

## A First Record of *Exserohilum rostratum* as a New Pathogen Causing Bean Blight in Egypt

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### ABSTRACT

Seedling blight of bean (*Phaseolus vulgaris* L.) was recorded in bean fields at five different localities in Beni Sweif Governorate, Egypt. Symptoms appeared as green dark to purplish-brown spots, with brown margins. The affected plant leaves were collected for mycological analysis. Percentage of disease incidence were 30%, 25%, 22%, 15% and 35% in El-Wasta, Nasser, Beni Sweif, Sumosta and Beba counties respectively. Leaf samples were surface sterilized and cultured on potato dextrose agar. The growing fungi were identified on morphological as well as on molecular basis. Microscopic examination revealed that the isolated organisms have the same characteristics of *Exserohilum rostratum* (Drechsler) Leonard & Suggs. Among the 30 fungal isolates collected from the five bean plantations, a representative isolate was grown for DNA extraction, PCR and rDNA sequencing. Universal primers targeting ITS regions of the rDNA were used for PCR and sequencing. Results confirmed that the sequences of these fungi showed close relationship with *E. rostratum* with 99.6% - 100% similarity. The obtained sequences were deposited in the GenBank with accession numbers MT075801, MT071830, MT071831, MT071832, and MT071834. Pathogenicity tests confirmed that *E. rostratum* strains were pathogenic showing the same disease symptoms previously observed on bean plants in the study areas. The minimum temperature for spore germination was 5°C, the optimum temperature was 35°C, and the maximum temperature was 50°C. On the other hand, conidial germination was stopped at both lower (3°C) and higher (55°C) temperatures. Studying the host range of the fungus showed that the pathogen was able to attack tomato, pepper, squash and potato beside common bean and watermelon.

**Keywords:** *Exserohilum rostratum*; Temperature; Bean; Symptoms; Pathogenicity; Host range

### INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is considered one of the most promising vegetable crops for local consumption as well as exportation. This crop is very important in the daily diet of more than 300 million people worldwide [1]. Bean plants are vulnerable to be attacked by a number of plant pathogens which can cause considerable losses in the yield and reduce the quality of seeds. Bean growers in Beni Sweif Governorate claimed that unknown disease began to attack bean plants just post-emergence of the seedlings above soil level up to the harvesting stage causing serious losses. Accordingly, the authors arranged several visits to bean plantations located in the five counties of

Beni Sweif Governorate and performed extensive surveys to determine the disease incidence and to collect diseased specimens for mycological and pathogenicity studies.

The genus *Helminthosporium* was discovered and classified by Link [2]. The type species, *H. velutinum*, is a saprobic dematiaceous fungus occurring on dead stems of *Alnus*, *Cornus*, *Salix* and numerous other plants. But currently less than 50 species are considered to represent true *Helminthosporium* species [3,4]. Refinements in the taxonomy of this genus resulted in there allocation of many of its members to other genera such as *Alternaria*, *Corynespora*, *Dendryphon*, *Septonema* and others [5].

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Later, Shoemaker [6] erected *Bipolaris* for taxa previously accommodated in *Eu-Helminthosporium bipolaris* was a heterogeneous entity which included two subgroups based on differences of the hilum morphology and associated with two different sexual morphs. The group that has conidia with non- or slightly protruding hila was often associated with the sexual morph *Cochliobolus*, characterised by filiform ascospores that often appear more or less coiled in a helix within the ascus [7-11]. On the other hand, the group that has conidia with a protruding hilum had sexual morphs with fusoidascospores enveloped in gelatinous sheaths, which was originally described in *Trichometasphaeria* [12-14]. Later Leonard & Suggs [15] erected *Exserohilum* to accommodate those *Bipolaris* s.lat. species with a distinctly protruding hilum, and *Setosphaeria* for the sexual morph. *Setosphaeria* differs from *Trichometasphaeria* by the production of non-clypeateascosmata which can be erumpent or superficial and produce larger ascospores.

