

First Record of *Colletotrichum* Stem Rot on Mungbean (*Vigna radiata* L.) in Egypt

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During 2009/2010, reddish brown lesions were observed on stems of some mungbean plants grown at the experimental research stations at Nubariya (El-Behera Governorate) and (Beni-Suef Governorate). The pathogen was identified as *Colletotrichum dematium*. Two isolates varied in morphological and cultural characteristic. Also, they varied in pathogenicity under greenhouse conditions and mycelial growth under laboratory conditions. Culture filtrate of isolates varied in their effect on percentage of seed germination and root length. Isolates varied in their ability to utilize carbon and nitrogen of sources. They also varied in mycelium dry weight and spores production. It was found that mycelial growth and spore production of two isolates was significantly affected by period of light and dark as well as temperatures.

Keywords: *Colletotrichum dematium*, mungbean, mycelia growth and stem rot.

Mungbean (*Vigna radiata* L.) is widely grown in South East Asia, Africa, South America and Australia. Mungbean for used as human food (as dry beans or fresh sprouts) and can be used as green manure crop and as forage for livestock. Mungbean seeds are sprouted for fresh use or canned for shipment to restaurants. Sprouts contain (21-28%) protein, calcium, phosphors and certain vitamins (Abu-Salium *et al.*, 1997). Rathaiah and Sharma (2004) reported cultivars viz., MLTG-2 and TARM-18 were highly resistant to *Colletotrichum truncatum*. Occurrence of *C. dematium* was observed on the sowing material of various plants species (Washington *et al.*, 2006 and Machowicz, 2010) so this species could be transported with seeds. Han and Lee (1995) reported that *C. truncatum*, *C. destructivum* and *C. gloeosporioides* are indicated as the causal agents of *Colletotrichum* diseases of mungbean. Thirty green gram genotypes were screened against *C. truncatum* under artificial conditions in greenhouse during 2006 where two genotypes were resistant three moderately resistant, while rest of genotypes were susceptible to highly susceptible (Sunil *et al.*, 2009). Mungbean (*Vigna radiata* L.) is one of the most important legumes of the arid and semiarid tropics (Chen *et al.*, 1987). Many fungal pathogens, some of which are seed transmitted, often reduce germination or kill the infected plants or substantially reduce the productive by Shamsur *et al.* (1999).

This research aimed to declare the definition of the extent of variability in morphology and ability to utilize different carbon and nitrogen sources, evaluation some mungbean entries and cultivars under greenhouse and field conditions and to study their relative importance and pathogenic potential. This work is the first record in Egypt on *Colletotrichum* of mungbean.

Materials and Methods

Isolation, Purification and Identification of pathogen:

For isolation of the pathogen from mungbean plants (cv. Giza-1) diseased samples were collected from El-Behera and Beni-Suef Research Stations. Diseased tissues were cut into 0.5 cm pieces, placed in 0.5% sodium hypochlorite solution for 3 min., rinsed in sterile distilled water, blotted dry and placed on Difco potato dextrose agar (PDA), containing streptomycin sulphate (50 mg/l.) and incubated at 25°C. Fungal colonies from the tissue pieces were then purified using single spore isolation method. The isolated fungi were examined with a light microscope and identified in Mycology Research & Disease Survey at the Agricultural Research Centre. The fungus was identified as *Colletotrichum dematium* according to Domsch *et al.* (1980).

Inoculum preparation:

Conidial spores inoculums of the isolates for pathogenicity experiments was produced on sorghum leaf medium (SLM) where conidial production was more abundant than on PDA. SLM medium was made as follow: 15g of green leaves of the sorghum at the 8-10 leaf growth stage were washed in water and cut into 2x3 cm pieces (Pande *et al.*, 1991). The pieces were then moistened, used to fill 250 ml flasks, and autoclaved at 121°C for 25 min. Each cooled flask was seeded with two mycelial plugs (0.5 cm²) cut from the margins of single colonies. Ten millilitres of 2% sucrose solution prepared in sterile distilled water was added to each flask at the same time of inoculation as an additional carbon source. Inoculated flasks were incubated for 10 days for conidial production. Conidial suspension was filtrated through four layers of cheesecloth to remove mycelial and leaf fragments. The filtered suspension was adjusted to 10⁶ conidial per ml using a haemocytometer. The following formula was used to adjust the inoculum to the desired concentrations after Beshir (1991):

$$V_o \times C_o = V_g \times C_g$$

Whereas: V_o : initial volume.

