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THE FIRST RECORD OF *ALTERNARIA TRITICINA* THE CAUSATIVE AGENT OF ALTERNARIA LEAF BLIGHT IN WHEAT AND BARLEY IN IRAQ

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A B S T R A C T

Alternaria leaf blight caused by *Alternaria triticina* can cause high yield losses at the severe infection in wheat and barley. It is first time for this pathogen to be recorded in Iraq. The investigation process of this disease included 25 locations from 12 sites in 5 provinces that plant wheat and barley in the south and middle of Iraq. The results revealed that the fungus was isolated from almost all examined locations with different frequency. Shethaif- Al Garbie and Sheikh Saad showed higher number of isolates with 60 and 40 in wheat fields, respectively; while, Shethaif- Al Garbie and Dabuni expressed the highest number of isolates in barley fields with 45 and 33, respectively. The highest isolation frequency in wheat fields was at Sheikh Saad and Ali Alsharqi with 100% followed by Ali Algharbie with 90% and the lowest was at Al- Kut and Al-Huria with 37.5 and 40%, respectively. The highest isolation frequency in barley fields was recorded at Dabuni with 82.5% followed by Shethaif- Al Garbie and Babil / Al-Huria with 75% and the lowest was at Al-Basrah/ Shatt al-Arab with 20%. The fungus was isolated from all plant parts (stem, leaf, and spike); however, spikes recorded the highest isolation frequency reaching 100% in some locations.

Keywords: *Alternaria triticina*, Alternaria leaf blight, wheat and barley.

INTRODUCTION

Wheat (*Triticum aestivum*) and barley (*Hordeum vulgare* L.) are the first and second crops in Iraq. Iraq produces about 3 million tons of wheat and 900,000 tons of barley annually according to FAO GIEWS, 2014. However, Iraq imports 1.7 million tons of wheat each year to cover people need. Iraq plants about 1,625 million hectares of wheat according to the Ministry of Agriculture statistics expressing the importance of this crop. *A. triticina* first recorded in India and it causes high yield losses in wheat and barley (Prasada and Prabhu, 1962). The fungus was also reported from Argentina, China, Turkey, Egypt, Greece, France, Lebanon, Nigeria, Middle East, Italy, Bangladesh, Mexico, and North Africa (Perelló and Sisterna, 2006, Özçelik and Özçelik, 1997, Beshir, 1994, Logrieco *et al.*, 1990, Wiese, 1987, Frisullo, 1982, Waller, 1981, Anahosur, 1978, Wiese, 1977). In the severe infection yield losses may reach about 60% of the planted area (Prabhu and Singh, 1974, Sokhi, 1974). It can cause significant decrease in seeds weight (Raut *et al.*, 1983).

This pathogen is considered as a soil borne and seed borne; however, soil borne does not play an active role in fungal transmission under hot conditions(Kumar and Arya, 1973, Kumar and Rao, 1979). Seeds can carry out the pathogen as conidia on the surface or as a mycelium inside the seed providing inoculums for the next season (Kumar and Arya, 1973, Kumar and Arya, 1976). It is difficult to distinguish *A. triticina* from other Alternaria species and it always a matter of confusion due to the close morphological characteristics despite of the developed recent studies (Dugan and Peever, 2002).

The main objective of this study is to investigate the occurrence and distribution of *A. triticina* in wheat and barley fields during growing season in the south and middle of Iraq.

MATERIALS AND METHODS

Samples Collection: Samples were collected from wheat and barley fields in the middle and south of Iraq particularly provinces that planting cereals from

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following sites (Al-Numaniyah, Kut, Dabuni, Shethaif- Al Garbie, Sheikh Saad, Amarah, Ali Algharbie, Kumayt, Ali Alsharqi, Al Diwaniyah, Shatt al-Arab, Al-Huria). Over 500 samples were collected from 12 sites from specific points according to Global Positioning System (GPS) during wheat seasons covering 5 Iraqi provinces in the middle and south (Table 1). Samples were gathered according to the targeted area and cereal type (wheat or barley). Generally, wheat is more popular in Iraq than barley; therefore, wheat fields are larger than barley ones which explain the higher number of wheat samples.