Rossmann et al. [16] recommended the name *Exserohilum* over *Setosphaeria* according to Article 57.2 of the International Code of Nomenclature for algae, fungi and plants [17]. MycoBank currently lists 38 taxa in *Exserohilum*, most of which are associated with diseases of grasses [18,19]. *Exserohilum* species attacking economically relevant crops include *E. pedicellatum* (causing root rot of maize and brown lesions on wheat roots), *E. prolatum* (producing leaf spots on maize), and *E. rostratum* (associated with leaf spot and foot rot of wheat, damping off of sugarcane seedlings, leaf spot of banana, and blackening and seed germination failure in many cereals) [20].

In Egypt as well as in other countries, no previous investigations were found in the available literature concerning bean blight therefore the present study was planned to shed lights on this disease and the causative pathogen. Experiments were run for isolation and identification of the causative fungus. The work was also expanded to study the pathogenic potentialities of the isolated fungus and its host-range.

## MATERIALS AND METHODS

### Disease survey in commercial bean fields

A total of 20 fields cultivated with bean were surveyed for bean blight during the growing seasons from October to December 2019 and February to April 2020. The incidence of plants showing typical disease symptoms was assessed in 10 randomly selected groups of 10 consecutive plants amounting to 100 plants/field. Incidence values were used to calculate a disease index. Percentage of disease incidence was recorded as the number of diseased plants relative to the number of growing plants for each treatment, and then the average of disease incidence was calculated.

### Sample collection and fungal isolation

Samples of infected bean plants were collected from thirty sampling locations during the planting season throughout Beni Sweif Governorate, Egypt. Fungi from leaf samples were isolated on potato dextrose agar medium (PDA) supplemented with an antibacterial antibiotic as described by Pitt and Hocking [21].

Spore suspension from each fungal isolate was streaked onto autoclaved 4% water agar (WA), and was then incubated for 24 h. at 28°C. A growing hyphal tip from a single spore was cut and transferred onto new PDA. Pure cultures of the fungus isolates were sub cultured and maintained as a conidial suspension in sterile 25% glycerol of water at -10°C.

### Phenotypic characterization of fungal isolates

The obtained isolates were identified macroscopically and microscopically on PDA. Macroscopic characteristics included colony features, color, and growth rate while microscopic characteristics included conidial shape and size using a microscope (Carl Zeiss, Germany). Morphological characterization was completed as described by Domsch et al. [22].

### Molecular identification of fungal isolates

Fungal isolates were grown in sterile Petri plates containing autoclaved potato dextrose agar (PDA) medium and incubated for 7 days at 28°C [21]. Cultures were sent to the Molecular Biology Research Unit, Assiut University for DNA extraction using Patho-gene-spin DNA/RNA extraction kit provided by Intron Biotechnology Company, Korea. The fungal DNA was then sent to SolGent Company, Daejeon South Korea for polymerase chain reaction(PCR) and rRNA gene sequencing. PCR was performed using ITS1 (forward) and ITS4(reverse) primers which were incorporated in the reaction mixture. Primers have the following composition: ITS1 (5' - TCCGTAGGTGAACCTGCG G -3'), and ITS4 (5'- TCCTCCGCT TAT TGA TAT GC -3'). The purified PCR products (amplicons) were sequenced with the same primers [23]. The obtained sequences were analyzed using Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information(NCBI) website. Phylogenetic analysis of sequences was done with the help of MegAlign(DNA Star) software version 5.05.

### Pathogenicity test

This part of study was conducted at Sids Agricultural Research Station. Seeds of common bean (var. Giza 6) were obtained from horticultural research institute, Giza Governorate. Under greenhouse conditions, seeds were sown in pots (20cm diameter) filled with a sterilized mixture of equal portions (v/v) of soil, sand and clay (3 seeds/pot). Five isolates of *E. rostratum* were used for pathogenicity tests on 2-wk-old bean seedlings. Ten days old cultures were used for inoculation. Inoculum was prepared by washing the conidia from cultures in Petri plates using sterile water with a surfactant, 0.05% Tween 20. Conidial suspensions were prepared and adjusted to  $1 \times 10^6$  spores/mL containing 0.05% Tween 20, and a sterile cell scraper. Fungal suspensions were sprayed onto the leaves of each bean plant. The inoculated plants were then covered with black plastic bags for 48 h to maintain high humidity. Three replicates of plants were sprayed with each tested isolate; un-inoculated plants served as a negative control. Nine days post-inoculation the same symptoms appeared. Pathogenicity was evaluated with disease incidence after inoculation and disease reaction was rated as described

before. Re-isolation of the pathogen from inoculated leaves was conducted as previously described. Morphological identification and comparison between the inocula and the re-isolated cultures was confirmed by microscopic examination.

