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Alternaria leaf blight of barley incidence in Benghazi district, Libya

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Abstract \

During the growing season (2019-2020), minute, irregular, and brown lesions appeared on barley (*Hordeum vulgare* L.) leaves. Therefore, the current experiment was conducted to accurately isolate and to identify the causative agent of leaf blight in barley's field in Benghazi plateaus. Results showed that *Alternaria* spp. was the causative agent based on morphological and cultural characters such as conidial color, septal enumeration, conidial dimension, and colony color on growth media. Detached leaf bioassay was used to fulfil pathogenicity test *in vitro*. Our finding suggests further studies should be directed to determine whether the fungus is a soil-borne or a seed-borne pathogen; hence, an effective disease management can be applied.

Key words: *Alternaria* complex, barley, leaf blight, Libya.

Introduction \

Barley (*Hordeum vulgare* L.) has been a central, and staple commodity crop in Libya with multiple usages including its grinded flour in various traditional Libyan cuisines, viz. Bazine, and bread, seeds and hay for feeding livestock, and malting. Libya's production of barley was significantly low compared with other neighboring countries' yields, 160,000 tons in

1997, therefore, the country relies completely on importing barley grains from foreign market.

Alternaria is a ubiquitous genus occupied different kinds of ecological niches, for example, soil rhizoshepere, and phyllosphere; and, indeed, its association with hosts varies from being saprophyte, endophyte or phytopathogen. Barley are attacked by several fungal, bacterial or viral diseases. Of importance, leaf blight caused by *Alternaria* sp. complex significantly reduces qualitative and quantitative barley yields annually. It has been well documented that *Alternaria* produces detrimental mycotoxins such as tenuazonic acid (TA) on barley grains which caused severe damage to livestock feeding (9). The pathogen *A. triticina* was first described in India by (11) and since then, it has been reported in Iraq (7), in Argentina (10), and in the United States of America (6). Reduction of seed weight (79%) and seed number per head (65%) of sunflower, *Helianthus annuus* L., infected with *A. alternata* was reported in Greece (8).

The objectives of the current study were to determine the causative agent of barley leaf blight, and to evaluate PDA as standard medium for isolation and identification.

Material and methods \

Description of study site: Four localities, namely Alrjema (1), Almagizha (2), Gerdina (3), and University of Benghazi's farm (4), were selected to conduct the present survey of barley leaf blight (fig. 1).

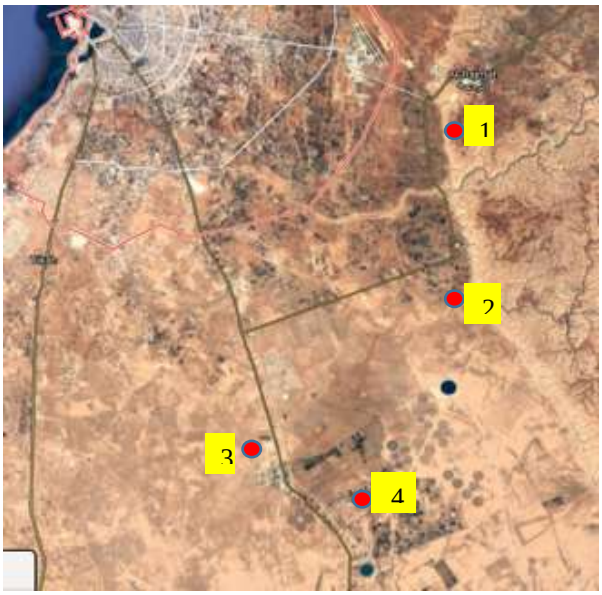

Fig. 1. Study locations

Sampling: We chose 30 diseased plants that showed typical leaf blight symptoms from each location, and five leaves were further selected out of 30 diseased plants for further analysis. Symptomatic barley leaves were collected and transported in plastic bags to the plant disease laboratory, faculty of agriculture, University of Benghazi for disease identification. Leaves were rinsed thoroughly with running tap water to remove dust particles and debris. Leaves then were cut into small fragments, soaked in 5 %

household bleach for 10 seconds, and rinsed thrice with deionized water for five minutes (4). After autoclaving potato dextrose agar(PDA) medium (Oxoid Ltd, UK) with Rose Bengal (0.3g/L) to inhibit fast-growing fungi, PDA additionally was amended with amoxicillin (0.5 g/ L) to eliminate bacterial contamination.

