLM test: $\beta = -2.33 \pm 5.38$ SE; *t-value* = -0.43; $p = 0.67$).

Second-year glasshouse trial

Infected *P. somniferum* samples were used in the second year of trial. Similar techniques, methods and identification procedures were applied as the first-year trial. Consequently, 7 new isolates, including Ghr5-2, Ghr5-4, Ghr18-3, Mr2-4, Mr5-3, Mr18-1 and Mr30-1, along with Mr28 and Ghr18 isolates from the previous trial, were used for the second-round glasshouse experiment (overall 9 isolates). Wilting symptoms were observed across the pots and based on the wilting severity the percentage in each MW and SW group were calculated. Similar to the first-year glasshouse trial, the wilting percentage of MW and SW groups were compared with each other and the results revealed a highly significant correlation (Pearson test: *t-value* = 4.02; $df = 28$; $r^2 = 0.6$; $P < 0.001$).

Among the 9 isolates, Mr28 (GLM test: $\beta = 55 \pm 7.69$ SE; *t-value* = 7.15; $P < 0.001$)., Ghr18, Ghr5-2 and Ghr5-4 caused severe infections on the poppy plants, therefore were the most virulent isolates (Fig. 3). An average of 73% of plants infected with Ghr5-2, 68% of plants infected with Ghr18, 60% of plants infected with Ghr5-4 and 55% of plants with Mr28 showed severe wilting symptoms and significant differences were observed compared to the control ($P < 0.001$). However, the remaining 5 isolates did not show any significant disease symptoms and therefore were not used in the further field analysis (Fig. 3).

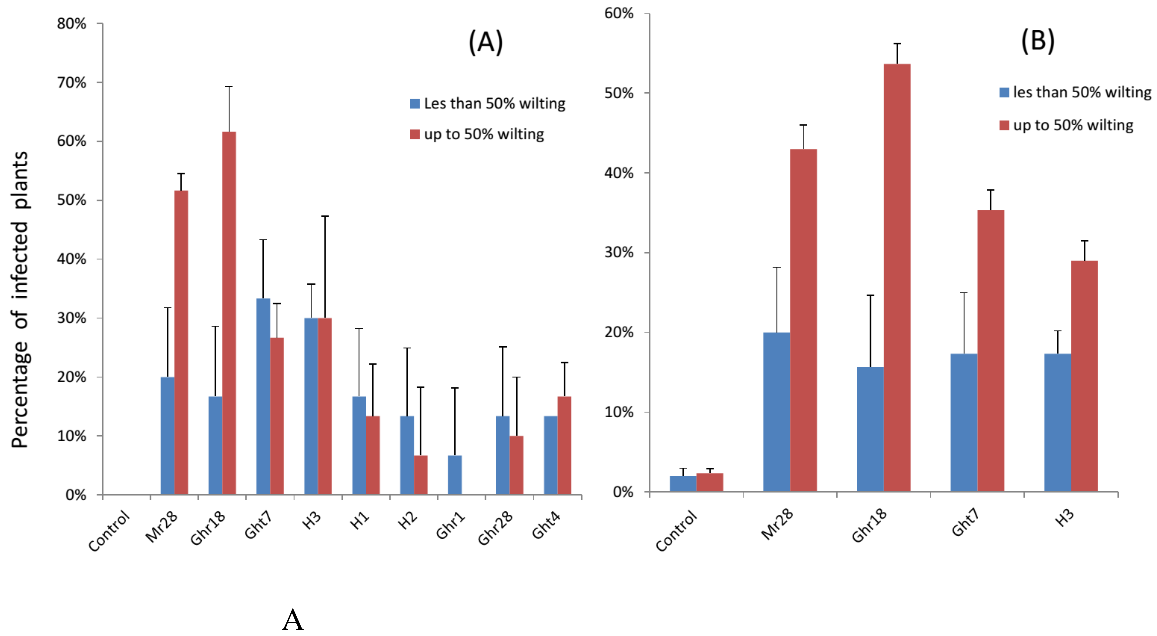

Fig. 3. The wilting percentage of poppy plants within each treatment in the first-year trials; (a) the glasshouse; (b) the field. Asterisks on top of the bars indicate significant differences between individual treatments (SW group) and the control, as tested with GLM test (**P < 0.01 and ***P < 0.001). Error bars represent SE of three replicates.

