which are toxic for humans as well as animals ( Bennett and Klich 2003 ; Senter et al. 1991 ).As a result of their numerous inhibitory impacts on eukaryotic cells,as the suppression of protein, DNA, and RNA synthesis, limitation of mitochondrial activity, and interference with cell division also membrane function, mycotoxins can have a suppressive effect on the immune system (Rocha et al. 2005). Most *Fusarium* species have the capacity to create one or more mycotoxins of varying potency(Bottalico and Perrone 2002).

Worldwide,a number of *Fusarium* species are causal of potato dry rot ( Cullen et al. 2005);examples *F. oxysporum* ( Schlechtend Fr.) *F. coeruleum* (Libert) (syn. *F. solani* var coeruleum ), *F. culmorum* ( Wm. G. Sm.) Sacc.,*F. sporotrichioides* ( sherb. and Corda) Sacc., *F. avenaceum* (Fr. : Fr.) Sacc., *F. sulphureum* (Schlechtend) ( syn. *F. sambucinum*( Fuckel), *F. semitectum* ( Berk & Ravenel),*F. acuminatum* (Ellis & Everh.)*F. equiseti* (Corda) Sacc.,,*F. crookwellense* (L. W. Burgess, P.E. Nelson & T. A. Toussoun), *F. scirpi* (Lambotte & Fautrey), *F. graminearum* (Schwabe).

The prevalent species in China was, *F. sambucinum* it was discovered that the larger percent of isolates which was 56% according to Du et al. (2012) belonged to it.In Iran a number of *Fusarium* species are defined as plausible causes of dry rot of potatoes,and the major ones were *F. sambucinum* and *F. solani* (Esfahani 2005), and in Tunisia *F. oxysporum*,*F. solani*,*F. graminearum* and *F*. sp. *tuberosi* have been most common( Daami- Remadi et al. 2006 a,b). while in USA, *Fusarium oxysporum* has been the most common species of dry rot (Tiwari et al. 2020) .*F. solani and F.oxysporum* are the two basic pecies related to dry rot in South Africa (Theron and Holz 1990).Also in China the most known fungus in major potato-growing areas is *F. sambucinum,* and includes four less well-known species: *F. oxysporum , F. equiseti, F. avenaceum, and F. acuminatum* (Du et al. 2012).Species fit into one of the two groups according to Duncan's test. One group included the three species that were the most dangerous,*F. oxysporum*, *F. sambucinum* and *F. avenaceum* while *F. equiseti* with *F. acuminatum* make the group of the least dangerous (Du et al. 2012).

Using primer sets ITS1/ITS4, the internal transcribed spacer (ITS) is enhanced by PCR ( White, et al. 1990 ), ITS region is advisable for the common fungal barcode sequence as a section of DNA has been sequenced in fungi's molecular ecology.In molecular systematics,it is frequently evidenced most facilitative for the species to genus level as well as within species (for instance, to determine geographic races) (Moussa et al. , 2017; Schoch et al. 2012 ).

The aims of this research was testing the pathogenicity of various *Fusarium* species that cause postharvest dry rot disease on potato tuber, morphology and molecular identification of *Fusarium* species.

## MATERIALS & METHODS

**- Sampling and isolation of *Fusarium* species.**

Potato tubers from 20 cold stores and 15 marketing stores all around Dohuk province having dry rot symptoms have been collected. Identified potato tuber samples were cleaned with tap water before surface sterilized for three minutes with sodium hypochlorite (1.5%).And washed twice in sterile distilled water.Infected tissue from the lesion's margin or the inside of the dry hollow was removed after cutting the tubers.In plates contained potato dextrose agar with 100 mg/ml ampicillin applied, this tissue was cultured.Plates incubating at 25 °C. Single spore cultures were prevailed by a series of dilutions . The single spore culture of 4 days, colony diameter was measured as well as morphological characters are recorded including these properties ,colony appearance, macroconidia, microconidia , conidiophore and chlamydospores (Leslie and Summerll,2006; Nelson et al. 1994, and Booth).