The excised barley leaves were dried with towel paper before transferring into sterilized plastic Petri dish (90 mm,16 mm, Bibby Sterilin Ltd, UK) with sterilized forceps for incubation. Five -seven days after incubation at 25°C with 12 hours photoperiod cycle, *Alternaria* pure culture was obtained via sub-culturing method (2).

***Alternaria* spore measurement:** *Alternaria* spores were photographed with a fixed camera attached to a compound microscope at 200 x magnification (Olympus microscope camera, Japan). Spores dimensions were measured with Image J software(Rasband).

Results \

Spore description: Club-shaped large, dark to light-brown, multi-celled with transverse (5-7) and longitudinal (1-2) septa; gradually tapering to beak (fig. 2.). Spore measurements were 49 µ in width, and 128 µ in length as shown in (fig.2).

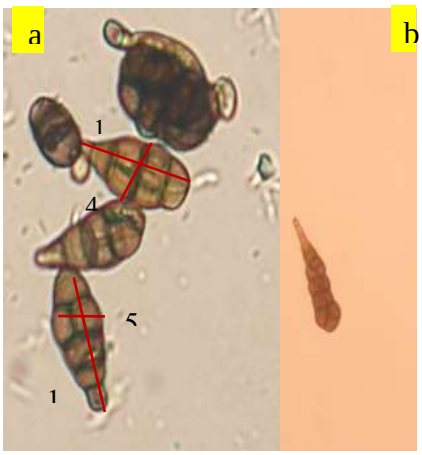

Fig. 2. *Alternaria* spores isolated from PDA medium (a). Conidium with short-beak (b)

Alternaria mycelium was effuse, fluffy, white first, and later turn to olivaceous color. Hypha was septate, hyaline, and brown to dark-brown (fig. 3).

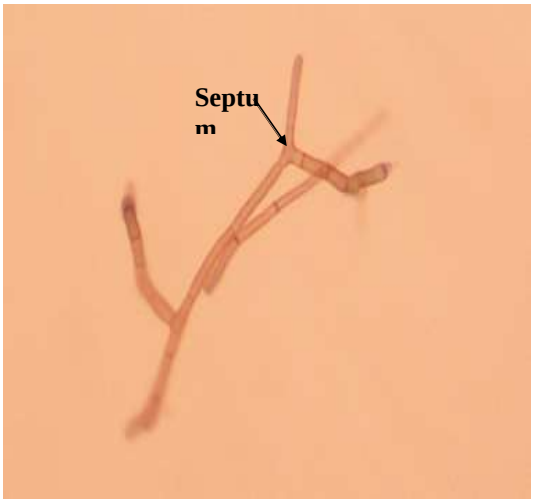

Fig. 3. *Alternaria* hypha under 20 x magnification.

Field symptoms first appear at the lower leaves, then begin spreading to the entire plant. Lesions were irregular, brown in color surrounded with yellow margin (fig. 4.). As the disease

progressed, these lesions enlarged and coalesced into each other, eventually causing leaves to drop prematurely.

Fig. 4. Barley leaves showing leaf blight symptoms in open field.

Pathogenicity assessment: Detached leaf bioassay was performed *in vitro* as described by Deadman and Cooke to satisfy Koch's postulates for pathogenicity. Three excised healthy barley leaves were placed on three sterile filter papers (Whatman) in Petri dish. Leaves were then inoculated with $1x10^4$ *Alternaria* spore suspension (with help of hemocytometer) obtained from fresh culture, whereas control leaves were sprayed only with sterile water. Seven days post inoculation, diseased symptoms were observed only on leaves inoculated with *Alternaria* suspension (fig. 5), while no diseased symptoms developed on control leaves. *Aletrnaria* spores were again re-isolated from barley leaves.

