

Research Article

Introduction of eight fungi isolated from potato golden cyst nematode in Iran

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Abstract

Introduction: The golden cyst nematode, *Globodera rostochiensis*, is considered as one of the most damaging potato pathogens in the world. Considering the skin composition of cyst nematodes and the ability of some fungi to produce enzymes that decompose it, this research was conducted to identify the fungi associated with potato cyst nematode. **Materials and Methods:** Eighty-six fungal isolates infecting potato golden cyst nematode in Hamedan province in western Iran were isolated and purified and identified based on morphological characteristics by valid keys. **Results:** Eight species of fungi belonging to three genera *Alternaria*, *Clonostachys*, *Fusarium* were isolated and identified from potato golden cyst nematode. The highest frequency was related to different species of *Fusarium*. **Conclusion:** The morphological characteristics of these eight fungi have been described and illustrated.

Key words: *Alternaria*, *Clonostachys*, *Fusarium*, *Globodera*

مقاله پژوهشی

معرفی هشت قارچ جدا شده از نماتد سیست طلایی سیب‌زمینی در ایران

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عباسی خ، افزالی‌نیا س (۱۴۰۱) معرفی هشت قارچ جدا شده از نماتد سیست طلایی سیب‌زمینی در ایران. دانش بیماری‌شناسی گیاهی ۱۱(۲): ۲۲-۳۱. Doi: 10.2982/PPS.11.2.22

چکیده

مقدمه: نماتد سیست طلایی *Globodera rostochiensis* به عنوان یکی از خسارت‌زاترین عوامل بیماری‌زای سیب‌زمینی در دنیا محسوب می‌گردد. نظر به ترکیبات پوست نماتدهای سیستی و توانایی بعضی از قارچ‌ها در تولید آنزیم‌های تجزیه‌کننده آن، این پژوهش برای شناسایی قارچ‌های همراه

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نماتد سیبست طلایی سیبزمینی اجرا شد. **مواد و روش‌ها:** هشتاد و شش جدایه قارچی آلوده کننده نماتد سیبست طلایی سیبزمینی در استان همدان در غرب ایران جداسازی و خالص‌سازی و بر اساس ویژگی‌های ریخت‌شناسی با کلیدهای معتبر شناسایی شدند. **یافته‌ها:** هشت گونه قارچ متعلق به سه جنس *Alternaria*, *Clonostachys*, *Fusarium* از نماتد سیبست طلایی سیبزمینی جداسازی و شناسایی شدند. بیشترین فراوانی مربوط به گونه‌های *Fusarium* بود. **نتیجه‌گیری:** خصوصیات ریخت‌شناسی این هشت گونه قارچ شرح و نشان داده شده است.

واژگان کلیدی: *Alternaria*, *Clonostachys*, *Fusarium*, *Globodera*

Introduction

مقدمه

Potato is one of the foremost dicotyledons that is utilized as a source of human nourishment and importantly, it gets to be the fifth most imperative item in the world after wheat, rice, corn, and grain (Zarghani et al. 2014). The golden potato cyst nematode *Globodera rostochiensis* Wollenweber, 1923, is one of the most damaging potato pathogens in the world, which can cause up to 100% damage and has been reported from potato growing areas in 65 countries (Gitty et al. 2011). Some species of fungi including: *Acremonium*, *Arthrotrichum*, *Aspergillus*, *Cylindrocarpon*, *Dactylella*, *Fusarium*, *Lecanicillium*, *Monacrosporium*, *Paecilomyces*, *Penicillium*, *Pochonia*, *Pyrenochaeta*, *Trichoderma*, *Verticillium* have been reported as the most common fungal genera antagonistic to nematode eggs (Verdejo et al. 2002). Considering the composition of eggshell in cyst nematodes and the ability of many fungi to produce a wide range of eggshell degrading enzymes, it is very important to identify nematode antagonistic fungi and their role in the biological control of nematodes, including cyst nematodes.

Materials and Methods

مواد و روش‌ها

Preparing fungal isolates

To study fungi associated with the golden potato cyst nematode (*G. rostochensis*) in Hamedan province in 2019, 86 fungal isolates were isolated from infected cysts of this nematode and subjected to taxonomic study. Accordingly soil samples infected with golden potato cyst nematode were prepared from potato fields, then the cysts were isolated in the laboratory according to the method of Fenwick (1940). From shriveled and sick nematode cysts, fungal isolates along with the nematode were isolated. To purify the isolates, a single spore and hypha tip methods were used.