**-Molecular identification of *Fusarium* spp.**

**- DNA Extraction**

Single conidial fungal isolates were subcultured,incubated for six days at $25^0$ C in potato dextrose broth in order to extract genomic DNA. Within aseptic environment, mycelia were cleansed and then frozen at -20°C. DNA have been extracted using a Jena (Jena Bioscience, Germany) yeast DNA extraction kit in accordance with the manufacturer 's manual. DNA purity as well as concentration have been calculated by Spectrophotometry Nanodrop ND 2000 (Nanodrop technologies Inc). To assess DNA purity, the optical density ratio at 260l280 nm has been used, the DNA sample purity would be considered acceptable if the ratio reading ranges between 1.7 to 2.0.

**-Polymerase chain reaction (PCR) amplification**

Amplification of ITS region has been done applying primers ITS1 and ITS4(White et al. 1990). ITS1 TCCGTAGGTGAACCTGCG. TCCTCCGCTTATTGATATGC. (550 bp). Macrogen (S. Korea Co. ) introduced forward and reverse primers ,it is made as a stock solution by dissolving freeze-dried powder with deionized distilled water to prepare a concentration of 100 pmol/µl.20 pmol/µl have been the work solution concentration for the forward and reverse primers. The PCR reaction have been prepared in a final volume of 40µl containing 20µl 2x Taq PCR Master Mix (Polymerase enzyme),2µl of both reverse and forward primers (20 pmol),6µl of genomic DNA (30-100 ng/µl)and 10µl of RNase – Free water. The process of cycling for *Fusarium* spp. Used for ITS1,ITS4 ,starts with the Initial denaturation of DNA that is done under conditions of 95℃for 4min and 1cycle,then denaturation at 94C for 30s at 10 rpm.Annealing at 60C for 45s,extension at 72C for 1:30s ,again repeating Denaturation at 94C for 30s and 25 rpm,Annealing at 55C for 45s ,Extension at 72C for 1:30s then the Final extension at 72C for 10 min 1 rpm,to get the process results Hold at4C for 1min and 1 rpm.

**- Sequencing alignment and phylogenetic analysis**

The BioEdit v7 sequence alignment editor have been applied to guarantee the purity of the DNA sequence.The sequence prevailed in this work is campared to other GenBank accessions, the BLAST tool was used. For comparing the selected sequences clustalW was used.Utilizing the Maximum Parsimony method was included in the evolutionary history. As the bootstrap consensus tree concluded from 1000 replicates is assumed to symbolise the history of evolution of the taxa reviewed (Felsenstein 1985).Subdivisions associated with divisions that were replicated in fewer than 50% of bootstrap replicates are squashed. the proportion of duplicate trees where, with in bootstrap test, the linked taxa bunched together (1000 replicates) are shown above the branches.and according to 95% maximum parsimony, it belongs to the same clade as *F. equiseti* strains 18, A577, 48, and MK7IA-FE (MP).

**- Pathogenicity test**

Obviously healthy potato tubers were chosen, surface sterilized about five minutes with 1% sodium hypochlorite, left to dry overnight, cleaned three times by sterilized water, and then infected by taking a plug of tissues (6mm-diam. And about 10mm deep) using sterilized cork borer, and replaced with a 6mm-diam. plug of the pathogen. Two inoculation methods were used Mycelial plug methods. Mycelial growth plug taken from cultures of a 6-day-old *Fusarium* species that are currently growing. Pipetting 50 µL of spore suspension (106 spores/ml) into a wound inoculates tubers. Measurements of the rotten areas were taken after the inoculated tubers had been cultured for 4 weeks in the dark at 25°C and 70% humidity in paper bags.PDA plugs that weren't inoculated were left in the injured tubers as a control measure (Estrada et al., 2010).The findings of the tuber dry rot test were analyzed using the lesion measures of each tuber one by one. Analysis of variance (ANOVA) was performed on the data. The significance of the mean differences for the lesion sizes was evaluated using the Duncan test by Genstat data analysis software, with a significance threshold of p = 0.05.