### Effect of temperature on conidial germination

Drops of *E. rostratum* spore suspension were placed on glass slides. The slides were then placed in moist chambers prepared by placing two moist filter papers in the inner surface of a Petriplate. Three replications were made for each treatment. The slides were incubated at 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C and 55°C for 24-48 hrs and the percentage of germination was calculated under a light microscope.

### Host range investigation

The pathogenicity of *E. rostratum* strain (AUMC 14371) was tested under greenhouse conditions across 13 plant species covering 4 families, i.e., Solanaceae (tomato, potato, eggplant, and pepper), Fabaceae (bean, cowpea and soybean) Cucurbitaceae (cucumber, watermelon, melon, squash, and cantaloupe) and Brassicaceae (cabbage). Seeds of all the test plants (except potato) were sown in 1L pots containing sterilized potting soil mix and distributed on 3 replicates. Potatoes used for host range studies were grown from tuber pieces each with a single shoot. All plants were inoculated at four-leaf stage for larger leaved plants (Cucurbitaceae and Brassicaceae plants) and at eight to ten-leaf stage for small leafy plants (Solanaceae and Fabaceae plants) [24]. Inoculums for all host range experiments were sprayed over the tested plants as described under pathogenicity test. Data were statistically analyzed for computing L.S.D. test at 5% probability according to the procedure outlined by Snedecor and, Cochran [25].

## RESULTS

### Blight symptoms on bean leaves

Primary symptoms of bean blight appeared on the cotyledonary leaves as green dark to purplish-brown spots. In general, the disease symptoms appear on the above ground parts of bean plant as small irregular lesions, dark green(gray) to dark brown with brown marquis. Later, the lesions enlarge and merge together to infect large areas (Figure 1). Severe infections result in premature defoliation and often caused plant collapse.

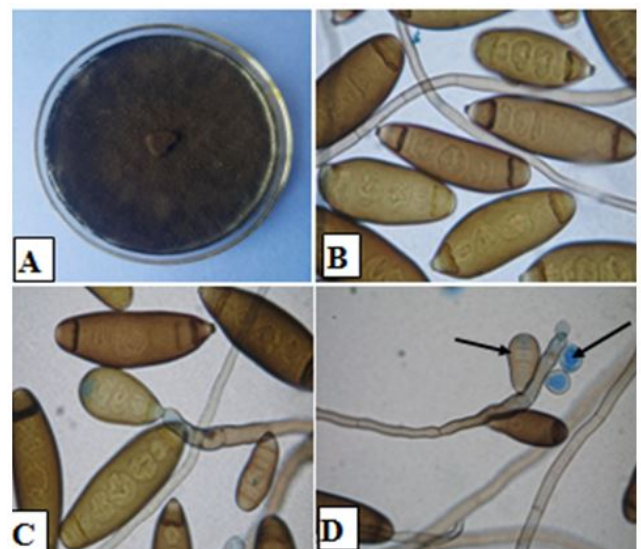
### Morphological characteristics of fungal isolates

The isolated fungi showed good growth on PDA medium. Colonies were circular, deep, the color of the colony was smoke grey fading to olivaceous (Figure 2). Conidia are dark brown, cylindrical each with a markedly protruding truncate hilum and 7 to 12 pseudosepta. Conidial dimensions range from 15.0- 80.0 µm in length and are 8.0-16.0 µm wide. A dark septum is usually observed at the polar cells of each conidium. Conidiophores erect or slightly curved, septate, geniculate, olivaceous-brown with swollen conidiogenous cells containing circular conidial scars. In the strains AUMC 14371, AUMC 14372, AUMC