Identification of the fungal isolates

Macroscopic and microscopic characteristics were examined for species identification of fungal isolates using valid identification keys (Simmons 1967, Seifert, 1996, Nagamani et al. 2006).

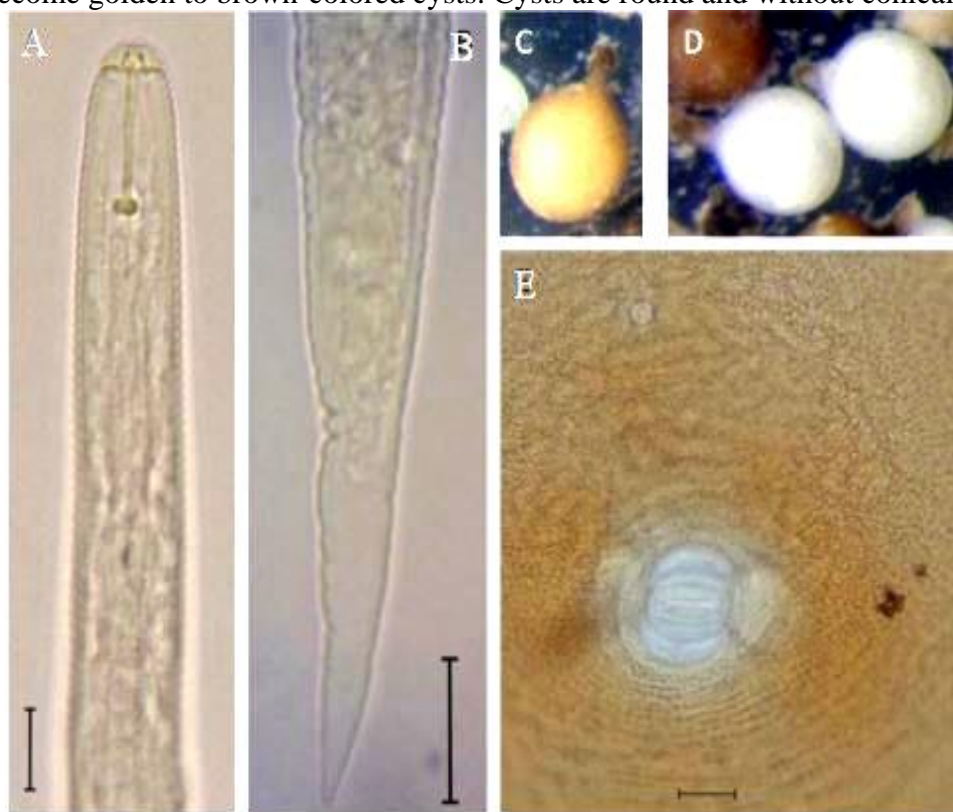
Results and Discussion

یافته‌ها و بحث

Identification of disease-causing nematode species

By examining the morphological characteristics of cysts and second-instar larvae and using valid identification keys and available resources (Mulvey and Stone, 1976). The

nematode isolates obtained were identified as *G. rostochiensis*. The young females in this species are milky at the beginning of their formation and then turn yellow. Finally, they become golden to brown-colored cysts. Cysts are round and without conical



شکل ۱. *Globodera rostochiensis*, A) سر و استایلت لارو سن دوم، B) دم لارو سن دوم، C) سیست، D) ماده جوان، E) انتهای بدن شامل مخرج و ناحیه ولوا. (مقیاس ۱۰ میکرومتر)

Figure 1. *Globodera rostochiensis*, A) Head and stylet of larvae, B) Larvae tail, C) cyst, D) young female, E) end of body including anus and vulva. (10 micrometer scale)

protrusions at the end of the body. In cysts, a circular window is formed due to the destruction of the cuticular membrane of the area around the genital slit, which is one of the characteristics of this genus.

The anus is below the end, and its distance to the genital slit (42-68) is 52 μm , the diameter of the genital slit (14-19) is 15.9 μm . And Gronk's ratio (it means dividing the distance from the closest range of the genital gap to the anus by the diameter of the genital gap) (2.8-3.6) was 3.16. The stylet in the second instar larvae is 21.2 μm long (20-23).