## RESULTS

**- Morphology identification**

1- ***Fusarium oxysporum*** Schltdl., Flora Berolinensis, Pars secunda: Cryptogamia: 139 (1824) [MB#218372]. *F. oxysporum* (Isolate No. B11T2) GenBank: OP379284, Consider as the first record in Iraq causing *Fusarium* dry rot on Potato tuber. Among many *Fusarium* species that have been isolated from dry rot infected potato tubers ,two specific *Fusarium* species are identified depending on molecular and morphological characteristics, *F. oxysporum* and *F. equiseti. F. oxysporum* isolate, at first produced white, later turns to pale pink colonies with short monophialides carrying numerous microconidia in false heads that are ranged from 5.5 to 7.9 × 3.9 to 4.8 µm. In addition macroconidia were largely 3-4 septates, curved somewhat, as it is ranged from 30.3 to 46.9 × 3.2 to 4.7 µm. While chlamydospores have been both terminal and intercalary,they can be solitary and also in short chains (2–4 elements), and ranged from 6.9 to 13.2 × 6.1 to 12.2 µm. (Fig. 1)

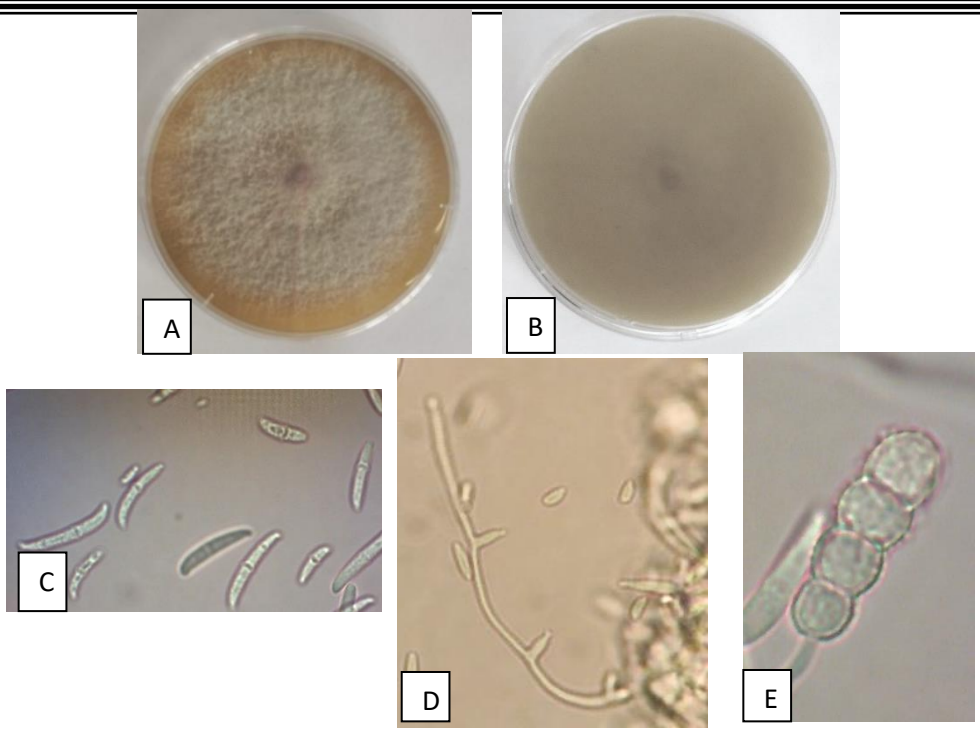


**Fig. (1):-** *Fusarium oxysporum* morphology on PDA A and B. C - Oval to kidney-shaped microconidia and slightly sickle shapes macroconidia D- short monophilides E- pairs chlamydospores

2- ***Fusarium equiseti***. *F. equiseti* (Corda) Sacc., Sylloge Fungorum 4: 707 (1886).*F. equiseti* (Isolate No. E13T4) Gen Bank: OP494711,consider as the first record in Iraq causing *Fusarium* Dry rot on a Potato tubers.

A pure culture grows to white colonies, then to beach orange color at agar of incubation at 25±1C°,absence of microconidia. There are many curved macroconidia with prominent foot cells, elongated and tapered apical cells, and 2–5 septate hyaline cells that range in size from 25.00 – 38.00 × 4.05-6.00 µm. Chlamydospores are formed intercalary, singly and in chains, nearly spherical, and hyaline. Their diameter measured 5.00- 11.00 µm (Fig. 2).

