Studies on Bottle Gourd Charcoal Rot Caused by *Macrophomina phaseolina* in Egypt

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Abstract: Bottle gourd (*Lagenaria siceraria* (Mol.) Standl.) is one of the oldest cucurbitaceae plants in the world. During summer 2012, growing season, bottle gourd plants showed wilted symptoms pre-maturely in August with patches similar to those of sudden death syndrome. Discoloration in cortex tissues of taproot and lower stems are typical. These symptoms often known by charcoal rot dry-weather wilt or summer wilt. The causal fungus was isolated and identified as *Macrophomina phaseolina* (Tassi) Goid. Pathogenicity test indicate the ability of *M. phaseolina* to cause the disease symptoms on bottle gourd plants. Seeds treated with different chemical inducers at 10 mM significantly reduced charcoal rot severity compared with the control. Seeds treated with oxalic acid showed the highest reduction of disease severity followed by $CaCl_2$. On the other hand, treatment with KH_2PO_4 and $CaCl_2$ as seed soaking demonstrated higher values for vegetative growth as total dry weight than the untreated control. According to the available literature, this is the first report of bottle gourd charcoal rot disease caused by *M. phaseolina* in Egypt.

Keywords: Bottle gourd, Cucurbitaceae, Charcoal rot**,** *Macrophomina*

INTRODUCTION

Bottle gourd, also known by the names of bottle squash, calabash gourd, doodhi and lowki (*Lagenaria siceraria* (Mol.) Standl.) is one of the oldest Cucurbitaceae plants in the world. This delicious vegetable can be considered as a rich source of vitamins, iron and minerals; it is an excellent diet for people having digestive problems. Bottle gourd is a great foodstuff for shedding extra calories and maintaining optimum health. It is an important rootstock for watermelon production in several countries such as Japan, China and Egypt. Unfortunately, survey bottle gourd diseases were conducted in Pakistan for occurrence of fungal diseases. Alternaria leaf blight and Cercospora leaf spot diseases were found to be prevalent during rainy season in bottle gourd (Maheshwari, *et al*., 2013). There are other reports of bottle gourd diseases eg., powdery mildew, downy mildew, fruit rot, antharacnose, root rot, root knot and viral diseases (Maholay, 1989; Hafiz, 1996 and Zitter *et al*., 1996). *Rhizoctonia bataticola* causing dry rot, leaf blight and charcoal rot in cucurbits (Maholay and Sohi, 1985; Maholay, 1989; Chandi and Maheshwari, 1992). In this respect, Dwivedi and Tandon (1976) reported many fungal species in bottle gourd viz. *Aspergillus flavus, A. niger, Alternaria alternata, Fusarium oxysporum* and *Rhizopus nigricans* associated with seed coat and cotyledons. *Macrophomina phaseolina* (Tassi) Goid. is a soilborne plant pathogenic fungus. Charcoal root rot, caused by *M. phaseolina* is one of the most frequently isolated from root and stalk rot disease of sorghum. The origin name of this disease is due to the charcoal appearance of infected areas where vascular bundles become covered with numerous tiny black microsclerotia of the pathogen. *Macrophomina phaseolina* can also infect seedlings under moist and high temperature conditions and cause seedling blight or damping-off of seedlings (Mughogho and Pande, 1984). The current trend in crop protection against diseases is to apply different chemical inducers that will stimulate the inherent defense mechanisms of the host plant. Such chemical inducers are assumed to be much more environmentally sound than synthetic fungicides, to have a lower economic cost for farmers, to lack environmental and toxicological risks, and to create induced systematic resistance in the hosts against several pathogens.

The present study was undertaken with the objective to document the occurrence of *M. phaseolina* charchoal rot on bottle gourd in Egypt. In addition, examine the effectiveness of nonconventional chemicals as inducers of resistance against charcoal rot.

MATERIALS AND METHODS

Field survey and collection of fungal isolates

During summer 2012, at Experimental Farm of Faculty of Agric. Suez Canal Univ., effect of milk whey as enhancing material for seed germination and plant growth was observed. Seeds treated with four enhancing treatments as seed soaking for two days in water, whey, whey + water (1:1, v: v) and whey + NaCl (5g/L) were evaluated in endemic soil with *Macrophomina phaseolina.* Stems and roots of symptomatic plants were sampled from the fields. Depending on the number of diseased foci in a trial, 3–5 plants were sampled per each treatment. All samples were deposited at the Laboratory for the further studies.