The knots are round, The cuticular network of the head is developed and distinct. Worm-shaped male nematode 890 to 1270 μm long, has a strong cuticular network of the head and a grown stylet 25-27 μm long, as well as a short round tail, and the male penis is located at the end of it. The species *G. rostochiensis* can be separated from the species *G. pallidia* with the mentioned features (Figure 1).

Description of fungi isolated from *Globodera rostochiensis* in Iran

Eight species of fungi belonging to three genera *Alternaria*, *Clonostachys*, *Fusarium* were isolated and identified from potato golden cyst nematode (Table 1).

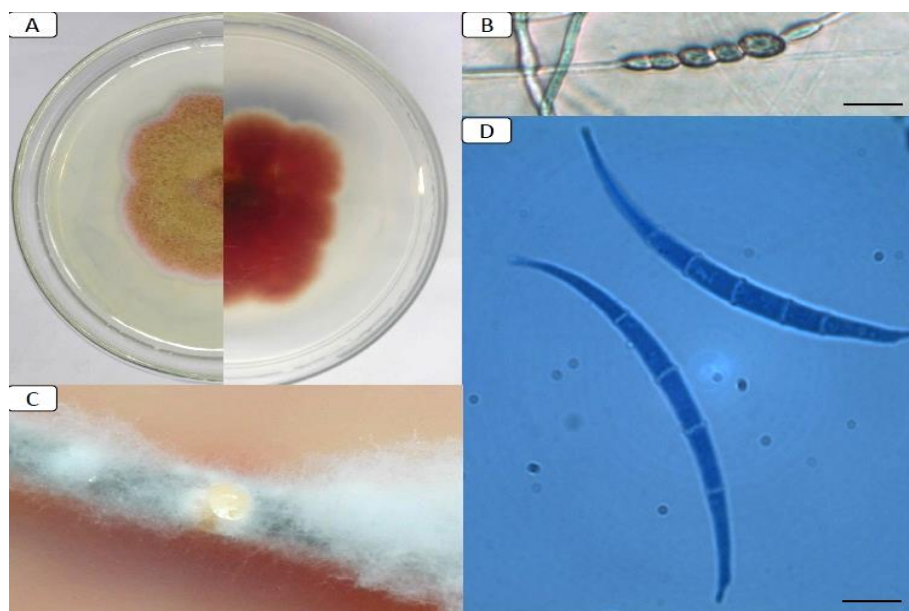
جدول ۱. قارچهای جدا شده از نماتد سیست طلایی سیبزمینی در ایران.

Table 1. Fungi isolated from golden potato cyst nematode in Iran.

جنس	گونه	تعداد
Genus	Species	Number
<i>Fusarium</i>	<i>F. acuminatum</i>	16
	<i>F. equiseti</i>	8
	<i>F. proliferatum</i>	9
	<i>F. solani</i>	19
	<i>F. verticillioides</i>	3
	<i>F. oxysporum</i>	13
<i>Alternaria</i>	<i>A. chlamydosporigena</i>	4
<i>Clonostachys</i>	<i>C. rosea</i>	14

1. *Fusarium acuminatum* Ellis & Everh.

Abundant macroconidia were formed in orange-colored spordochia found on carnation leaf agar (CLA) medium. Macroconidia are sickle-shaped and have 3-5 transverse septate with a tapered apical cell and a distinctly foot-shaped basal cell. (Figure 2). There was no microconidium in this isolate. The presence or absence of microconidium in this species is not a criterion for classification. Observations showed that chlamydospores are formed in clusters or chains and slowly. However, their presence is not a suitable taxonomic criterion. From this species, 16 isolates were isolated from nematode cysts and larvae.