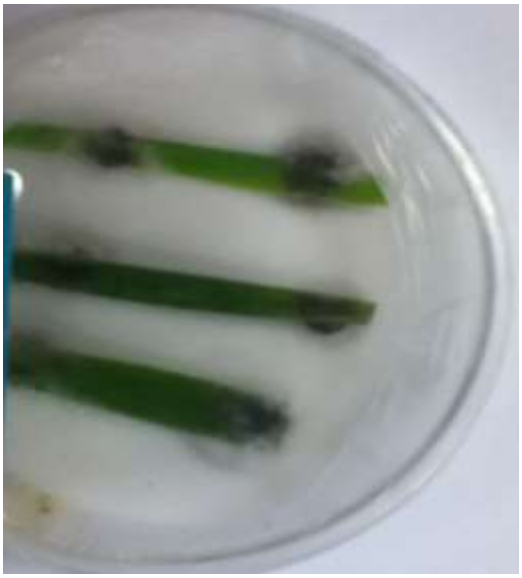

Fig. 5. Barley leaves inoculated with *Alternaria* suspension.

Discussion \

Leaf blight is considered globally an important fungal disease that decreases barley yields and wheat alike. Losses due to leaf blight infection vary depending on various factors such as plant accession, environmental factors and fungal aggressiveness. In a study done by(12), wheat plants at the flag leaf stage inoculated with *Alternaria triticina* had to up to 24% losses in grain number per ear. The result from the present experiment clearly showed that *Alternaria* complex sp. was the causative agent of leaf blight of barley in all surveyed areas. Surprisingly, *Alternaria* infection was comparatively high in all surveyed areas, except in site 1 in which the infection rate was

relatively low (fig1). Low infection rate can sometimes lead to a serious disease outbreak in the subsequent year. For example, *Pyrenosphora* leaf stripe begun as a minor and sporadic disease of barley crops, the following year the disease was present in approximately 40 % of barley's tiller (3).

The population samples were quite small and cannot be fully represent the surveyed sites, but it unquestionably provides us preliminary data regarding leaf blight's existence and incidence in Benghazi district. In addition, spore morphological features recorded in this survey were in full agreements with description provided by (1،13). Furthermore, *Alternaria* spore dimensions reported here matched those reported by (10). It can be hence deduced from this experiment that *A. triticina* might be the responsible species for this disease based on both host association, barley, and the disease symptoms. However, we tried not to draw this clear-cut identification based upon mere morphological features, as recommended by (13) and (5) .

Environmental condition in the eastern region of Libya favors plant fungal diseases occurrence in general, and leaf blight in particular, as this disease was reported in countries have similar semi-arid environment (7). To the best of author's knowledge, this is the first report of *Alternaria* leaf blight of barley in Benghazi district.

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الملخص العربى /

لفحة *Alternaria* علي محصول الشعير في سهل بنغازي، ليبيا

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خلال موسم النمو (2019-2020)، تم ملاحظة بقع صغيرة غير منتظمة الشكل ذات لون بني علي أوراق نبات الشعير(*Hordeum vulgare* L.). لذلك تم اجرا التجربة الحالية لغرض عزل و تعريف المسبب المرضي علي محصول الشعير المزروع في سهل بنغازي. النتائج المتحصل عليها من هذه التجربة أظهرت ان *Alternaria* sp. هو المسبب الفطري بناء علي الصفات الظاهرية و المزرعية ومن هذه الصفات لون الكونيديا و عدد التقسيم الكونيدي و ابعاد الكونيديا (الطول و العرض) وأخيرا لون المستعمرة الفطرية النامية علي اطباق بتري. تم إعادة عزل *Alternaria* ثلاث مرات معمليا من أوراق مصابة. تم استخدام بيئة مسحوق البطاطا مع مضاد حيوي(PDA) كبيئة عزل لهذا الفطر من أوراق الشعير المصابة.

نتائجنا المتحصل عليها توصي بضرورة اجراي أبحاث مستقبلية وذلك لمعرفة هل الفطر محمول في التربة او محمول في البذور و علي ذلك يتم اختيار الطريقة المناسبة للمكافحة.

كلمات مفتاحية: معقد *Alternaria* ، محصول الشعير، لفحة الأوراق، ليبيا.