Disease Note

First report of Didymella Glomerata (Corda) and Penicillium Polonicum Zaleski on Wheat (*Triticum aestivum* L.) Seeds in Iraq

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ABSTRACT

In the present study, two fungal species, *Didymella glomerata* and *Penicillium polonicum*, were isolated from seeds of four wheat cultivars, named Mahmoodia (MHD), Adena (ADN), Eba 99 (EBA), and Wefia (WAF), and determined by using phenotypic characteristics and molecular sequencing. Molecular diagnosis for both fungi was applied based on internal transcribed spacer primers (ITS1 and ITS4). This is the first record of *D. glomerata* and *P. polonicum* as wheat seed-borne fungi in Iraq.

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Introduction

Wheat *Triticum aestivum* L. is one of the most necessary grain crops playing a vital role in attaining global food security [1]. Wheat grains supply more than 18% of calories and 50% of carbohydrates consumed in the world. It also contains a high amount of various phytochemicals, including tannins, alkali, flavonoids, saponins, steroids, terpenoids, and glycosides, that support the conventional system of healthcare for humans [2]. In Iraq, this crop is considered a main staple of nutrition for most if not all population. Moreover, its local production was more than three million tonnes [3]. The wheat crop is attacked by different fungal species including seed-borne fungi whether at pre-harvest or at post-harvest resulting in economic losses in production [4]. Seeds could be passive carriers of fungal pathogens from stores to agrarian areas. Those infected seeds become the source of primary infection when grown in the fields causing seedling damage, seed abortion, seed necrosis, elimination or reduction germination, and seed rot leading to disease development by systemic infections during the growth of crop [5, 6]. Because of the limitation of data on both fungal species, the aim of current report is to determine particularly exact *Didymella glomerata* and *Penicillium polonicum* associated with wheat seeds, as the first record in Iraq.Initially, two species were isolated from sterilized seeds of selected wheat cultivars cultured on full-strength PDA media. Then, phenotypic and molecular characteristics for each fungal genera were identified after purification process of fungal cultures. Identification of the fungal isolates was used to correspond with that mention in related studies of *D. glomerata* [7, 8] and *P. polonicum* [9, 10].In their research, Valenzuela-Lopez *et al*. [11] focused on the species of the families Didymellaceae and Cucurbitariaceae including *D. glomerata* described phenotypically and molecularly. Moreover, Alidadi *et al*. [6] recorded *D. glomerata* as a phytopathgen and examined its phenotypic and molecular features. Khalil *et al*. [8] confirmed that *P. polonicum* could produce reproductive and vegetative structures isolated from investigated plant samples. They also found that the fungus was mycotoxigenic fungal pathogen being able to synthesize fatal mycotoxin such as citrinin, cyclopiazonic acid and penicillic acid. Chen *et al*. [12] also identified that the fungus could produce Ochratoxin A.Regarding *D. glomerata*, colonies were dark brown at the center and white at edges, fluffy and grew on PDA ranging 74 mm in diameter after nine days at 25°C. The mycelia were brown, soft and loose. Conidia were hyaline, single cell and ellipsoidal measured 3.4-3.7 µm in width and 6.5-6.8 µm in length (Fig.1 A and B). Concerning *P. polonicum*, colonies on PDA were smooth, fluffy and bluish green with white margins reaching

8.2 µm in diameter after nine days at 25°C. Conidiophores were septate and straight with smooth to rough walls. Phialides (conidiogenous cells) were ampulliform. Conidia were sub-globose to globose, smooth walls, and generated in columns. Moreover, conidial measurements were 2.7-3.8 µm in length and 2.4-3.7 µm in width (Fig. 2 A and B).