Field trails

However, the four most virulent isolates from the previous experiments were used in the second field trial. Similar to the first-year field trial, no significant differences were observed across field and glasshouse environments (GLM: *t-value* = 0.33; *df* = 28; *r* = 0.6; *p* = 0.745) (*P* = 0.745). The results of this experiment also confirmed isolates, Mr28, Ghr18, Ghr5-2 and Ghr5-4, highly virulent with varying degree of wilting (P < 0.001). Based on the results presented in Figure 3, 62% of plant samples in Ghr5-2 isolates and 55% in Ghr18 indicated severe wilting, and consequently had the most negative impacts on opium plants (Fig. 4). Moreover, an average of 50% and 42% of plants in Ghr5-4 and Mr28 isolates, respectively, were classified as SW group.

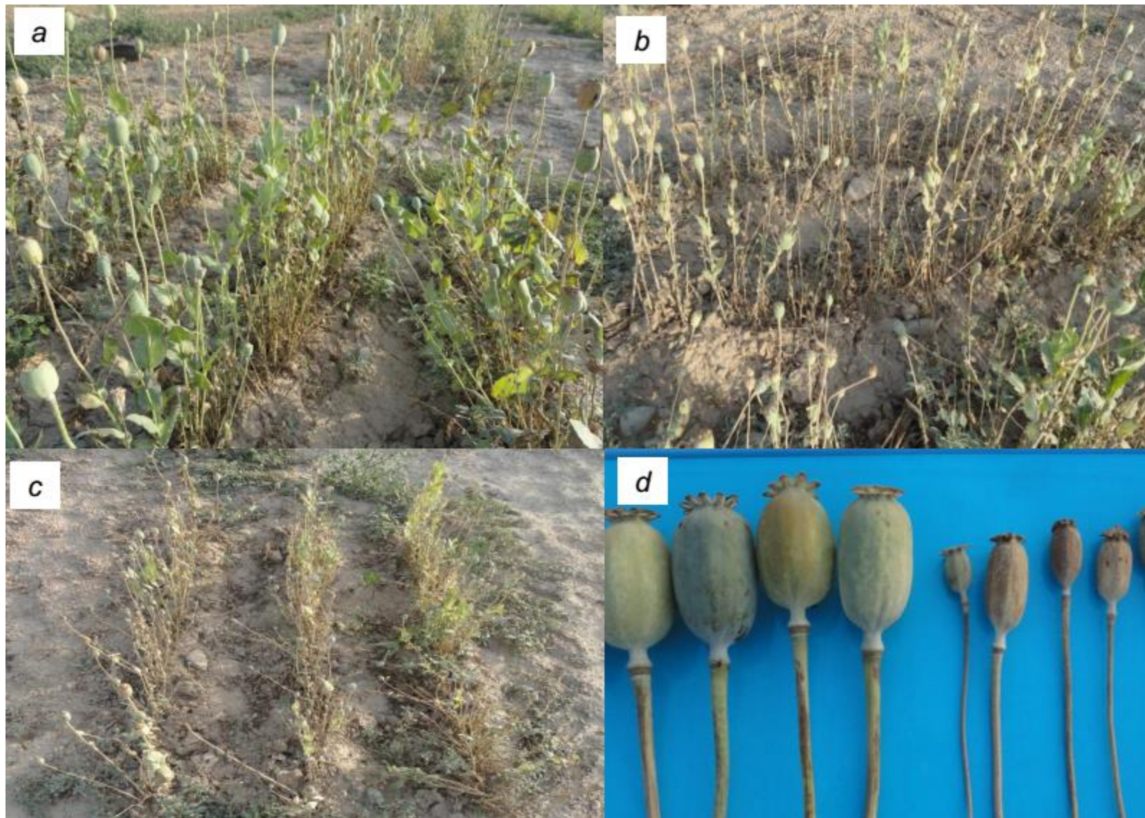

Fig. 4: Effect of *F. oxysporum* on papaver plants on the field, control A), infected plants B) using Ghr18 . D isolate, infected plants with Mr28 .C isolate C), healthy and infected fruit D).

In credit of *F. oxysporum* host specificity, the four virulent isolates (Mr28, Ghr18, Ghr5-2 and Ghr5-4) were infected to saffron, zucchini, beans and lambsquarters. No significant disease symptoms were observed between the infected non-host species and the control ($P > 0.05$).

Discussion

Genrrally, the medicinal uses of poppy were described by the ancient Greeks and opium, as an addictive agent, was identified by Arabic physicians more than 900 years ago. Because of the medicinal importance of morphine derivatives, efforts have been made to identify a species of Papaver that contains high levels of a suitable starting compound for the commercial synthesis of codeine (Bailey, et all. 2010). However the application of biological control agents on poppy plants where legal policy surrounding the growing of this plant is attempted recently. For instance the capacity of *Fusarium oxysporum* as a potential biocontrol agent against poppy opium has been demonstrated. Based on the results of the 2-year experiments, among 16 selected

isolates, Mr28, Ghr18, Ghr5-2 and Ghr5-4 were found to be the highly virulent isolates and the highest pathogenicity within these isolates was caused by Ghr5-2 and Ghr18 ($P < 0.001$). The infected plants with these isolates showed rapid symptoms of wilting and necrotic spots on surfaces, while *P. somniferum* affected by the remaining isolates appeared partially wilted (mild wilt symptoms).