Isolation and identification of *Macrophomina phaseolina*

A soil-assay technique based on Alabouvette (1976) was used as to isolate the causal organisms.

Isolation from diseased plant samples, collected plants were rinsed well under running tap water for 1 h, and dried with a sterile paper towel. Separate subsamples of roots and stems of each plant were sterilized by immersion in ethyl alcohol 70% for 2 min to follow by rinsing for 2 min in sterile distilled water. Tissues enucleate from dying roots and stems were cut into small segments (5 mm) and placed on Petri dishes containing PDA-medium. Plated Petri dishes were incubated at 28±2°C. Each colony was observed under the microscope for the presence of fungal growth and then propagated further onto PDA for 10 days, and stored at 5°C.

Pathogenicity tests

Pathogenicity test was conducted under greenhouse conditions, to confirm that *M. phaseolina* is the causal agent of root rot of bottle gourd plants. Sterilized corn meal sand medium (Kumar *et al.,* 2007) was used to prepare fungal inoculum. The medium was inoculated with 5mm disc of 15 days old culture of *M. phaseolina* and incubated for 15 days at 28±2°C. Pots (15-cm in diam.) were filled with 1:1 sand:clay soil. The potted soil was infested with the fungal isolate at the rate of 3% (w/w) from the soil weight. Control pots were inoculated with the equivalent amount of sterile corn meal sand medium. The infested soil was watered and mixed thoroughly for one week to insure even distribution the inoculum. Three seeds per pot were sown and five replicates were used with each isolate. Another five replicates were used as a control treatment. Disease incidence was calculated at 60 days after sowing as infection percentage of root rot and disease severity according to Haware and Nene (1980).

Induce resistance against charcoal rot

The chemical inducers used in this study include salicylic acid, oxalic acid, ascorbic acid, di-potassium hydrogen phosphate (K_2HPO_4), potassium di-hydrogen phosphate (KH_2PO_4) and calcium chloride ($CaCl_2$) were obtained from Sigma Aldrich Chemical Co., (St. Louis, MO, USA). Aqueous solutions were prepared at concentrations of 10 mM (10 millimoles) for each inducer. Solutions were prepared by dissolving the known amount of inducers in sterile distilled water at room temperature (25-28 °C) and mixed on a stirrer for few minutes to ensure complete solubalization. In the field experiments, the inducers were tested as seed soaking treatments. The seeds of bottle gourd (seeds obtained from the Horticulture Department, Fac. of Agric., Suez Canal Univ.) were soaked in aqueous solutions of different chemical inducers and in distilled water as control for 24 h and allowed to dry up on filter paper for one hour.

Greenhouse studies

Seeds soaking in each of the different inducers or in water as a control were prepared. The potted soil was infested with *M. phaseolina as* mentioned above. Five pots free from the pathogen were sown to use as control. The pots were sown with treated seeds at the rate of three seeds pot^{-1} for each replicate and irrigated when needed. Other group of pots was sown with soaking seeds in non-infested soil. The experiment was arranged in a complete randomized block designed with five replicates. Percentage of infected plants with charcoal rot and healthy plants was recorded at 60 days after sowing as described before according to Haware and Nene (1980).

Determination of total phenols content

Total soluble phenols in bottle gourd roots of treated or non-treated with chemical inducers were determined by using colorimetric method described by Folin and Ciocaltu (Anonymous, 1985). The concentration of phenols was calculated from a standard curve of pyrogallol according to Coseteng and Lee (1987). The relationship between total phenols content and root rot severity recorded in the field trials was determined by using regression analysis.

Statistical analysis:

Obtained data were exposed to the statistical analysis using complete randomized block design described by (Gomez and Gomez, 1984) and treatment means were compared using L.S.D test at %5.

RESULTS AND DISCUSSION

Bottle gourd seeds treated with enhancing materials as seed soaking showed different responses. Data presented in Table (1) showed significant ($P<0.05$) differences in shoot growth. Seed soaked in the water showed high seed germination percent. In the contrary, soaking seeds in the water showed the least vegetative plant weight. On the other hand, seed soaking in whey + salt showed high percent in vegetative weight compared with other treatments.

Growing bottle gourd plants showed different symptoms on pre-maturely in August with patches similar to those of sudden death syndrome. Discoloration in cortex tissues of taproot and lower stems is typical. When stems are split, piths of diseased plants have brown rot like browning in the lower part of the stem. These symptoms often known by charcoal rot dry-weather wilt or summer wilt.