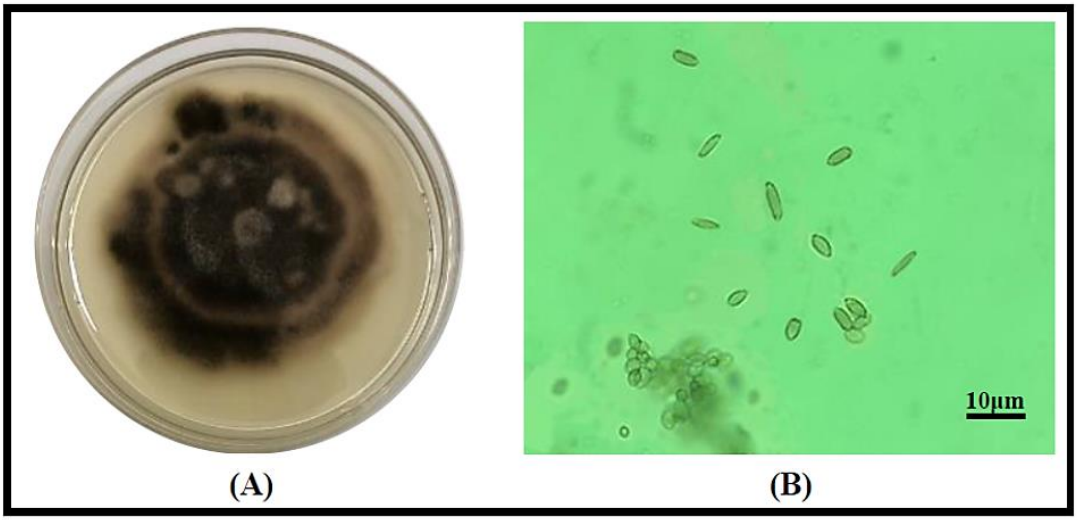

Fig. 1: Morphological traits of *D. glomerata*

(A) Morphology of colony on PDA after nine days, (B) Conidia with a magnification of 40x. Bars: B = 10 µm.

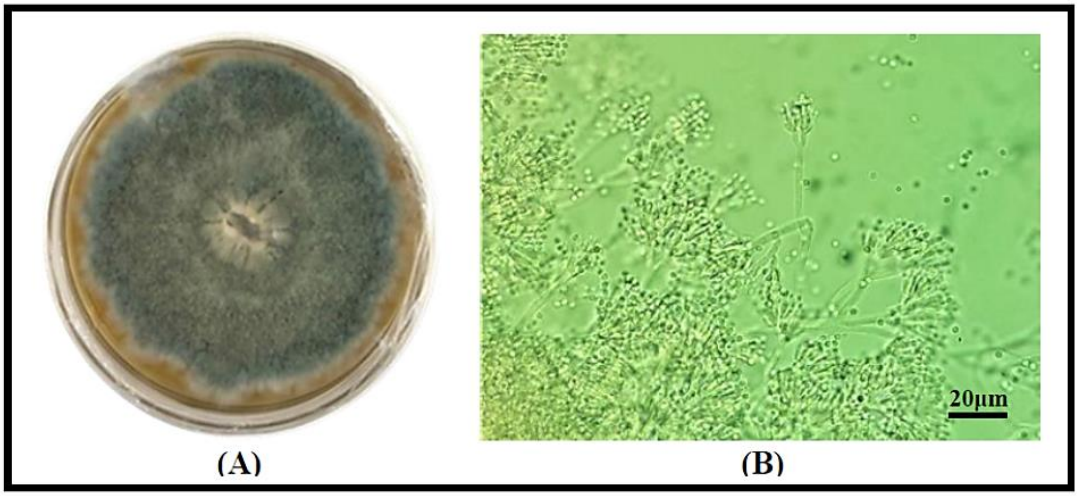

Fig. 2: Morphological traits of *P. polonicum*

(A) Morphology of colony on PDA after nine days, (B) Conidia, conidiophores and phialides with a magnification of 40x. Bars: B = 20 µm.

Both examined fungi were identified molecularly by applying ITS primers (ITS1 and ITS4) [13], the results of phylogenetic analysis revealed the similarity index of 99% for both *D. glomerata* (LT6033041.1) and *P. polonicum* (MT487786.1). The phylogenetic trees were constructed to show the similarities as illustrated in Figure (3 and 4).