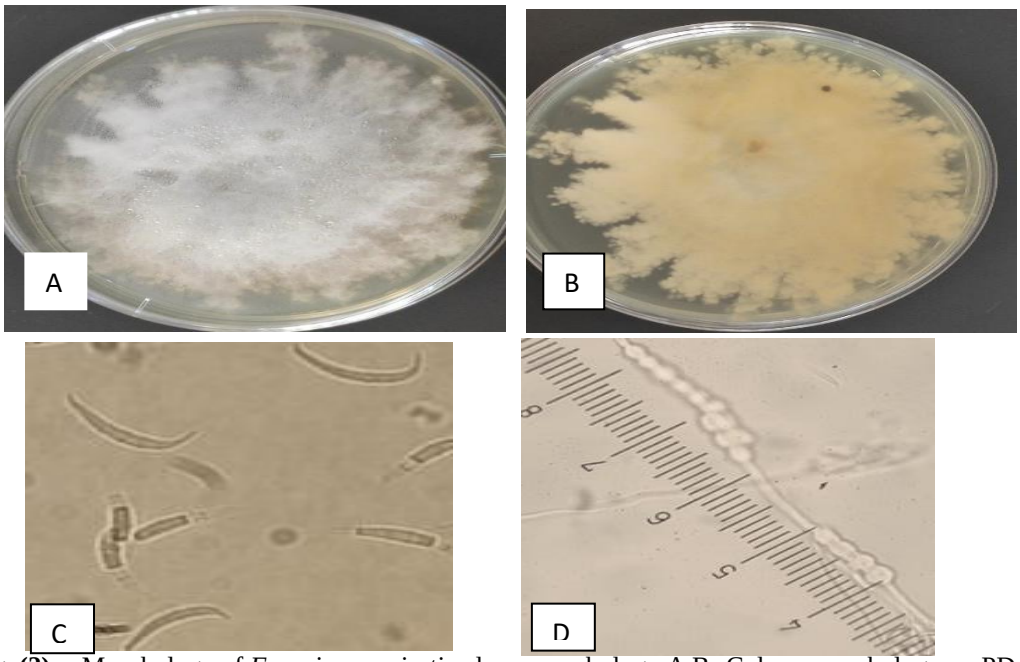


**Fig. (2):-** Morphology of *Fusarium equiseti* colony morphology A,B- Colony morphology on PDA, C – Macroconidia D- Pairs chlamydospores

**- Molecular identification and Phylogenetic analysis**

B11T2 isolate was identified as *Fusarium oxysporum* ,with 99% MP,as it is grouped with in the same clade with *F. oxysporum* strain BMS ,*F. oxysporum* isolate EPOOL and with *F.oxysporum* voucher RAB1113317. While all are claded with an out group species *Alternaria solani* isolate SX300-4. (Fig. 3).

E13T4 isolate was identified as *Fusarium equiseti* ,and according to 95% maximum parsimony, it belongs to the same clade as *F. equiseti* strains 18, A577, 48, and MK7IA-FE (MP).