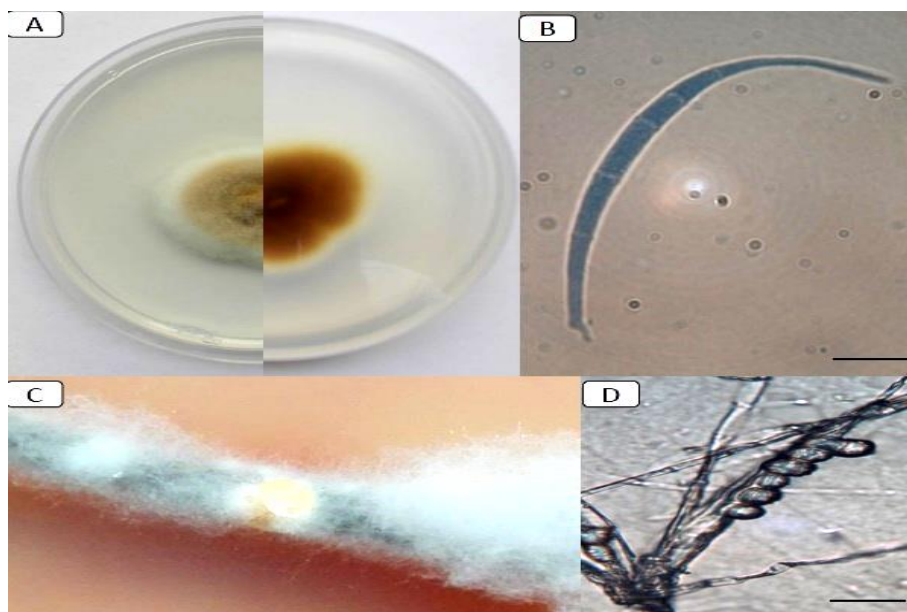
شکل ۲. *F. acuminatum* (A) ریخت شناختی پرگنه در محیط کشت PDA، (B) کلامیدوسپور،

(C) هاگودوخیوم روی برگ میخک در محیط (CLA) و (D) ماکروکنیدیوم. (مقیاس ۱۰ میکرومتر)

Figure 2. *F. acuminatum*, A) Morphology of mycelium on PDA, B) Chlamydospore C) Sporochium on clove leaves on CLA medium and D) Macroconidia. (10 µm).

2. *Fusarium equiseti* (Corda) Sacc.

Macroconidia arise from single phialides on branched conidiophores in sporodochia. The color of sporodochium is orange and it appears around the found carnation leaf pieces in CLA medium and sometimes it may be hidden under the mycelium. Macroconidia have thick, highly curved walls that have five to six septate. The apical cell is tapering and elongates. on the basal cell is a well-developed foot shape. Microconidium was not seen in this species and many chlamydospores are formed in chains and clusters on the aerial filaments or around the culture medium. Also, the chlamydospore wall is often wart-like and below it (Figure 3).



شکل ۳. *F. equiseti* (A : پرگنه در محیط کشت PDA، B) (ماکروکنیدیوم، C) هاگودوخیوم روی محیط برگ میخک آگار (CLA) و (D) کلامیدوسپور. (مقیاس ۱۰ میکرومتر) (اصلی)

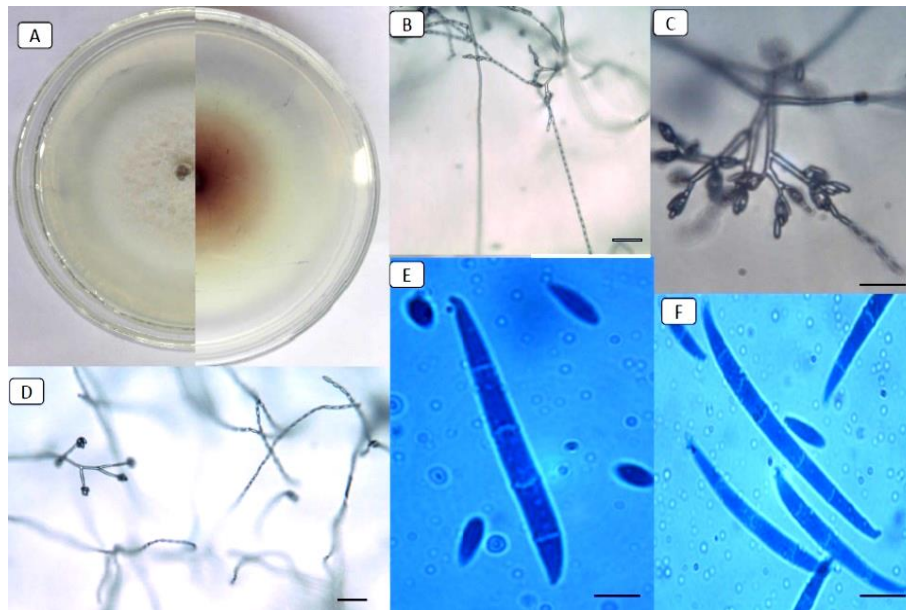
Figure 3. *F. equiseti*, A) Morphology of mycelium on PDA, B) Macroconidia. C) Sporodochium on clove leaves on CLA medium and D) Chlamydospore. (10 μ m)

3. *Fusarium proliferatum* (Matsush.) Nirenberg

Microconidia were abundantly formed in chains and false heads from monophialid and polyphialid conidiophores on aerial hyphae. Microconidia are pear-shaped to oval and without septate.