Significant ($P<0.05$) differences in shoot growth was recorded, which ranged from 20 gm (Control) to 25gm (whey + salt) as presented in Table (1). Control plants yielded the smallest shoot growth, suggesting growth stimulation by different whey treatments.

Table (1): Effect of whey as seed soaking on seed germination and growth of bottle gourd plants

Seed soaking treatment	Seed germination %	Transplant height (cm)	Vegetative weight(gm)
Water (control)	100	20	20
Whey	50	15	23
+ water (50%)	60	17	21
Whey + salt (5 gm/L)	80	18	25
L.C.D 5%	8.3	3.7	1.6

Charcoal root rot and wilted symptoms of bottle gourd plants were observed and recorded. Data presented in Table (2) proved that the tested bottle gourd as seed soaking treatment was reacted differently throughout the natural infection in the Experimental Farm of Fac. Agric., Suez Canal Univ. Bottle gourd showed high susceptible with wilt, stunting and dead plants under natural infection. These results are in agreement with Mughogho and Pande (1984), Farr *et al.* (1995) and Saleh *et al.* (2010).

Disease symptoms:

Charcoal rot or dry weather wilt caused by the widespread soilborne fungus *Macrophomina phaseolina* (synonyms: *Rhizoctonia batiticola, Sclerotium batiticols*). Charcoal rot affected bottle gourd plants. First symptoms was yellowing and death of crown leaves followed by water-soaked lesions on the stem at the soil line (Fig. 1, A and B and Fig. 2, B). As the disease progresses, the stem of infected plants ooze amber-colored gum (Fig. 1, A and Fig 2, C), and the stem eventually becomes dry and tan-to-brown in color (Fig. 1, B and Fig 2, E). The stem may be girdled by the lesion, resulting in plant death (Fig. 1, C and D & Fig 2, D and E). Charcoal rot is favored by hot, dry weather especially in combination with fertility deficient soils or other unfavorable growing conditions. A similar symptom was described by Hoes, 1985; Kolte, 1985 and Khan, 2007.

Isolation and identification of the causal pathogens:

Isolation from collected samples of wilted bottle gourd yielded *M. phaseolina* reached 62.1% of total isolated fungi. *M. phaseolina* were isolated from the internal basal stem parts with high percentage followed by internal root segments and from the soil surrounding the root system, (27.3, 21.7 and 13.1 %, respectively).

Colonies of this fungus were identified as *M. phaseolina* based on the presence of growth parameter and present microsclerotia according to (Clude and Rupe, 1991). In this regard, other pathogenic fungi at the rate 16.2 % from the total isolates were obtained from infected plant parts at low percentage. Some other genera such as *Cladosporium*, *Aspergillus* and *Alternaria* were isolated from the soil and external parts of the roots with low frequency.

Table (2): Disease survey with different symptoms on bottle gourd plants treated with enhancing germination materials under natural soil infestation.

Seed soaking materials	Percentage of different observed symptoms			
	Wilted plants	Stunted plants	Dead plants	Healthy plants
Whey (crude 100%)	11.1	27.8	16.7	44.4
Whey + water (50%)	22.2	22.2	16.7	38.9
Whey + salt (5 gm/L)	11.1	22.2	22.3	44.4
Water (control)	33.3	9.1	24.3	33.3
L.C.D %5	2.6	1.4	2.1	3.7

A B C D

Fig. (1): A: Healthy plant, B: affected plant by charcoal rot, C: disease symptoms on the shoot system and D: affected bottle gourd field under natural infection.