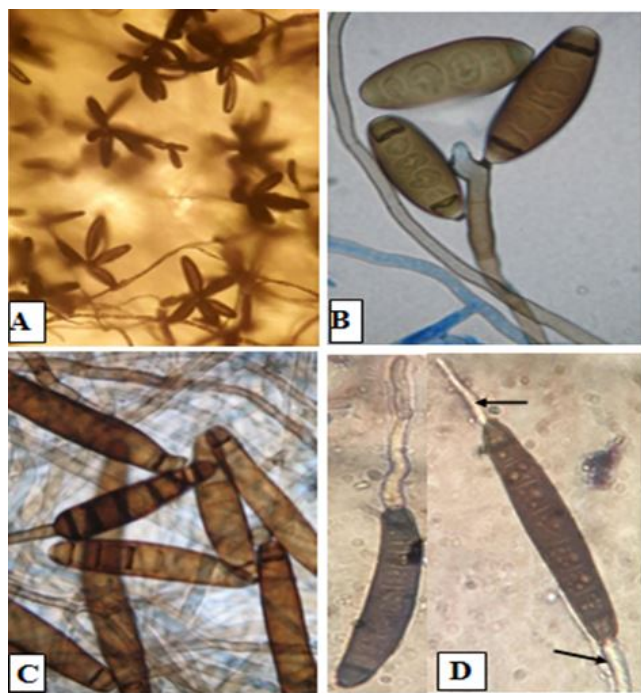
14373, and AUMC 14374 compared with strain AUMC 14375 was tallest and dark brown which isolated from Beba County. Conidia germinate predominantly from the two opposite poles (Figure 3) and occasionally from intermediate cells. These morphological characteristics are consistent with those of *Exserohilum rostratum* (Drechsler) Leonard & Suggs.



**Figure 1:** Blight symptoms on bean leaves infected with *E. rostratum* isolates. Lesions varied from dark green (gray) to brown, with brown margins on the leaves.



**Figure 2:** *Exserohilum rostratum*: Colony on PDA at 35°C after 5 days (A); Mature conidia of strain AUMC 14371 (B and C 400x), Pre mature spores emerge from the tip of the conidiogenous cell (arrowed D 200x).



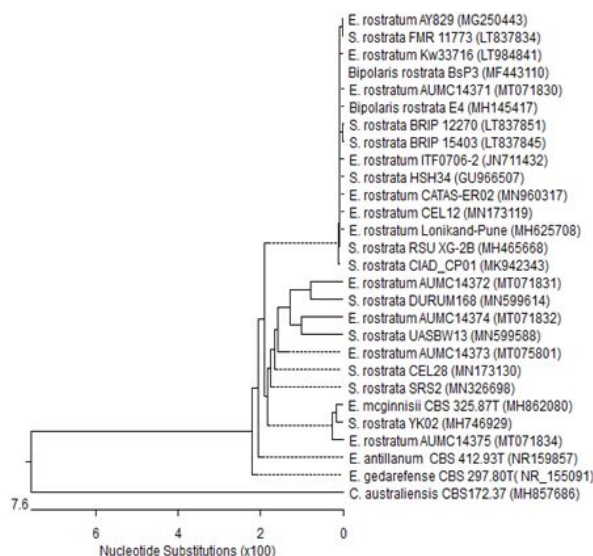
**Figure 3:** *Exserohilum rostratum*: Conidia on the conidiophores of strain AUMC 14374(A 100x), Conidia of strain AUMC 14371(B 400x), Spores on conidiophores of strain AUMC 14375(C), and Bipolar conidial germination(arrowed D 400x).

### Disease survey in commercial fields

An accurate extensive survey of blight was carried out on bean plantations during 2019 and 2020 at Beni Sweif Governorate. The inspected counties were El- Wasta, Nasser, Beni Sweif, Sumosta and Beba. In general, the percentages of disease incidence on bean plants during 2020 season were much higher than those recorded during 2019 season while the reverse was recorded for Beba county was record the opposite. Data obtained during the season of 2019 revealed that the highest infection on bean was recorded at Beba, being 35%. However, during the season of 2020 the highest disease incidence on bean plants was recorded at El-Wasta County, being 35%, in Table 1.