Polyphialidity is the main factor for distinguishing this species from *F. verticillioides*. The sporodochium in this species is pink in most isolates and is formed on the surface of the environment. A limited number of isolates formed carnation leaf pieces in CLA medium.

Macroconidia were formed on monophyalides and polyphyalides in sporodochium. Macroconidia are almost straight and long and narrow without bending and have a thin wall and curved apical cells and a slightly developed and foot-shaped basal cell. Macroconidia have 3-5 septate (Figure 4). Of this species, nine isolates were isolated and identified.



شکل ۴. *F. proliferatum* (A : ریخت شناختی پرگنه در محیط PDA، B) زنجیره میکروکنیدیوم، C و D) کنیدیوفورها، E و F) ماکرو و میکروکنیدیوم ها. (مقیاس ۱۰ میکرومتر)

Figure 4. *F. proliferatum* A) Morphology of mycelium on PDA, B) Microconidia chain. C, D) Conidiophore, E, F) Macroconidi and microconidia. (10 μ m)

4. *Fusarium solani* (Mart.) Sacc.

Macroconidia are abundantly formed in orange-colored sporodochiums And they often have three thick transverse septate which rarely have 4-5 septate. The apical cell of macroconidium is relatively pointed and the foot cell is not very clear in them which is seen as a depression.

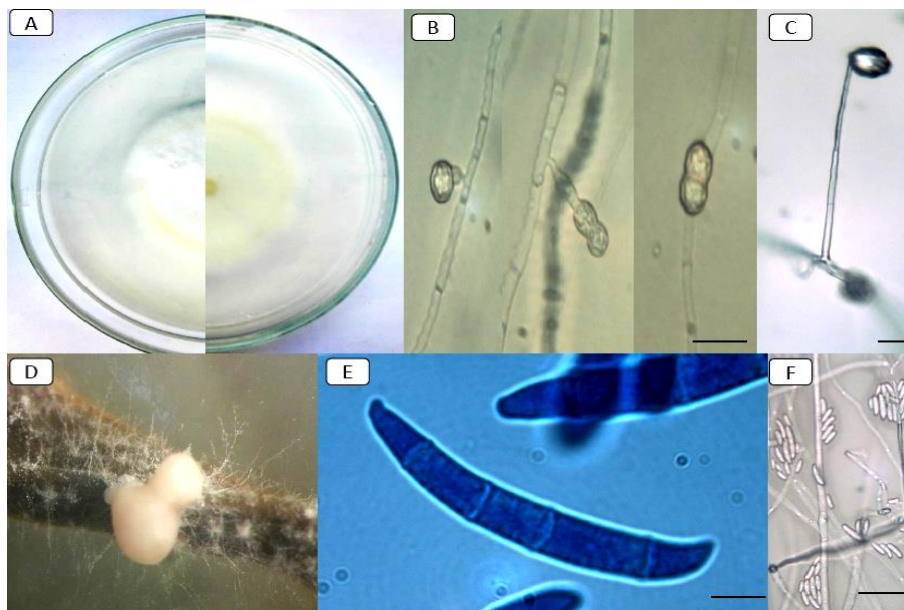
Conidiophores are formed as long monophialides. Chlamydospores are spherical with smooth walls, which are formed terminally or medially in small chains or pairs in filaments (Figure 5). Unlike *F. oxysporum*, this species produces long phialides, and this difference can be used to distinguish between them. Of this species, 19 isolates were isolated and identified.

5. *Fusarium verticillioides* (Sacc.) Nirenberg

This fungus easily produces spores on PDA and CLA medium. microconidia are produced by lateral phialides on aerial hypha but over time, macroconidium is also seen in the environment.