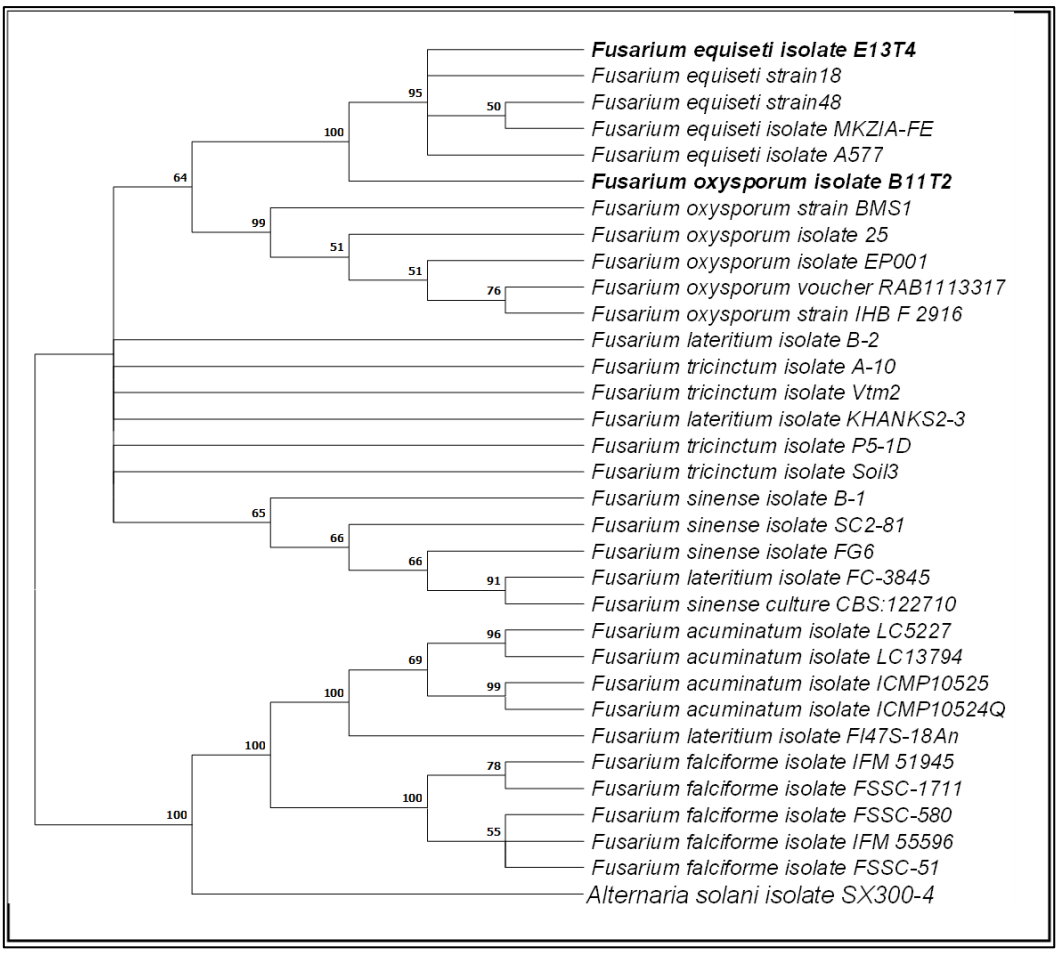


**Fig. (3):**- Maximum phylogeny analysis of ITS generated in Megax and 1000 replication bootstrapping for the two new *Fusarium* species ( *F. oxysporum* (No. B11T2) and *F. equiseti* (No. E13T4) ) causing *Fusarium* dry rot on a potato tuber.

**-Pathogenicity test**

Regardless of the *Fusarium* isolates used for the inoculation, dry rot symptoms, including brown with dry decay developed on the incubated potatoes.On the control potato tubers that weren't inoculated, there were no signs of dry rot. Infections initiate at wound sites. As soon as the infection begins, it slowly grows wider in every direction. Due to the fungus drying out the tuber's contents, the infected region skin rots and wrinkles,and may cause concentric circles .The internal infected spots range from light brown to black as the pathogen destroys cells the tuber.Typically, vibrant fungal mycelium can be found in the dry rot cavities (Fig. 4).

Depending on the pathogenicity test results on Potato tubers (Arizona cv.), *F. oxysporum* was the most significant aggressive species on the potatoes from markets,with wound measurements were (23.86mm depth,21mm width), *F. equiseti* was less aggressive as the wound measurements were (12.86mm depth,8.14 mm width. This result concerning highly virulent *F. graminearum* is approved because numerous researches perfomed all around the world are showning that the most prevailing aggressive *Fusarium* species on potatoes are *F. oxysporum*, *F.*

*sambucinum* and *Fusarium solani* (Tivoli and Jouan, 1981;Desjardins et al.,1992; Theron and Holz, 1989, 1990; Wastie *et al*., 1989;Tivoli et al.,1986a,1988; Choiseul, 1996; Carnegie *et al*., 2001;Ayed et al., 2006a; Vitale *et al*., 2004; Daami-Remadi and El Mahjoub, 2004).According toChoiseul (1996),it appears that the potato cultivars may be benefitable more than fungicides utilized to control the dry rot of *Fusarium*.