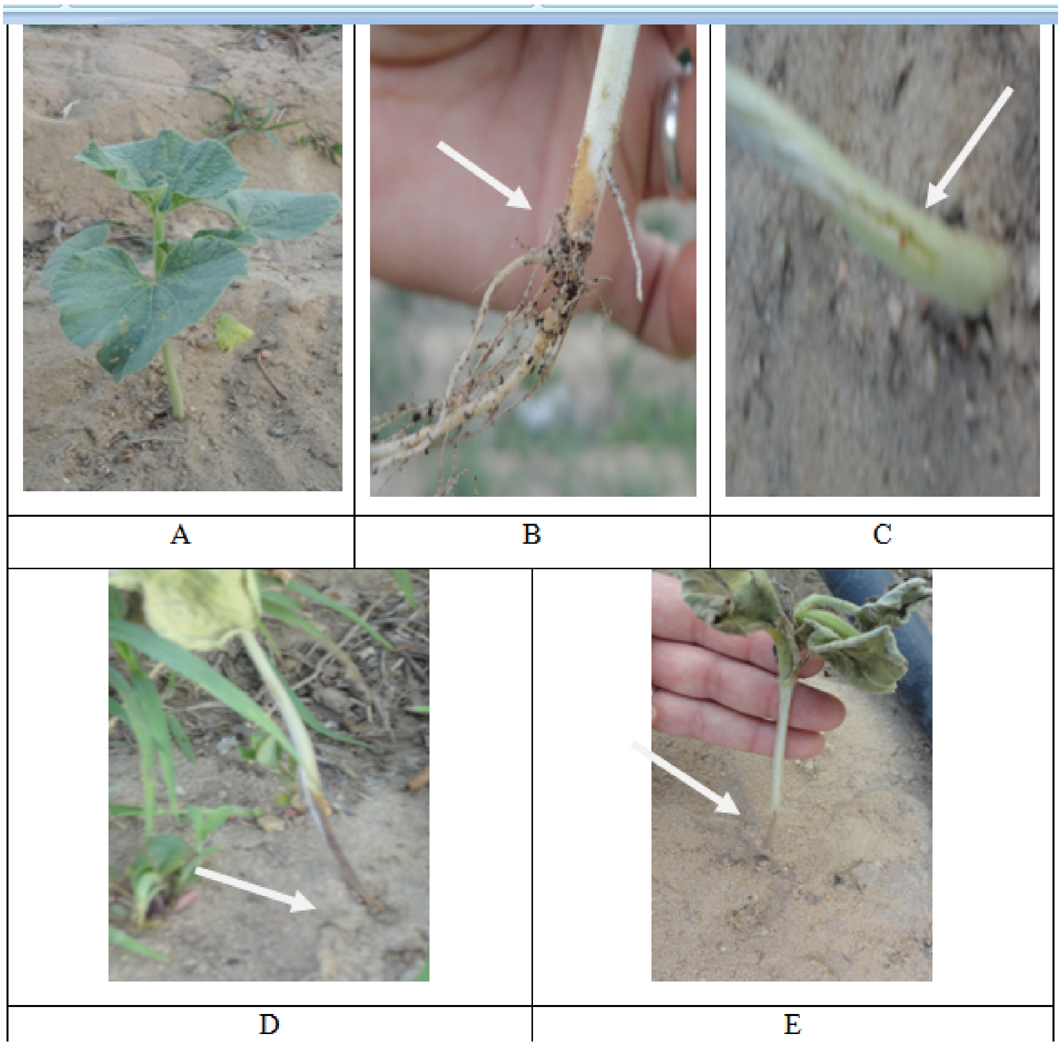

Fig. (2): Different disease symptoms of *M. phaseolina* or charcoal rot on bottle gourd plants under artificial inoculation, A: non-inoculated seedling, B: water soaked lesion C: stem ooze gum and D, E: stem girdling lesion.

Table (3): Isolation of the causal pathogens from rotted parts or wilted plants and sowing soil

Fungi	Frequency of isolates from different parts			
	Soil	Roots	Basal stem	Total
Macrophomina phaseolina	13.1	21.7	27.3	62.1
Fusarium solain	8.7	8.7	4.3	21.7

The obtained results are in agreement with those (Cordell, *et al.,* 1989) as they noted that a similar disease, black root rot, is caused by a complex of organisms of which *M. phaseolina* and *Fusarium oxysporum* are the most important pathogens.

Pathogenicity tests

Pathogenicity test with high frequency of isolated fungi *i.e. M. phaseolina* and *F. solani* were studied under greenhouse conditions. Data presented in Table (3) show that *M. phaseolina* caused the highest destructive on bottle gourd seedlings. In addition, *M. phaseolina* showed different symptoms associated with charcoal rot on the bottle gourd plants and these include root rot, soft stalks at the high rate (66.7%) compared with *F. solani* (26.6%). In addition *M. phaseolina* caused stunted plants, smaller stalks with premature drying as a result of seedling blight. These results are in agreement with those reported by Smith and Carvil 1997 and Jha and Dubey, 1998

Induce resistance

Comparative evaluation of the six chemical inducers, KH_2PO_4, K_2HPO_4, $CaCl_2$, ascorbic acid, salicylic acid, and oxalic acid were tested for their efficiency in reducing charcoal rot severity of bottle gourd under field conditions. Seeds treated with different chemical inducers at 10 mM significantly ($P \leq 0.05$) reduced charcoal rot severity compared with control (Table, 4). However, seeds treated with oxalic acid showed the highest reduction of disease severity followed by $CaCl_2$. Whereas, Ascorbic acid and KH_2PO_4 showed lower effect in reduction the charcoal rot compared with the control. In this regard, healthy survival plants recorded at the high value when seeds treated with oxalic acid, $CaCl_2$ and KH_2PO_4 and salicylic acid, respectively. On the other hand, Ascorbic acid and Salisilic acid showed the less effect in controlling damping off and charcoal rot severity.