C_o : initial concentration obtained by counting on haemocytometer.

V_g : final volume for inoculation.

C_g : final concentration.

$$V_g = V_o \times C_o / C_g$$

Two drops of Tween 20 was added to 100 ml of conidial suspension as a wetting agent before inoculation.

Pathogenicity tests and varieties reaction:

Five mungbean varieties, *i.e.* Giza-1, VC-1000, King, VC-2719 and Kawmey-1. These varieties were originated from Legume Dept., Field Crops Res. Inst., Agric. Res. Centre, Giza. Sterilized plastic pots 30-cm-diam., were used. The pots were potted with autoclaved light loam soil. Eight seeds were sown in each pot under greenhouse condition four pots were used for each variety. Four weeks after seeding, plants at the 10-12 node stage were inoculated with conidial suspension (10⁶ conidial/ml) of two isolates separately with an atomizer until there was run-off point (150 ml/pot). The control plants were sprayed with sterile distilled

water. All plants were covered with polyethylene bags for 24 hr in a tent of polyethylene at 100% relative humidity necessary for fungal infection. Symptoms on stems were rated two weeks after inoculation. Disease severity was measured using the equation proposed by Al-Kherb *et al.* (2002). Whereas, 0= no symptom, 1= 1-20 %, 2= 21-40%, 3= 41- 60%, 4= 61-85% of stem area affected. Disease severity (%) was calculated using the equation proposed by (Al-Kherb *et al.*, 2002) as follows:

$$DS (\%) = \frac{(n \times r)}{4 N} \times 100$$

Whereas, n = Total of the symptomatic plant and their corresponding scale.

r = Disease severity rating.

N= Total number of sampled plant.

4 = Highest score scale.

Detection of seed borne fungi in mungbean seeds:

The coloured or irregular and atrophied seeds were chosen. Seeds were plated on Czabek's agar media (Al-Abdalall, 2010). Ten seeds were placed in each Petri dish (12 cm) with eight replicates and incubated at 28°C for five days. The species and number of fungi were recorded using the hyphal tip technique suggested by Dhingra and Sinclair (1985). The isolated fungi were identified according to their morphological characters under stereo-binocular microscope and the pathogens confirmed after preparing slides identified in Mycol. Res. & Dis. Survey Dept.

Effect of culture filtrate of C. dematium on seed germination and root length of the tested cultivars:

The two isolates were grown on Czapek's medium containing NaNO₃ (3.0g), K₂NPO₄ (1.0 g), MgSO₄ (0.5 g), KCl (0.5 g), FeSO₄·7H₂O (0.01 g) and Sucrose (30 g)/litre distilled water. Each 100 ml of the liquid medium was transferred into 250ml Conical flask. Six flasks were used as replicates for each isolate. Conical flasks were individually inoculated with one disc (5-mm-diam.) from an activity grown (7-day-old) culture and incubated at 28±1°C for 2 weeks in the dark. Culture filtrates were sterilized by Seitz filter. Tested seeds were sterilized by 2% sodium hypochlorite for 3 min., and then dried. Fifteen seeds were plated per Petri dish (12-cm-diam.). Ten ml of each culture filtrate was transferred into each dish. Ten ml of uninoculated Czapek's medium was added to each control dish. Four dishes were used as replicates for each treatment. Germination percentage of each tested seed cultivar and root length were calculated after incubation at 27±1°C for 7 days.