**Table 1:** Evaluation the disease incidence of bean seedling blight (*E. rostratum*) in different counties of Beni Sweif governorate during the two growing seasons 2019 and 2020.

County	Disease incidence (%)	
	1st season	2nd season
El-Wasta	30	35
Nasser	25	27
Beni Sweif	22	24
Beba	35	32
Sumosta	15	20
L.S.D. at 0.05	4.8	5.4



**Figure 4:** Phylogenetic tree based on ITS sequences of rDNA of the fungal strains isolated in the present study (AUMC No. 14371, 14372, 14373, 14374 and 14375) aligned with closely related sequences accessed from the GenBank. These isolates showed 99.65% - 100% similarity with several strains of *Bipolaris rostratum* *Exserohilum rostratum* and *Setosphaeria rostrata*. *Curvularia australiensis* is included as an outgroup strain. (E. =*Exserohilum*, S. = *Setosphaeria*, GenBank accession Numbers are shown between parentheses).

Five isolates representing the five counties at Beni Sweif governorate were selected for molecular studies. Molecular identification of the five isolates, i.e., AUMC 14371, AUMC 14372, AUMC 14373, AUMC 14374 and AUMC 14375 was confirmed using the internal transcribed spacer (ITS) region of ribosomal DNA, amplified with universal primers ITS1/ITS4, and sequenced directly. An NCBI-BLAST search showed the greatest identity (100% similarity) with the ITS sequences of *Exserohilum rostratum*. The sequences were deposited in NCBI GenBank with accession numbers MT071830, MT071831, MT075801, MT071832 and MT071834 respectively. Based on microscopic, morphological, and molecular characteristics, the fungal isolates were identified as *Exserohilum rostratum*. Representatives of closely related strains of *E. rostratum* are included in the phylogenetic tree Figure 4.

### *Exserohilum rostratum* AUMC 14371 (MT071830) (Length: 598)

TAGGTGAACCTGCGGAGGGATCATTACACAACAAAAT  
 ATGAGGGTGTGGTTTGCTGGCAACAGCGTCCGCCCC  
 AAGTATTTTTTACCCCATGTCTTTTGC GCACTTTTTGTTT  
 CCTGGGCGAGTTCGCTCGCCACCAGGACCCAACCATA  
 AACCTTTTTTATGCAGTTGCAATCAGCGTCAGTATAAT  
 AATTCAATTTATTA AAAACTTTCAACAACGGATCTCTGG  
 TTCTGGCATCGATGAAGAACGCAGCGAAATGCGGATAC  
 GTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCT  
 TTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGC  
 ATGCCTGTTTCGAGCGTCATTTGTACCCTCAAGCTTTCG

TTGGTGTGGGGCGTCTTTTTGTCTCTCCCCTTGTTGG  
GGGAGACTCGCCTTAAAACGATTGGCAGCCGACCTAC  
TGGTTTTCGGAGCGCAGCACAAATTTGCGCCTTCCAA  
TCCACGGGGCGGCATCCAGCAAGCCTTTGTTTTCTAT  
AACAAATCCACATTTTGACCTCGGATCAGGTAGGGATA  
CCCCTGAACTTAAGCATATCAATAAGCGGAGGA

***Exserohilum rostratum* AUMC 14372 (MT071831)**  
(Length: 577)

TGCGGAGGGATCATTACACAACAAAAATATGAGGGTGT  
GGTTTGCTGGCAACAGCGTCCGCCCAAGTATTTTTTC  
ACCCATGTCTTTTTCGCACTTTTTGTTTCCTGGGCGAG  
TTCGCTCGCCACCAGGACCCAACCATAAACCTTTTTTTT  
ATGCAGTTGCAATCAGCGTCAGTATAATAATTCAATTTA  
TTAAAACCTTTCAACAACGGATCTCTTGTTCTGGCATC  
GATGAAGAACGCAGCGAAATGCGATACGTAGTGTGAA  
TTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAC  
ATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTT  
GAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTG  
GGCGTCTTTTTGTCTCTCCCCTTGTTGGGGGAGACTC  
GCCTTAAAACGATTGGCAGCCGACCTACTGGTTTTTCG  
GAGCGCAGCACAAATTTGCGCCTTCCAATCCACGGGG  
CGGCATCCAGCAAGCCTTTGTTTTCTATAACAAATCCA  
CATTTTGACCTCGGATCAGGTAGGGATACCCGCTGAA  
CTTAAGCATATCAA