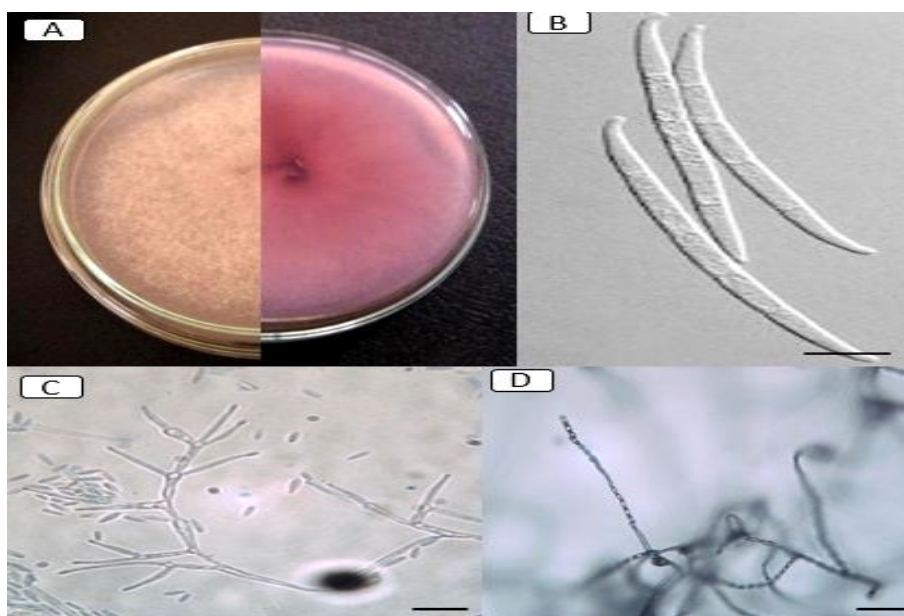
Microconidia are abundant and form chains with false heads and they are often unicellular. Sporodochiums are seen in orange color. Conidiophores are monophialid They are branched and non-branched. Conidial cells branched with a maximum of three branches but up to five branches were seen in them. Most microconidia are ovoid or almost pear-shaped.

However, conidia with one side wide and the other side flat or oval were also observed. Macroconidia are thin, sickle-shaped, and relatively straight. Their apical cells are narrow and curved and their basal cells are distinctly foot-shaped. Most macroconidia are seen as three to five septate. The size of one or two-celled microconidia is about 2.5



شکل ۵. *F. solani*, A) ریخت شناختی پرگنه روی محیط PDA، B) کلایمیدوسپور، C) فیالید و تشکیل سر دروغین، D) هاگودخیوم E و F) ماکروکنیدیوم ها. (مقیاس ۱۰ میکرومتر)
Figure 5. *F. solani*, A) Morphology of mycelium on PDA, B) Chlamidospore. C) Phialid and false head, D) Sporodochium, E, F) Macroconidia and microconidia. (10 μ m)

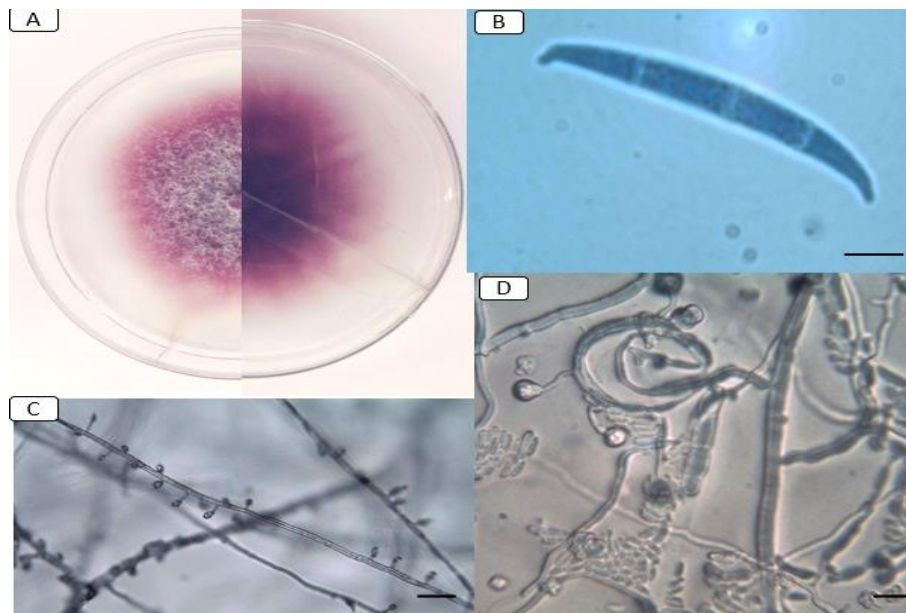
$\times 11 \mu$ m and the three-walled macroconidia are $3.7 \times 35 \mu$ m (Figure 6). Of this species, three isolates were isolated and identified.