Effect of different carbon and nitrogen sources on mycelial growth and sporulation:

Effect of different sources of carbon as well as nitrogen on mycelial growth and spore production was studied. Richard's medium was used as the basal medium. The carbon and nitrogen nutrition was studied by replacing the sucrose and potassium nitrate in the basal medium with various carbon and nitrogen compounds. Twenty five millilitres of each medium were transferred into 100 ml flask, plugged with non-absorbent cotton and autoclaved at 121°C for 15 minutes. The cultures were filtered through filter paper and the mycelial was dried in a hot air oven at 60°C for 24 h. Mycelial weight was measured by subtracting the initial weight of the filter paper from the weight of the filter paper along with the mycelia mat.

a- Effect of different carbon sources:

Four different carbon sources were used as well as fructose, lactose, maltose and manitol which were added to the basal medium at 21.053 g/l. Richard's medium without adding sucrose was used as a control. Each of the treatments was replicated four times. All flasks, containing different carbon sources, were individually inoculated with 5mm mycelial disk of one week old fungal cultures and incubated at $27\pm 1^{\circ}\text{C}$ for 10 days. The fungal mycelial mat was filtered through filter paper and the dry weight was recorded after drying it in hot air oven at 60°C for 24 h. The effect of carbon sources on sporulation was determined. Data were recorded as (-) for no sporulation, (+) for poor sporulation, (++) for moderate sporulation and (+++) for heavy sporulation. Recorded data were analyzed (Manjunatha and Rawal, 2002).

b- Effect of different nitrogen sources:

Four different nitrogen sources, *i.e.* sodium nitrate, potassium nitrate, asparagines and ammonium nitrate were added to Richard's liquid medium at 1.3855g/litre. Richard's broth free of potassium nitrate was used as control. Each treatment was replicated four times. All flasks containing different nitrogen sources were individually inoculated with 5 mm mycelial disk of one week old fungal cultures and incubated at $27\pm 1^{\circ}\text{C}$ for 10 days. Fungal mycelial mat was filtered and data recorded as mentioned by Sangeetha and Rawal (2008).

Effect of light and temperature on mycelial growth and sporulation:

a- Light effect:

Six Conical flasks (250 ml), each contained 100 ml of Richard's liquid medium, were used for each tested isolate. These flasks were inoculated and exposed to different lengths of light and/or darkness, *i.e.* cycles of twelve hours of light and twelve hours of darkness as well as continuous light and continuous darkness in an environmental chamber. Conical flasks were inoculated with 5mm discs taken from the periphery of one week old pure culture. Each treatment was replicated four times and incubated at $27\pm 1^{\circ}\text{C}$ for 10 days. Dry weight of mycelial and sporulation was obtained as described by Vinod and Benagi (2009).

b- Effect of different temperatures:

Richard's liquid medium was used in this experiment. Six temperature degrees, *i.e.* 10, 15, 20, 25, 30 and 35°C , were tested for their effect on the growth and sporulation of *C. dematium*. Mycelial discs (5mm) taken from 10 days growing cultures. Treatments were replicated 4 times. The fungus was inoculated under aseptic conditions and incubated at $27\pm 1^{\circ}\text{C}$ for 10 days. Dry mycelial weight and sporulation was obtained as described by Vinod and Benagi (2009).

Statistical analysis:

Data were subjected to ANOVA test, using MSTATC program. Means for the two factors as well as the interaction were separated by LSD test when ANOVA revealed $P < 0.05$.

Results and Discussion

Pathogenicity test and varieties reaction:

Two isolates were tested against five mungbean cultivars for diseases severity, yield per plant, weight 100 seeds and plant height. Data in Table (1) indicate that cv. Giza-1 recorded the shortest length with the two isolates, while Kawmey-1 recorded the highest length followed by cv. King. Also, cvs. Vc-1000 and Vc-2719 recorded lesser yield with the two isolates than the other tested cultivars. Rathaiiah and Sharma (2004) stated that cvs. MI-TG-2 and TARM-18 were found to be highly resistant to *C. truncatum*.