***Exserohilum rostratum* AUMC 14373 (MT075801)**  
(Length: 577)

TGCGGAGGGATCATTACACAACAAAAATATGAGGGTGT  
GGTTTGCTGGCAACAGCGTCCGCCCAAGTATTTTTTC  
ACCCATGTCTTTTTCGCACTTTTTGTTTCCTGGGCGAG  
TTCGCTCGCCACCAGGACCCAACCATAAACCTTTTTTTT  
ATGCAGTTGCAATCAGCGTCAGTATAATAATTCAATTTA  
TTAAAACCTTTCAACAACGGATCTCTTGTTCTGGCATC  
GATGAAGAACGCAGCGAAATGCGATACGTAGTGTGAA  
TTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAC  
ATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTT  
GAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTG  
GGCGTCTTTTTGTCTCTCCCCTTGTTGGGGGAGACTC  
GCCTTAAAACGATTGGCAGCCGACCTACTGGTTTTTCG  
GAGCGCAGCACAAATTTGCGCCTTCCAATCCACGGGG  
CGGCATCCAGCAAGCCTTTGTTTTCTATAACAAATCCA  
CATTTTGACCTCGGATCAGGTAGGGATACCCGCTGAA  
CTTAAGCATATCAA

***Exserohilum rostratum* AUMC 14374 (MT071832)**  
(Length: 588)

TGCGGAGGGATCATTACACAACAAAAATATGAGGGTGT  
GGTTTGCTGGCAACAGCGTCCGCCCAAGTATTTTTTC  
ACCCATGTCTTTTTCGCACTTTTTGTTTCCTGGGCGAG  
TTCGCTCGCCACCAGGACCCAACCATAAACCTTTTTTTT  
ATGCAGTTGCAATCAGCGTCAGTATAATAATTCAATTTA  
TTAAAACCTTTCAACAACGGATCTCTTGTTCTGGCATC  
GATGAAGAACGCAGCGAAATGCGATACGTAGTGTGAA  
TTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAC  
ATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTT  
GAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTG

GGCGTCTTTTTGTCTCTCCCCTTGTTGGGGGAGACTC  
GCCTTAAAACGATTGGCAGCCGACCTACTGGTTTTTCG  
GAGCGCAGCACAAATTTGCGCCTTCCAATCCACGGGG  
CGGCATCCAGCAAGCCTTTGTTTTCTATAACAAATCCA  
CATTTTGACCTCGGATCAGGTAGGGATACCCGCTGAA  
CTTAAGCATATCAATAAGCGGAGGA

***Exserohilum rostratum* AUMC14375 (MT071834)**  
(Length: 574bp)

GCGGAGGGATCATTACACAACAAAAATATGAGGGTGT  
GGTTTGCTGGCAACAGCGTCCGCCCAAGTATTTTTTC  
ACCCATGTCTTTTTCGCACTTTTTGTTTCCTGGGCGAG  
TTCGCTCGCCACCAGGACCCAACCATAAACCTTTTTTTT  
ATGCAGTTGCAATCAGCGTCAGTATAATAATTCAATTTA  
TTAAAACCTTTCAACAACGGATCTCTTGTTCTGGCATC  
GATGAAGAACGCAGCGAAATGCGATACGTAGTGTGAA  
TTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAC  
ATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTT  
GAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTG  
GGCGTCTTTTTGTCTCTCCCCTTGTTGGGGGAGACTC  
GCCTTAAAACGATTGGCAGCCGACCTACTGGTTTTTCG  
GAGCGCAGCACAAATTTGCGCCTTCCAATCCACGGGG  
CGGCATCCAGCAAGCCTTTGTTTTCTATAACAAATCCA  
CATTTTGACCTCGGATCAGGTAGGGATACCCGCTGAA  
CTTAAGCATATC