The mode of action of chemical inducers for controlling plant diseases may include: (1) acting as second messengers in enhancing the host defense mechanism (Geetha and Shetty 2002); (2) activating resistance by increasing the activity of peroxidase, the synthesis of new peroxidase isoforms, or the accumulation of the phenolic compound (Sarma *et al.,* 2007); (3) activating resistance through inhibition of some antioxidant enzymes and catalases, thereby leading to production of elevated amounts of H_2O_2 accumulation (Radwan *et al.,* 2008); and (4) enhancing resistance by direct effects on multiplication, development, and survival of pathogens or indirect effects on plant metabolism, with subsequent effects on the pathogen food supply (Reuveni and Reuveni 1998; Khanam *et al.,* 2005). As evident from the differential mode of action of the chemical inducers, the varying efficiencies among these chemicals in protecting bottle gourd against charcoal rot have been observed in this trial.

Results of the present study revealed the role of chemical inducers in the accumulation of total phenols. Salicylic acid, KH_2PO_4 and oxalic acid treatments showed the maximum accumulation of total phenols when compared with the control treatment (Table 5). An important finding of this study indicated that, some chemical inducers had adverse effects on the plant growth. In this regard, Kruger *et al.* (2002) noted that the synthesis of phenols occurs as an early response of plants to attempt infection by pathogens, as antimicrobial compounds, signal molecules, and cell wall strengthening components. In the same trend, Sarma *et al.* (2007) revealed a positive correlation between the accumulation of phenolic compounds and the reduction of plant mortality in chickpea when exposed to Sclerotinia stem rot.

In addition, plants treated with KH2PO4 and CaCl2 as seed soaking demonstrated higher values for vegetative growth as total dry weight than the untreated control or compared with other chemical inducers. In this regard, Raskin (1992) suggested that the inducing chemicals did not cause a major expenditure of energy. However, the decreases in growth of plants treated by salicylic acid and oxalic acid might be related to the differential mode of action of both chemicals in controlling plant disease. For example, salicylic acid is presumed to play a role in controlling ion uptake by roots and stomatal conductivity.

In general, a tendency for reduced bottle gourd charcoal rot severity with increased total extractable phenols in roots was indicated that higher phenols content resulted in higher resistance towards charcoal rot.

In this study, moderate relationship between the disease severity (Fig. 3) and bottle gourd root content of total phenols was recorded. The closest relationship was recorded of coefficient value (R^2) =0.439 between the total phenols content in root tissues and observed charcoal rot severity under greenhouse conditions. Previous studies have indicated a relationship between charcoal rot resistance in sorghum with sugar and phenol levels (Anahosur *et al.,* 1985), but this relationship between host biochemistry and disease resistances does not appear to apply to root rots and may only be relevant to the stalk tissues and the specific pathogen.

Table (4): Pathogenicity of the isolated fungi on bottle gourd plants

Inoculated fungi	Root rot severity%	Healthy survive plants%
Macrophomina phaseolina	66.7	33.3
Fusarium solani	26.6	73.4
Control	0	100
L. S .D 5%	2.8	3.3

Table (5): Effect of some chemical inducers on damping off and charcoal rot percentage under natural soil infestation

Chemical inducers	Charcoal rot%	Survival plants%
K H2PO4	58.7	41.3
Salicylic acid	60.3	39.7
Ca Cl2	55.4	44.6
K2 HPO4	61.5	38.5
Oxalic acid	51.8	48.2
Ascorbic acid	65.4	34.6
Control	83.3	16.7
LSD 5%	3.5	3.1

Table (6): Effect of different chemical inducers as seed soaking of bottle gourd plants on total phenols content and total dry weight