Table 1. Pathogenicity test and varieties reaction

Isolate	Cultivar	D.I (%)	D.S	Plant height (cm)		M	Yield/plant (g)		M	Weight of 100 seed (g)		M
				A	B		A	B		A	B	
1	Giza-1	70.44	2.60	67.65	56.40	62.03	18.39	15.01	16.70	6.92	6.89	6.91
	Vc-1000	79.16	2.75	64.06	60.48	62.22	10.68	7.23	8.96	4.53	4.36	4.45
	King	49.61	1.95	79.92	66.36	73.14	20.84	16.53	18.69	7.33	7.16	7.25
	Vc-2719	65.03	2.23	74.64	61.01	67.83	12.45	8.36	10.41	5.11	4.92	5.05
	Kawmey-1	58.78	2.00	86.92	78.70	82.81	21.10	17.89	19.50	4.41	4.32	4.37
	Mean			74.64	64.59		16.69	13.00		5.66	5.53	
2	Giza-1	75.53	3.25	67.65	52.12	59.89	18.39	14.30	16.35	6.92	6.81	6.87
	Vc-1000	82.00	3.30	64.06	65.10	64.58	10.68	8.18	9.43	4.53	4.30	4.32
	King	54.43	2.15	79.92	65.50	72.71	20.84	17.40	19.12	7.33	7.02	7.18
	Vc-2719	68.66	2.43	74.64	57.32	65.98	12.45	7.58	10.02	5.11	4.83	4.97
	Kawmey-1	63.30	2.30	86.92	75.30	86.92	21.10	16.43	18.77	4.41	4.30	4.36
	Mean			74.64	60.01		16.69	12.78		5.66	5.45	

Detection of seed-born fungi in mungbean seeds:

Various pathogenic fungi isolated from different varieties of mungbean seeds are presented in Table (2). *Rhizoctonia solani* recorded the highest frequency (21.95%) followed by *Alternaria* spp. and *Colletotrichum dematium* (19.51 and 17.07%, respectively). Meanwhile, *Verticillium albo-atrum* recorded the less frequency (2.44%). Seed born fungi percentage was maximum in Giza-1 followed by Vc-1000 (being 29.27 and 24.39, respectively), while Kawmey-1 was the least (12.20%). Edema (1995) mentioned that the most frequently isolated fungi from cowpea in Uganda were *Colletotrichum* spp., *Ascochyta phaseolorum*, *Cladosporium vignae*, *Fusarium solani*, *F. oxysporum* and *Tracheiphilum lindemuthianum*. *Colletotrichum dematium* was observed on the sowing material of various plant species and be transmitted with seeds (Washington *et al.*, 2006). The most common pathogenic fungal genera which infect bean seeds are *Colletotrichum*, *Sclerotinia*, *Alternaria*, *Fusarium*, *Rhizoctonia*, *Pythium*, *Ascochyta* and *Botrytis* (Elmer *et al.*, 2001). The highest percentage of fungal infection associated with mungbean seeds reached (34.12%) when isolated from 10 legumes varieties (Al-Abdalall, 2010).

Table 2. Frequency of isolated fungi associated with seeds of five different mungbean cultivars

Isolated fungus	Mungbean cultivar					Total	Frequency (%)
	Giza-1	VC-1000	King	VC-2719	Kawmey-1		
<i>Alternaria</i> spp.	3	2	1	2	8	19.51
<i>C. dematium</i>	1	3	1	2	7	17.07
<i>Fusarium</i> spp.	2	1	1	1	5	12.20
<i>F. oxysporum</i>	1	1	1	3	7.32
<i>M. phaseolina</i>	2	1	2	1	6	14.63
<i>R. solani</i>	3	2	1	3	9	21.95
<i>S. sclerotiorum</i>	1	1	2	4.88
<i>V. albo-atrum</i>	1	1	2.44
Total	12	10	5	8	6	41
Frequency (%)	29.27	24.39	12.20	19.51	14.63

Effect of culture filtrate of C. dematium on seed germination and root length of tested cultivars:

Results summarized in Table (3) and Fig. (1) indicate that culture filtrates significantly decreased percentage of seed germination and root length. The lowest percentage of seed germination and root length were recorded with isolate No.1 for Giza-1 (55.65%, 20.33mm) while the lowest percentage of seed germination and root length was obtained with Vc-2719 and Vc-1000 (58.89%, 21.33mm, respectively) with isolate No.2. While the highest percentage of seed germination were (83.84, 77.78%) with cv. King followed by cv. Kamey-1 (73.33 and 66.67%) with two isolates, respectively.