Fungal Isolation and Purification: Three 2 mm pieces of tissue from each stem, leaf and spike were subjected to fungal isolation. The pieces were surface sterilized in bleach (1% available chlorine) for 5 minutes, and washed twice in sterile water for 5 minutes. Then, the pieces were dried by placing them on sterile paper towel. Subsequently, tissue pieces were transferred onto quarter-strength potato dextrose agar (PDA) plates which contain 100 µg/ml streptomycin sulphate and 10 µg/ml tetracycline hydrochloride. Plates were incubated at ambient temperature and placed under standard white fluorescent light (35098 F18E/33 General Electric, USA) for 24 hours for 5 -7 days. Spore suspension was made by adding 3-4 drops of sterile distilled water on the fungal colony that were grown around the plated wheat tissues using sterile flame-sterilized loop. This spore suspension was streaked onto 2% water agar media by using a flame-sterilized metal loop and plates were incubated under laboratory conditions for 24h. A single germinated conidium was transferred onto full-strength PDA media plate and incubated at ambient temperature according to Scott and Chakraborty (2010).

***Alternaria triticina* Identification:** *Alternaria* isolates were identified according to (Prasada and Prabhu, 1962, Dugan and Peever, 2002, Mercado Vergnes *et al.*, 2006, Simmons, 2007). Isolates obtained from different plant parts (leaf, stem, and spike) from wheat and barley at the end of season. There is a clear overlap between *A. triticina* that causes *Alternaria* leaf blight and *A. alternata* that causes black mold on wheat especially the symptoms were discovered just before harvesting stage and the fungus was isolated from different plants parts (leaf, stem, and seeds).

Table 1. Collected samples from different locations of middle and south Iraqi provinces and their GPS points.

Province	Site	Longitude	Latitude	Locations	Samples
Wasit	Al-Numaniyah	45° 24'E	32° 35' N	2	40
	Kut	45° 49' E	32° 30' N	1	33
	Dabuni	45°21' E	32° 21' N	3	60
	Shethaif- Al Garbie	45°11' E	32° 87' N	3	144
	Sheikh Saad	46° 12' E	32° 66' N	3	85
Amarah	Amarah	46° 69' E	31° 26' N	1	22
	Ali Algharbie	46°66' E	32° 49' N	2	35
	Kumayt	46°94' E	32° 10' N	3	56
	Ali Alsharqi	46° 43' E	32° 70' N	2	40
Al-Qadisiyyah	Al Diwaniyah	44° 55' E	31° 59' N	2	44
Al-Basrah	Shatt al-Arab	47° 77' E	30° 44' N	2	45
Babil	Al-Huria	44° 39' E	32° 84' N	1	52

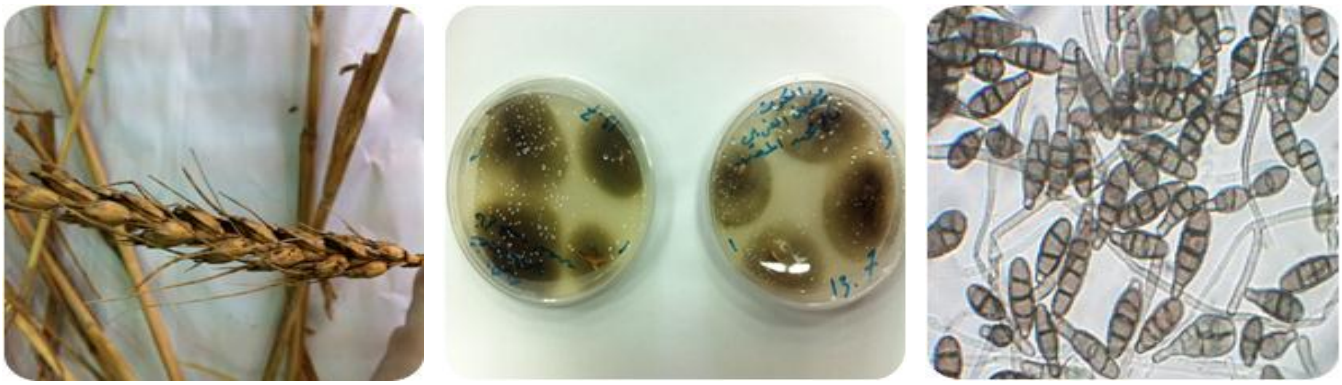

Figure 1. a. Alternaria leaf blight symptoms on stems and spikes b. *A. triticina* colony shape and color on PDA medium c. *A. triticina* young conidia and mycelia shape and color.