Researchers have explored ITS to be limited and insufficient in identifying complex and variable genes and suggest the include of additional gene sequences, such as intergenic spacer (IGS), translation elongation factor 1α (TEF-1α) and β-tubulin genes (TUB), for the differentiation of species (e.g. Nilsson et al., 2008; O'Donnell and Cigelnik, 1997). However, with the use of high-throughput technologies, several other studies have found ITS region to be highly effective in discriminations among species (e.g. Badotti et al., 2017; Detinger et al., 2011; Oechsler et al., 2009). In this study, the sequence data analysis of the ITS along with the traditional morphological classification and the phylogenetic analysis, provided enough resolution and sufficient genetic scaffolding to reliable detection and identification of the *Fusarium* species.

In order to determine the ecological host range of a potential biological control agent various experiments must be performed under variety of environments (Hopper, 2001). In our study, on interactions between pathogens and *P. somniferum* under various field and glasshouse conditions (differences in temperatures (glasshouse= 20 °C; field= 14 °C), in relative humidity (glasshouse= 20%; field= 40%), and in soil types (glasshouse= sand/maize; field= clay loam), the environmental conditions had no significant impact on the results ($P > 0.5$) and within both sites similar wilting symptoms were observed. Although, comparable results were obtained in both glasshouse and field trials, further investigation and measurements are necessary to see whether this approach can operate across different soil types and microclimates.

Risk assessment methodologies and host specificity testing are necessary to prevent the detrimental impacts of pathogens on non-target plants or on environment (Elzein et al., 2008). Here, the host specificity of pathogenic *F. oxysporum* was confirmed by testing on the non-host inoculated plants, where no wilt symptoms were detected. The host range testing approach can quantify and assess potential risks toward nontarget

organisms. Furthermore, the strains of *Fusarium* used in this study were derived from the native opium poppy and since they were not transferred between locations, they remain native to the environment.