Table 3. Effect of culture filtrate of two *C. dematium* isolates on seed germination and root length on five mungbean cultivars

Tested cultivar	Isolate No. 1		Isolate No. 2		Control	
	S.G. (%)*	R.L. (mm)**	S.G. (%)	R.L. (mm)	S.G. (%)	R.L. (mm)
Giza - 1	55.56	20.33	64.45	23.67	100	43.33
VC - 1000	66.89	32.67	61.11	21.33	100	46.00
King	83.84	39.67	77.78	28.33	100	56.67
VC - 2719	63.33	35.33	58.89	30.00	100	53.33
Kawmey-1	73.33	40.00	66.67	45.33	100	60.00
Mean	68.58	33.60	65.78	29.73	100	51.87
L.S.D at 5% for: * Seed germination (S.G.): Isolates= 0.49 Varieties= 0.45 I x V= 0.79						
**Root length (R.L.): Isolates= 0.39 Varieties= 0.88 I x V= 0.77						

Culture filtrate of *Aspergillus niger*, *Fusarium culmorum*, *Penicillium* sp. and *Rhizoctonia solani* reduced percentage seed germination of soybean seeds (Haikal, 2008). Culture filtrate of *F. oxysporum* f.sp. *udum* inhibited seed germination and seedling growth after 7 days of incubation. Germination (%) was very poor (10%) in var. ICP-2376 and root-shoot length of tested pigeon pea vars. (Jalander and Gachande, 2011).



Fig. 1. Effect of culture filtrate of two isolates (1 and 2) of *C. dematium* on seed germination and root length on five mungbean cultivars

Effect of different carbon and nitrogen sources on C. dematium mycelial growth and sporulation:

The two isolates varied in its ability to utilize by different carbon and nitrogen sources. Data in Table (4a) indicated that fructose gave maximum dry mycelial weight (1.214 and 1.044 g) with the two isolates followed by manitol (1.081 and 0.976 g) while maltose was less utilize. Maltose and fructose greatly increased sporulation for both isolates while lactose was less effective. Also isolates varied in its ability to utilize by different nitrogen sources (Table 4b) where sodium nitrate and ammonium nitrate were more supported on mycelial growth and sporulation of isolate No. 1 (0.864 g) while ammonium nitrate and potassium nitrate were more supported on mycelial growth and spores production with isolate No. 2 (0.985 g) while asparagine was less effective with two isolates. Kumara and Rawal (2008) found that fructose was the best source of carbon for the growth and sporulation of most of isolates. Sangeetha and Rawal (2008) reported that manitol gave maximum dry mycelial weight and ammonium supported good growth and sporulation.

Table 4a. Effect of different carbon sources on mycelial growth and sporulation of two *C. dematium* isolates

Carbon source	Dry weight (g)		Mean	Sporulation*	
	Isolate			Isolate	
	1	2		1	2
Fructose	1.214	1.044	1.129	++	+++
Lactose	1.011	0.849	0.93	+	+
Maltose	1.062	0.924	0.993	+++	++
Manitol	1.081	0.976	1.029	+	+
Control	0.202	0.228	0.215	+	+
Mean	0.914	0.804			
LSD at 5% for:	Isolates (I) = 0.02,		Carbon (C)= 0.05,	Ix C= 0.07	

* (-): No sporulation, (+): 1-10, (++) : 11-50 and (+++) : > 100 spores per microscopic field.

Table 4b. Effect of different nitrogen sources on mycelial growth and sporulation of two *C. dematium* isolates

Nitrogen source	Dry weight (g)		Mean	Sporulation*	
	Isolate			Isolate	
	1	2		1	2
A. nitrate	0.695	0.840	0.840	+++	+
Asparagine	0.515	0.564	0.564	+	+
P. nitrate	0.622	0.669	0.669	+	+++
S. nitrate	0.864	0.635	0.635	+	+
Control	0.348	0.377	0.377	+	+
Mean	0.609	0.668			
LSD at 5% for: Isolates (I)= 0.01, Nitrogen (N)= 0.02, IxN=					

* As described in footnote of Table (4a).