RESULTS

Morphological characteristics of the fungus such as mycelium shape and color; conidium shape, color, and size; and conidiophores shape, were used to identify the exact causative *Alternaria* species. Mycelia and conidiophores are spetated, brunched, and have the same color. Mycelium width is 2-7µm. The conidia vary in length from 17-85 × 7.5 -29, 15.8 -68 × 7-13.5 and 18 -88 × 7-18 µm on malt extract agar, standard nutrient agar, and the host, respectively (Prabhu and Prasada, 1970). *A. triticina* produce black to brown colored colony on PDA medium reaching 80 mm after five days at 25 ˚C.

Samples were collected from randomly chose wheat fields and the number of samples was determined according to the planted area. Some province sites revealed high number of isolates in wheat fields such as Shethaif- Al Garbie and Sheikh Saad in Wasit with 60 and 40 isolates respectively (Table 2). However, some locations expressed high isolation frequency such as Sheikh Saad and Ali eastern with 100% followed by Ali Algharbie with 90%. Dabuni, Shatt al-Arab, Shethaif- Al Garbie, and Kumayt showed high isolation frequency ranging from 82.5-75%. The lowest isolation frequency rate was recorded at Al- Kut and Al-Huria with 37.5 and 40% respectively (Table 2).

Wasit in Shethaif- Al Garbie and Dabuni showed the highest number of isolates in barley fields with 45 and 33, respectively (Table 3). The highest isolation frequency rate was recorded at Dabuni with 82.5% followed by Shethaif- Al Garbie and Babil / Al-Huria with 75%. The lowest isolation frequency rate was reported at Al-Basrah/ Shatt al-Arab with 20% (Table 3). Low number of locations of barley fields was examined to determine fungal isolation frequency due to the less planted area.

Table 2. Number of examined samples and the percentage of isolation frequency from wheat fields.

Location	No. of examined samples	No. of Isolates	% Isolation frequency
Wasit/ Shethaif- Al Garbie	80	60	75
Wasit/Al- Kut	16	6	37.5
Wasit/Sheikh Saad	40	40	100
Wasit/ Al-Numaniyah	25	12	48
Dabuni	40	33	82.5
Al-Qadisiyyah/ Al Diwaniyah/Dahghar	40	20	50
Babil / Al-Huria	30	12	40
Amarah/ Kumayt	24	18	75
Amarah/ Ali Algharbie	20	18	90
Amarah/ Ali Alsharqi	16	16	100
Al-Basrah/ Shatt al-Arab	22	17	77.2

Table 3. Number of examined samples and the percentage of isolation frequency from barley fields.

Location	No. of examined samples	No. of Isolates	% Isolation
Wasit/ Shethaif- Al Garbie	60	45	75
Wasit/Sheikh Saad	22	16	72.72
Dabuni	40	33	82.5
Babil / Al-Huria	20	15	75
Amarah/ Kumayt	22	15	68.18
Amarah/ Ali Algharbie	16	8	50
Amarah/ Ali Alsharqi	16	12	75
Al-Basrah/ Shatt al-Arab	10	2	20

A. triticina isolates were collected from different plant parts (stem, leaf, and spike). The distribution of isolates among plant parts showed that spike recorded the highest isolation frequency rate at all locations and samples as compared to other tested parts (Table 4). Al-Qadisiyyah/ Al Diwaniyah-Dahghar and Al-Basrah/ Shatt al-Arab showed 100% of the isolates from spikes. Amarah/ Ali Algharbie and Dabuni highest percentage of isolates was from spike with 88.88 and 81.18% respectively followed by Wasit/Sheikh Saad, Wasit/ Kut, and Amarah/ Kumayt with 70, 66.66, and 66.66% respectively. Stem as well revealed high isolation

frequency especially at Wasit/ Kut and Wasit/ Al-Numaniyah with 33.33% followed by Amarah/ Ali Alsharqi and Wasit/ Shethaif- Al Garbie with 25 and 21.66% respectively. Leaf highest isolation frequency was recorded from Amarah/ Kumayt with 33.33% followed by Dabuni with 18.18% (Table 4).