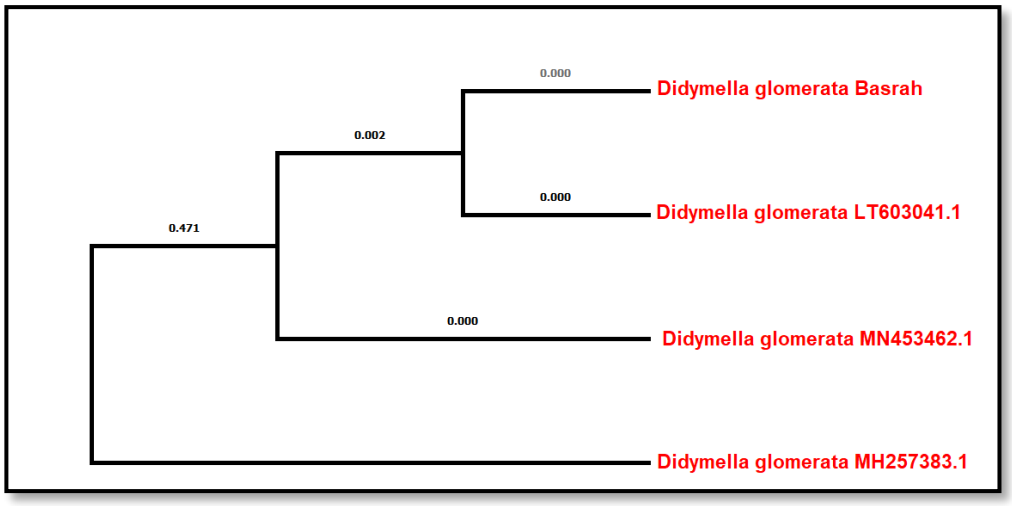

Fig. 3: Phylogenetic analysis of *Didymella glomerata* Basrah isolate.

Maximum likelihood analysis (MLA): phylogenetic tree deduced using Internal Transcribed Spacer (ITS). The nearest three *Didymella glomerata* isolates published in GenBank (https://www.ncbi.nlm.nih.gov/genbank/samplerecord/) were used in ClustalW program in MEGA-11 to construct the tree.

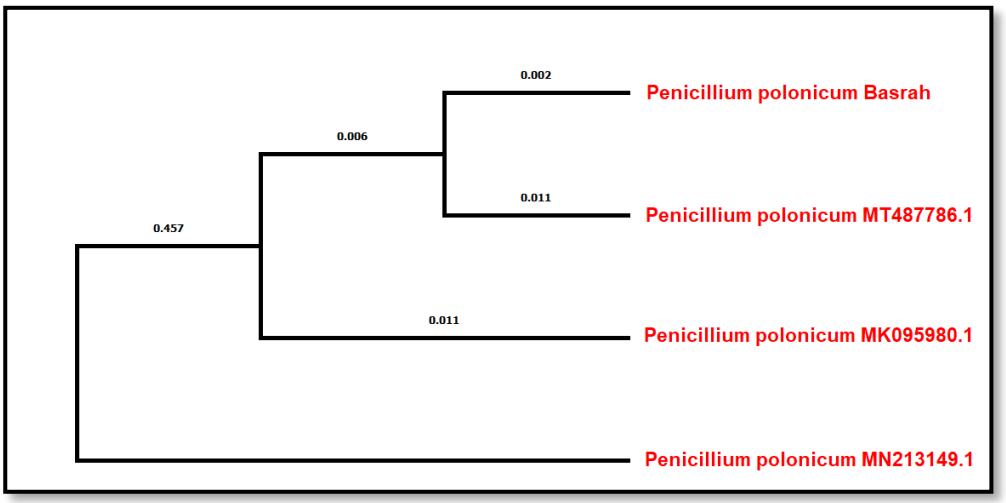

Fig.4: Phylogenetic analysis of *Penicillium polonicum* Basrah isolate.

Maximum likelihood analysis (MLA): phylogenetic tree deduced using Internal Transcribed Spacer (ITS). The nearest three *P. polonicum* isolates published in GenBank (https://www.ncbi.nlm.nih.gov/genbank/samplerecord/) were used in ClustalW program in MEGA-11 to construct the tree.

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التسجيل الاول للفطرين *Didymella glomerata* (Corda) Zaleski و *Penicillium polonicum* على بذور (*Triticum aestivum* L.) الحنطة في العراق

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المستخلص

في هذه الدراسة، تم عزل الفطرين *Didymella glomerata* و *Penicillium polonicum* من بذور أربعة أصناف حنطة وهي المحمودية (MHD) ، أدنا (ADN) ، إباء 99 (EBA) ووفية (WAF) ، وتشخيصهما على اساس الصفات المظهرية والمجهرية وكذلك باستخدام التتابع الجزيئي. وتم تطبيق التشخيص الجزيئي لكلا الفطرين اعتماداً على الواسمات (ITS1 و ITS4) والتي تُعد أول تسجيل لـ *D. glomerata* و *P. polonicum* كفطريات محمولة ببذور الحنطة في العراق.