Effect of light and temperature on mycelial growth and sporulation of C. dematium:

a- Light effect:

Data in Table (5) show that maximum mycelial growth and sporulation (1.263 and 0.896g) was recorded when *C. dematium* incubated under continuous light followed by incubation for 12h light/dark (0.839 and 0.796g), while continuous darkness was less effective (0.584 and 0.558 g). These results indicate that tested isolates were not sensitive to darkness and were more sensitive to light. Russo and Pappes (1993) found that incubating *C. dematium* under light significantly increased mycelial elongation and sporulation over dark cultured colonies. Yi Ding *et al.* (2007) reported that maximum colony diameter was observed when exposed continuous darkness (6.80 cm) followed by (6.63 cm) at 12h light/dark photoperiod and (5.83 cm) at continuous light after 7 days. Vinod and Benagi (2009) reported the maximum radial growth (89.29mm colony diameter and 593.71 mg dry mycelial weight) was observed when exposed to alternate cycles of light and darkness followed by continuous light and followed by continuous darkness.

Table 5. Effect of light/dark on mycelial growth and spore production of two *C. dematium* isolates

Treatment	Dry weight (g)			Sporulation*	
	1	2	Mean	1	2
Light	1.263	0.994	1.129	+++	++++
Dark	0.584	0.558	0.571	+	+
12 / 12 hr	0.839	0.796	0.818	++	++
Mean	0.895	0.750			
L.S.D at 5% for: Isolates (I)= 0.402, Treatment (T)= 0.248, I x T = 0.031					

* As described in footnote of Table (4a).

b- Effect of different temperatures:

Different isolates of *C. dematium* responded differently to various temperature regimes as shown in Table (6). Data indicate that maximum mycelial growth and sporulation was recorded by isolate No.1 at range of 25-30°C, while maximum mycelial growth and sporulation of isolate No. 2 was recorded at 25°C. Sangeetha and Rawal (2008) Found that maximum growth of different mango isolates of *C. gloeosporioides* at a temperature range of 25-30°C, while good sporulation was maximum at optimum temperature (28-30°C) when grown on Richard's agar medium.

Table 6. Effect of temperature on mycelial growth and spore production of two *C. dematium* isolates

Temperature (°C)	Mycelial dry weight (mg)			Sporulation*	
	1	2	Mean	1	2
10	0.289	0.267	0.278	-	+
15	0.392	0.376	0.384	+	+
20	0.613	0.622	0.618	+	++
25	0.836	0.841	0.839	+++	+++
30	1.263	0.869	1.066	+++	++
35	0.497	0.468	0.483	+	+
Mean	0.648	0.574	-----		
L.S.D at 5% for: Isolates (I)= 0.63, Treatment (T)= 0.60, I x T= 0. 85					

* As described in footnote of Table (4a).

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التسجيل الأول لفطر *Colletotrichum*

مسبب عفن ساق فول المانج في مصر

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معهد بحوث أمراض النباتات - مركز البحوث الزراعية - الجيزة -

شوهدت بقع بنيه محمرة على سيقان بعض /
(جيزة -) المزروع لتجارب بحثيه في محطة بحوث النوبارية
(محافظة البحيرة) (محافظة بنى سويف)
وتم تعريف الفطر المعزول على انه

Colletotrichum dematium.

وأظهرت اختبارات القدرة المرضية أنها ممرضة لنباتات فول المانج.
أن العزلتين
العزلتين تحت ظروف الصوبة.
العزلتين تحت ظروف الصوبة.
العزلتين اختلفا من حيث تأثيرهما على النسبة المئوية لإنبات بذور الأصناف
المختبرة وكذلك طول الجذير. أيضا أن العزلتين اختلف
منهما من مصادر الكربون والنيتروجين المختلفة وتأثيرها على الوزن الجاف
للميسليوم وإنتاج الجراثيم. أن النمو الميسليومى وإنتاج الجراثيم للعزلتين
في