شکل ۶. *F. verticillioides*, A) ریخت شناختی پرگنه روی محیط PDA، B) ماکروکنیدیوم، C) فیالید و زنجیره میکروکنیدیوم. (مقیاس ۱۰ میکرومتر)
Figure 6. *F. verticillioides*, A) Morphology of mycelium on PDA, B) Macroconidia, C) Phialid and D) Microconidia chain. (10 μ m)

6. *Fusarium oxysporum* Schltdl.

Macroconidia are short to medium in size, slightly curved with thin septate, and elongated and curved apical cells. The basal cell has a foot and three septate. Microconidia were formed as false heads on short monophialides which is the difference between this species and *F. solani* species. Microconidia are ovoid and often non-septate. Chlamydospores were formed singly, double, or chain in the CLA medium. Chlamydospores have a thick wall with a spiny or smooth surface and sometimes a terminal (Figure 7). From this species, 13 isolates were identified.



شکل ۷. *F. oxysporum* (A) ریخت شناختی پرگنه روی محیط PDA، (B) ماکروکنیدیوم، (C) فیالید و (D) کلایمیدوسپور. (مقیاس ۱۰ میکرومتر)

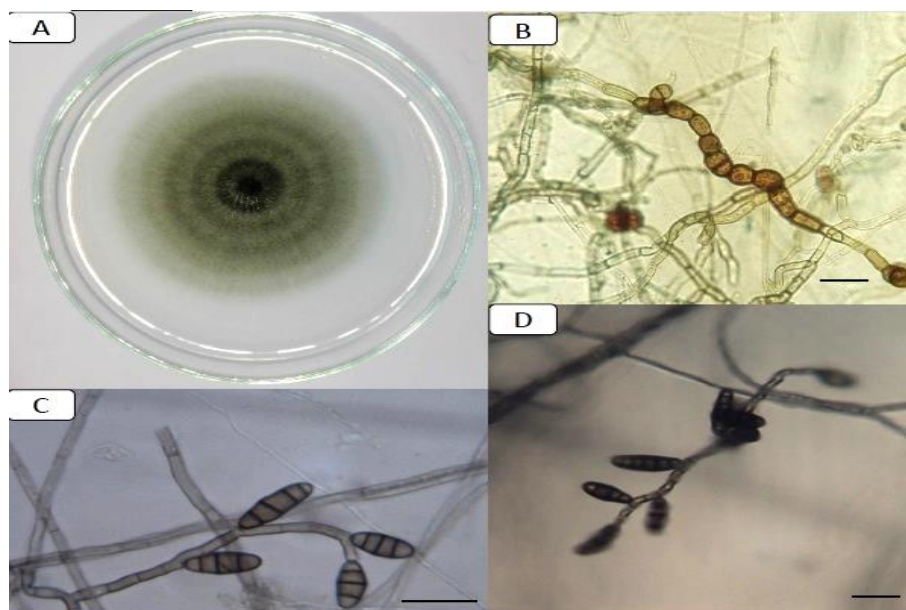
Figure 7. *F. oxysporum*, A) Morphology of mycelium on PDA, B) Macroconidia, C) Phialid and D) Chlamydospore. (10 μ m)

7. *Alternaria chlamydosporigena* Woudenh. & Crous

Conidiophores are single, straight, or rarely branched, straight or 3-1 bent at the end. The dimensions of conidiophores are $23-36 \times 3.5-4 \mu$ m and nine transverse septate. Conidia are formed singly. They are formed in the holoblastic form at the end of conidiophores and they have an egg-like appearance, elongated oval or semi-cylindrical, and straight with relatively curved and smooth surfaces. The dimensions of conidia are $22.5-27 \times 7-7.5 \mu$ m. The dimensions of aerial chlamydospores are $14-25 \times 35-55 \mu$ m (Figure 8). Four isolates were identified from this species.