Chemical inducers	Under artificial infested soil conditions		Under noninfested soil conditions	
	Total phenols ($mg\ g^{-1}$)	Total dry weight ($g\ plant^{-1}$)	Total phenols ($mg\ g^{-1}$)	Total dry weight ($g\ plant^{-1}$)
$K\ H_2PO_4$	0.584	33.5	0.475	44.7
Salicylic acid	0.591	14.7	0.501	22.9
$Ca\ Cl_2$	0.471	35.2	0.397	45.5
$K_2\ HPO_4$	0.354	18.1	0.328	26.1
Oxalic acid	0.573	14.5	0.514	21.3
Ascorbic acid	0.532	25.4	0.457	35.2
Control	0.325	16.2	0.243	23.7
LSD 5%	0.13	3.2	0.14	2.4

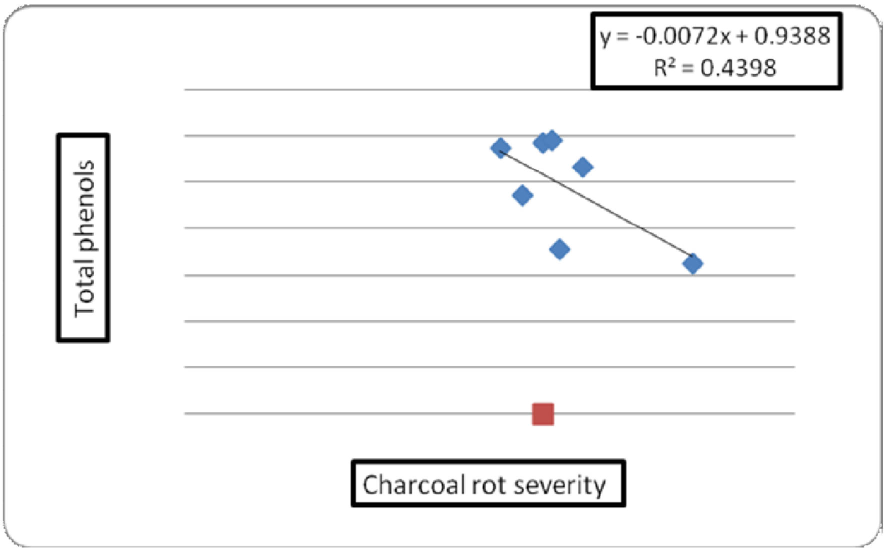

Fig. (3): Regression coefficient between charcoal rot severity recorded for 7 chemical inducers in a greenhouse trial and total phenols content of roots.

CONCLUSION

Bottle gourd one of cucurbit plants, during summer growing season plants are wilted and showed charcoal rot disease symptoms. The causal fungus was isolated and identified as *Macrophomina phaseolina*. Pathogenicity test indicate the ability of the isolated fungus to cause the disease symptoms. Seeds treated with different chemical inducers significantly reduced charcoal rot severity. Using bottle gourd as rootstock should be carefully in infested soil with *M. phaseolina* .

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دراسات على عفن الجذر الفحمى فى نبات اليقطين المتسبب عن فطر *Macrophomina phaseolina* فى مصر

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اليقطين *Lagenaria siceraria* يعتبر احد اقدم نباتات العائلة القرعية فى العالم. اثناء موسم النمو فى صيف ٢٠١٢ اظهرت نباتات اليقطين اعراض الذبول عند نضج النباتات فى بقع منتشرة تشبة اعراض الموت المفاجىء. بالاضافة لظهور تلون فى القشرة الخارجية للجذر الرئيسى وقاعدة الساق. توصف هذه الاعراض يعفن الجذر الفحمى او الذبول الصيفى ا وذبول الطقس الحار. تم عزل وتعريف المسبب المرضى لهذه الاعراض على انه هو الفطر *Macrophomina phaseolina.* اظهر اختبار القدرة المرضية ان فطر *M. phaseolina* ادى لظهور اعراض العفن الفحمى على نبات اليقطين. معاملة البذور ببعض المواد الحاثة للمقاومة بتركيز ١٠ ملمول اظهر انحفاض معنوى فى شدة مرض عفن الجذر الفحمى. معاملة البذور بحامض الاوكساليك ادى الى انخفاض فى شدة المرض يلية المعاملة بملح كلوريد الكالسيوم. من جهة اخرى, اادت معاملة البذور بفوسفات البوتاسيوم وكلوريد الكالسيوم الى زيادة فى انتاج المجموع الخضرى فى صورة اجمالى الوزن الجاف مقارنة بباقى المعاملات. وفقا للمراجع المتاحة، يعتبر هذا هو التقرير الغول عن مرض عفن الجذر الفحمى المتسبب عن فطر *M. phaseolina* فى نبات اليقطين فى مصر.