Table 4. The distribution of fungal isolates according to plant part (stem, leaf, and spike) in wheat fields.

Location	Number of isolates	Plant part		
		Stem%	Leaf%	Spike%
Wasit/ Shethaif- Al Garbie	60	21.66	13.33	65
Wasit/ Kut	6	33.33	0	66.66
Wasit/Sheikh Saad	40	20	10	70
Wasit/ Al Numaniyah	12	33.33	16.66	50
Dabuni	33	0	18.18	81.81
Al-Qadisiyyah/ Al Diwaniyah/Dahghar	20	0	0	100
Babil / Al-Huria	12	16.66	25	58.33
Amarah/ Kumayt	18	0	33.33	66.66
Amarah/ Ali Algharbie	18	0	11.11	88.88
Amarah/ Ali Alsharqi	16	25	12.5	62.5
Al-Basrah/ Shatt al Arab	17	0	0	100

Barley isolates revealed almost the same trend as wheat ones with highest isolation frequency from spikes (Table 5). The highest isolation frequency rate was from spike at Dabuni, Amarah/ Kumayt, and Al-Basrah/ Shatt al-Arab with 100% followed by Amarah/ Ali Algharbie, Amarah/ Ali Alsharqi, and Wasit/Sheikh Saad with 81.25, 68.75, and 68.18% respectively. Stem highest frequency isolates was at Wasit/ Shethaif- Al Garbie with 35.48 followed by Babil / Al-Huria with 15%. The highest rate of leaf isolates was at Babil / Al-Huria with 25% followed by Wasit/ Shethaif- Al Garbie with 24.19%. Some of plant parts such as leaf and stem did not express fungal isolates (Table 5).

Table 5. The distribution of fungal isolates according to plant part (stem, leaf, and spike) in barley fields.

Location	Number of isolates	Plant part		
		Stem	Leaf	Spike
Wasit/ Shethaif- Al Garbie	62	35.48	24.19	40.32
Wasit/Sheikh Saad	22	13.63	18.18	68.18
Dabuni	40	0	0	100
Babil / Al-Huria	20	15	25	60
Amarah/ Kumayt	22	0	0	100
Amarah/ Ali Algharbie	16	0	18.75	81.25
Amarah/ Ali Alsharqi	16	12.5	18.75	68.75
Al-Basrah/ Shatt al Arab	10	0	0	100

DISCUSSION

This is first time for the fungus *A. triticina* to be recorded in wheat and barley fields in Iraq causing leaf blight. The olive-buff symptoms were noticed on wheat and barley leaves in the Iraqi middle and south fields especially at the mature stage. After first isolation of the fungus a larger screening was initiated to estimate infection distribution and density. The fungus was isolated from almost all the inspected fields and from all plant parts (stem, leaf, and spike); however, the infection severity varied from province to another and from location to another.

The emergence of this pathogen in Iraq might be because of the change in climate in the last three years with the increase of late rain during the planting season providing favorable conditions. Perelló and Sisterna (2006) also suggested the same reasons when *A. triticina* first recorded in Argentina. Moreover, agriculture practices such as conservation tillage, nitrogen fertilization, irrigation may increase this disease severity (Perelló and Sisterna, 2006). Another important explanation to the emergence of *A. triticina* is the use of seeds that were not certificated by Iraqi

Ministry of Agriculture as Iraq Ministry of Agriculture normally does not allow farmers to plant unauthenticated wheat and barley seeds. Seeds can transmit this pathogen via the surface inoculation by conidia or via grown mycelia inside the seeds (Kumar and Arya, 1973, Kumar and Arya, 1976).

Another justification of the emergence of this disease is: it might be existed before but it was not recognizable due to the low infection rate because of the unfavorable climate conditions. Nevertheless, when the weather became favor to the pathogen, it turns out to be a pest that can cause remarkable yield losses. As in Argentina *A. triticina* reported in (1992) by Perelló *et al* but it emerged as a big problem on wheat by 2006 and one of the expected reasons was suitable weather conditions (Perelló and Sisterna, 2006). Wider studies on this pathogen should be established measuring the expected yield losses and host reaction. Moreover, it is important to investigate other Iraqi provinces particularly the north of Iraq to know the distribution of the pathogen and it is reaction to climate change.

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