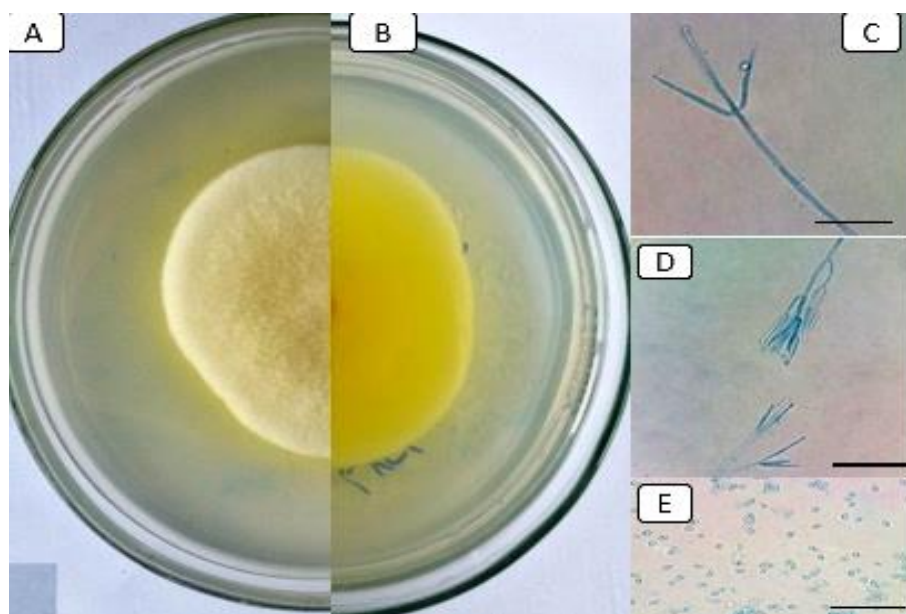
8. *Clonostachys rosea* (Link) Schroers, Samuels, Seifert & W. Gams

Aerial mycelium and two types of conidiophores (primary and secondary) are formed in this fungus. *Verticillium*-like primary conidiophores, single-stranded and branched, and delicate and elongated phialides with dimensions of $27-34 \times 2-2.6 \mu$ m are visible. Secondary *penicillium*-like and branched conidiophores and dense phialides were formed with dimensions of $2-2.7 \times 14-15.5 \mu$ m. Conidia are flat at the base, spherical and wider at the apex, colorless, straight to slightly curved, and measuring $2-4 \times 5-10 \mu$ m (Figure 9). Fourteen isolates of this species were identified.



شکل ۸. *A. chlamydosporigena*. A) ریخت شناختی پرگنه روی محیط PCA، B) اجتماعات و تورمات هیفی، C و D) کنیدیوفور و کنیدیوم. (مقیاس ۱۰ میکرومتر)

Figure 8. *A. chlamydosporigena*, A) Morphology of mycelium on PCA, B) Swollen hyphae, C, D) Conidiophore and conidia. (Bars=10 μ m)



شکل ۹. *C. rosea*. A) ریخت شناختی پرگنه روی محیط PDA، B) کنیدیوفور اولیه ورتیسیلیوم مانند، C و D) کنیدیوفور ثانویه پنی-سیلیوم مانند و E) کنیدیوم. (مقیاس ۱۰ میکرومتر)

Figure 9. *C. rosea*, A) Morphology of mycelium on PDA, B) Primary conidiophores of *Verticillium*-like, C, D) Secondary conidiophores of *Penicillium*-like E) Conidia. (10 μ m) .

Conclusion

نتیجه‌گیری

The golden potato cyst nematode is considered one of the most important parasitic nematodes of this product in the world. Although the nematode egg is one of the most resistant biological structures it is sensitive to the attack of pathogenic fungi. Therefore, the identification of fungi associated with nematodes and their role in the biological control of nematodes, including cystic nematodes, is very important. In this research, a total of 86 isolates belonging to three genera and eight species based on morphological characteristics including *Fusarium acuminatum*, *F. equiseti*, *F. proliferatum*, *F. solani*, *F. verticillioides*, *F. oxysporum*, *Alternaria chlamydosporigena*, and *Clonostachys rosea* were identified. The highest frequency was related to different species of *Fusarium* (75.31%). Therefore, it can be concluded that filamentous fungi, as producers of various enzymes, have a suitable potential for biological control of golden potato cyst nematode.

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