While this study has demonstrated the virulence of pathogenic strains of *F. oxysporum* against *P. somniferum,* various researchers in this field have repeatedly attempted to improve the virulence of the biocontrol agents. There are many ways that can enhance the effectiveness of *F. oxysporum*, including genetic transformation and amendments such as amino acids and organic matter. Tiourebaev et al. (2001) enhanced biocontrol efficacy of *F. oxysporum* f. sp. *cannabis* isolates, obtained from diseased cannabis plants, through selection and use of amino acid excreting strains. Such practices can also be used as methods in order to improve efficacy of *F. oxysporum* and reduce the production costs involved, however further work is needed to verify this.

Researchers believed that various *F. oxysporum* pathotypes can survive successfully in soil as well as in plant residues between seasons. The crop residues in the soil are the most important source of *F. oxysporum* inoculum (primary inoculum) for infecting crops as they produce chlamydospores which can survive periods between host crops (e.g. Haware et al., 1996; Vakalounakis and Chalkias, 2004). Although, in our study, evidence of *F. oxysporum* f. sp. *papaver* inoculum survival was presented throughout the year, further study is required to determine the persistence of this biological control agent. McCain and Noviello (1985) reported that *F. oxysporum* f. sp. *cannabis* inoculum as a biocontrol agent against C. sativa survived in the soil for at least one growing season.

This study is the first report on the control of opium poppy with *F. oxysporum* under both field and glasshouse conditions in Iran; consequently, the long-term effects of such approaches have not been examined adequately. Although, contaminated irrigation water (adding spore suspension) and the ability of *F. oxysporum* to grow and survive for extended periods in soil, holds great promise to control or regulate the numbers of unwanted opium plants; successful implementation of this procedure into commercial use should be priorities of future research. Satellite imagery and equipment's such as drones or unmanned aerial vehicles (UAVs) can be very useful in monitoring and surveying fields and

furthermore be used as delivery mechanism for biological control agents.

Despite the advances in scientific research on biocontrol, there are still certain concerns that some misidentified strains of biocontrol agents such as *F. oxysporum* could be intentionally used as "agro-terrorist weapons" against crop production around the world (Avedi et al., 2014). The likelihood of a successful bioterrorist attack is very slim, considering the technical difficulties, constrains, lack of skills and expertise. Knudsen (2013) stated that the use of biocontrol approaches has mostly been regarded as benign without much scrutiny. However, regulatory cautions must be taken as the impact of a bioterrorist attack can still be high.

Afghanistan's poppy cultivation and opium production must be interpreted in terms of globalization and fragmentation, as the flow of drugs toward Europe and the rich markets from the neighboring states such as Iran. Although Iran policies and proposed regulations for drug trafficking and illicit crops cultivation (e.g. barbed wire and wall with trenches along the border, seizures and heavy penalties), prevent huge amounts of drugs from reaching to other countries, this country face security concerns (UNODC, 2014). Due to the escalation of violence, thousands of Iranian border police officers have lost their lives fighting better-equipped Afghan and Pakistani drug gangs (Erdbrink, 2012). The use of *F. oxysporum* agents can be an act of defense and a legitimate response towards the people engaged in drug plant cultivation, production and trafficking.

There is a great need to make biological control research applicable to local political and social situations. Hallett (2005) has stated that sociocultural and political factors are the primary considerations toward the development and implementation of bioherbicides for the control of illicit crops rather than biological. Considering that many families, particularly from poor socioeconomic backgrounds, are engaged in illicit crops, it is suggested to collect background information and useful analysis of the region to provide crop substitutes and economic alternatives. In Iran and neighboring countries, saffron can be selected as an alternative plant for low-input agriculture, as it is cultivated in a wide range of environments with mild to dry climates and in places with low water availability. The knowledge of this economical viable alternative crop can encourage farmers in low-fertility areas to increase their income with saffron cultivation instead of illicit crops.

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