**Pathogenicity**

Cultivar of bean (Giza 6) was used to compare five isolates of *E. rostratum* (AUMC 14371, AUMC 14372, AUMC 14373, AUMC 14374 and AUMC 14371). These isolates collected from El-Wasta, Nasser, Beni Sweif, Sumosta and Beba respectively in Beni Sweif governorate. Data observed in Table 2 revealed that, isolate (AUMC 14371) recorded lowest effect of disease incidence 26.3% however isolate (AUMC 14375) obtained from Beba was scored the highest of bean blight disease incidence 36.7%. These symptoms were similar to the characteristic lesions of bean blight that were observed in the field. The re-isolated fungal cultures from the artificially infected leaf lesions showed the same morphological characteristics as the inocula, fulfilling Koch’s postulates.

**Table 2:** Pathogenicity test of *E. rostratum* isolates on bean plants (var. Giza 6).

N	Isolates	Disease incidence %
1	AUMC 14371	26.3
2	AUMC 14372	34.0
3	AUMC 14373	29.0
4	AUMC 14374	32.0
5	AUMC 14375	36.7
L.S.D 0.05		3.8

### Host range

Data presented in Table 3 demonstrate six plant hosts namely Tomato, Pepper, Potato, Watermelon, squash in addition to Bean, showed distinct disease responses upon inoculation with *E. rostratum*. Irregular gray patches are formed on leaves and pods, which finally cover the whole leaf surface and young stems. In comparison with egg plant, soybean, cowpea, muskmelon, and cucumber which showed no visible infection. Under favorable environmental conditions for disease development symptoms. Finally, the lesions become necrotic and the whole leaves die.

**Table 3:** Susceptibility of various species infection by *Exserohilum rostratum*.

N	Family	Plant species, Common name	Disease reaction
1	Solanaceae	<i>Solanum melongena</i> L in Egg plant	-
		<i>Lycopersicon esculentum</i> Mill. Tomato	+
		<i>Capsicum annuum</i> Linn. Pepper	+
		<i>Solanum tuberosum</i> Linn. Potato	+
2	Fabaceae	<i>Phaseolus vulgaris</i> Common bean	+
		<i>Glycine max</i> Soybean	-
		<i>Vigna unguiculata</i> sub sp. <i>unguiculata</i> Cowpea	-
		<i>Cucumis melo</i> Naud Muskmelon	-
3	Cucurbitaceae	<i>Cucurbita pepo</i> L. Squash	+
		<i>Citrullus lanatus</i> Watermelon	+
		<i>Cucumis sativus</i> L. Cucumber	-
		<i>Brassica oleracea</i> var. <i>Capitata</i> Cabbage	-

### Effect of temperature

Effect of temperature levels on spore germination from 5-55°C (Table 4) as were used to study the effect of temperature on conidial germination in the presence of high relative humidity. Data revealed that conidia are able to germinate in a wide temperature range. The average of spore germination was the highest after 48 hours incubation (61.9%) at 35°C. meanwhile the germination was not recorded at 55°C even after 48hours incubation. On the other hand, the lowest spore germination as 2.7% on the average at 5°C.

**Table 4:** Effect of temperature on spore germination of *E. rostratum*.

N	Temperature (A)	Spore germination %		
		Incubation period (B)		Mean(A)
		24h	48h	
1				
2	5°C	1.7	6.3	2.7
3	10°C	4.7	12.0	5.6
4	15°C	18.0	39.0	19.0
5	20°C	31.0	56.0	29.0
6	25°C	32.7	67.7	33.4
7	30°C	45.0	91.0	45.4
8	35°C	86.7	99.0	61.9
9	40°C	61.7	88.0	49.9
10	45°C	21.7	39.3	20.3
11	50°C	3.0	9.0	4.0
12	55°C	0.0	0.0	0.0
Mean (B)		27.8	46.1	~
LSD at 5%		A=2.9 B=1.5 AB=5.0		

### DISCUSSION

In this study, the fungus isolated from infected bean leaves with seedling blight symptoms was identified as *Exserohilum rostratum* based on morphology and the phylogenetic properties based on ITS1 andIT4 that placed all isolates in the same clade with high support and apart from the other *Exserohilum* or *Setosphaeria* species. There was no significant variation in ITS and IT4 sequences among the *Exserohilum rostratum* isolates collected from different localities throughout Beni Sweif governorate,

Egypt and were added to the GeneBank with new accession number..