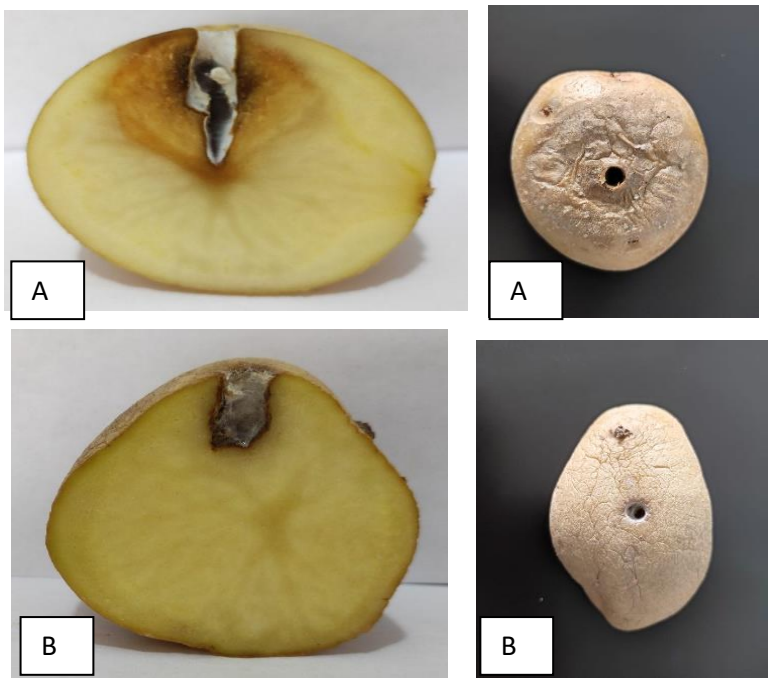


**Fig. (4):** Potato tubers (Arizona cv.) infected with A- *Fusarium oxysporum* B) *Fusarium equesti*

## DISCUSSION

Global potato output is seriously threatened by the *Fusarium* species disease known as potato dry rot. Diseases that are soil- and the seed-borne affect the crop through preventing potato sprouts growth also causing severe rots on seed tubers, the table potatoes, as well as during cold storage. Furthermore, utilizing the least demonstrative or low nucleotide sequence modification of the ITS region, it has previously been possible to clearly identify a number of complicated taxa (Oechsler et al. 2009; Wang et al.2011).The ITS section from the rDNA may be amplified using a thermal cycler, and when the right primer pairs are used, it is dependable, accurate, and quick to identify *Fusarium* species. In the ITS section, *Fusarium* spp. show polymorphisms, according to Duggal et al. (1997).That supports the findings of the earlier studies(White et al. 1990) as well as(O'Donnell 1992) , that refers to the benefit gained from the ITS section as a molecular marker to determine *Fusarium* spp.

The pathogenicity of two isolates of *Fusarium* species obtained of the tissues from the infected plant varied just similarly to the previous studies (Ray and Hammerschmidt 1998;El-Hassan et al.2007).The primary plant parts which are adversely impacted with dry rot are the tubers and the roots. On the outer surface of tubers and roots, lesions typically develop in a pattern of shrinkage and shriveling. At the same time the interior tissues also experience black or brown degeneration . The infections primarily enter the body through wounds, where they cause internal tissue to deteriorate and turn black, brick orange, white, or brownish in color.

The evaluation of local potato cultivars in the current research against two *Fusarium* species that are truly associated with the dry rot development is original since that has never been done in Iraq. Due to the numerous differences in pathogen aggressivity as well as plant material between different countries, the originality has been partly linked with the site-specific nature of this type of research.It is crucial to

evaluate the available cultivars as well as the newly created germplasm against the disease in order to rank them for susceptibility and resistance. Because a cultivar's sensitivity or resistance varies based on the *Fusarium* species and storage temperature,so for creating a breeding program that should be concerned .

In the present study, two species of *Fusarium* (*F. equiseti* and *F. oxysporum*) have been isolated of infected potato tubers collected from different warehouses and local markets in Duhok province. Pathogenicity test confirmed their virulence to cause dry rot on Potato tuber. Both *Fusarium* species were identified based on molecular identification of ITS region.Since the resistance of potato against every *Fusarium* species is complex,as this standard is affected by the date of plantation, irrigation fertilizer, soil quality,temperature and preservation period, more experiments are needed to incorporate the physiology of the tuber for the susceptibility of dry rot . Moreover, the Fusarium complex's varying aggressiveness was caused by its interaction with the incubation temperature (Daami- Remadi et al. 2006).

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