Pathogenicity tests showed that *Exserohilum rostratum* is able to make a significant impact on bean productivity at this time, especially, the pathogen can infect and cause the disease in a wide range of temperature and altering agronomic practices, climate change or the appearance of virulent genotypes [26,27].

*Exserohilum holmii*, originally described as *Helminthosporium*, was the cause of leaf blight of *Dactyloctenium aegyptium* in Georgia, USA. Luttrell [13] obtained the sexual morph in culture by mating compatible conidial strains on Sach's agar supporting sterilized barley grains.

*Exserohilum rostratum* is by far the most commonly recorded and known species of the genus. This cosmopolitan species has been recorded from numerous hosts, especially Poaceae and other monocots, causing leaf spot and foot rot of wheat and other grasses, blight, damping-off, rots including leaf spot of banana [19,28]. All these symptoms and several hosts correspond to our results in the host range test with infection in plant species belong to Solanaceae, Fabaceae, and Cucurbitaceae. At any rate, knowledge about bean blight and the causal pathogen of the disease in Egypt is still scanty and uncertain and needs more further studies.

*E. rostratum* is a fungus with a broad ecological niche and shows considerable diversity in nature. The fungus causes disease symptoms on more than 30 plant species covering 28 genera in 11 families, including many economically important crops such as corn, rice, sugarcane, sweet sorghum, tomato and wheat [29-33]. In some cases, *E. rostratum* is known to cause severe yield losses, for example, corn seed infection has been recorded at the rate of 85% and corn leaf incidence up to 18% [30,34]. Eight plant species have been reported as natural hosts of *E. rostratum* in China [35,36], while approximately another 20 plant species were confirmed to be hosts of *E. rostratum* through artificial inoculations [37]. *E. rostratum* was found to be the causal agent of leaf blight of the ornamental plant *Caryota mitis Loureiro* (Arecaceae) in Argentina [38], leaf spot of tiger grass *Thysanolaena maxima* (Roxb.) Kuntze in South Florida [39], boll and lint rot disease of cotton in Nehbandan region, South Khorasan, Iran [40], banana leaf spot in China [20] and leaf spot diseases of pineapple in China [41]. In south western Iran, a new sugarcane leaf spot disease caused by *E. rostratum* was reported by Ahmedpour et al. [42]. *E. rostratum* was recorded to be associated with leaf spot of rice in Malaysia [43,44] and a pathogen of rice brown spot in Pakistan [45]. *E. rostratum* has been reported as causal agent of leaf spots on *Zea mays*, *Ananas comosus*, *Musa paradisiaca* and *Hevea brasiliensis* in China [46].

In reports from India, Choudhary et al., showed that *E. rostratum* was responsible for leaf spot disease of *Lagenaria siceraria* (a medicinal and nutritious domesticated cucurbit vegetable named as Bottle gourd) [47]. More recently *E. rostratum* was recorded as a cause of Petal Spot of *Phalaenopsis* Orchid in Taiwan [48].

In Egypt, *E. rostratum* was reported a rare fungus from the phyllosphere and phylloplane fungi of broad bean leaves [49] and was less frequently isolated from the air over banana

plantations [50]. Moreover, *E. rostratum* was recorded among the endophytic fungi of the Red Sea aquatic plant *Avicennia marina* [51] and was considered a causal pathogen of lettuce root rot in combination with *Fusarium oxysporum* [52].

## CONCLUSION

As the authors are far aware, there is no available information on bean blight disease and also *Exserohilum rostratum*, the causative of the disease, was isolated herein from diseased common bean plants for the first time in Egypt.

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