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First Record of Cercospora Leaf Spot Disease on Okra Plants and its Control in Egypt

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Abstract: During June to September 2008, okra plants (*Hibiscus esculentus* L.) in Kafr El-Sheikh Governorate exhibited typical symptoms of Cercospora Leaf Spot (CLS) at different locations. Symptoms of infected okra leaves firstly started as light brown spots then turned to purple and varying in size. The spots spread to cover large areas of infected leaves. In case of severe infection, spots joined together and formed patches. Later, leaves were dry and remained intact with stem of plant. Samples of diseased leaves were collected to isolate the causal organisms. Isolated fungi were purified using single spore culture technique. Developed fungus was identified as *Cercospora* sp. Fresen based on cultural and morphological characteristics after light microscope examination. Also, *Alternaria alternate* and *Aspergillus niger* were isolated as associated fungi. Pathoginicity test confirmed efficiency of *Cercospora* sp. to induce typical symptoms on okra plants compared with other fungi. Foliar application using different concentrations of Topsin M-70WP and lemongrass oil was significantly reduced disease incidence compared with control. According to the available literature, this is the first record of CLS on okra in Egypt under natural infection in the field.

Key words: Okra, disease, cercospora leaf spot, pathoginicity test, fungi

INTRODUCTION

Okra (*Abelmoschus esculetus* L.) is a nutritious and delicious annual vegetable crop grown in the tropical and sub-tropical regions. Tender green fruits of okra used as vegetable are fairly rich in vitamins and minerals. It's an important vegetable crop of Egypt grown commonly in summer season. Various factors responsible for low yield of the crop especially diseases which play a vital role. The most relevant ones are fungal diseases. The following specific common diseases, Cercospora leaf spot (*Cercospora abelmoschi*), Damping-off (*Pythium* sp. and *Rhizoctonia* sp.), Powdery mildew (*Oidium* sp.), Southern blight (*Sclerotium rolfsii*), Verticillium wilt (*Verticillium albo-atrum*), wet rot (*Choanephora cucurbitarum*) (Raid and Palmateer, 2006), Alternaria leaf spot (Atia and Tohamy, 2004) and Okra leaf curl virus (Atiri and Fayoyin, 1989). Species belonging to genus *Cercospora* sp. Fresen are distributed worldwide and cause CLS disease on most of the major plant families (Crous and Braun, 2003). CLS is an important disease of cowpea (Amadi, 1994), maize (Crous *et al*., 2006), safflower (Lartey *et al*., 2005), sugar beet (Hashem and Farrag, 2005), okra (Chauhan *et al*., 1980), *Hibiscus cannabinus* (Prasad *et al*., 1960), avocado (Darvas, 1977), groundnut (Ambang *et al*., 2011) sesame (Enikuomehin, 2005) and *Acanthus mollis* (Rooney-Latham *et al*., 2011). CLS is mainly controlled with foliar applied chemicals (Jacobsen *et al*., 2000). Other control measures include clean plough down of crop residues, use of resistant cultivars and two-to-three year rotation with non-hosts (Ruppel, 1986). The present investigation was undertaken with the following objectives: (1) Survey for CLS in major okra growing areas of Kafr El-Sheikh governorate (2) Isolation, identification and pathogenicity studies (3) Bioassay of fungicides and lemongrass oil for control CLS incidence.

MATERIALS AND METHODS

Survey of CLS disease: Survey was conducted to know the percent incidence of CLS disease in okra growing areas at Kafr El-Sheikh Governorate during 2008. Five locations were surveyed during four months starting from June to September. The plants were examined every 15 days and quantitative assessments (number of plants/leaves infected) were made. Assessment of the number of infected plants was done in two randomly quadrates (1×1 m) per plot. The total number of plants and number infected in a quadrate were counted then the percentage disease incidence was calculated. Number of leaves infected was obtained from five randomly plants per plot and was expressed as percentage of the total number of leaves.

Isolation of causal pathogen and associated fungi: The causal organism *Cercospora* sp. was isolated from okra

leaves showing typical leaf spot symptoms. Single separated lesions on leaf materials were incubated in a humid chamber at 25°C for 24 h and then examined for sporulation under a binocular microscope. Conidia from separate conidiophores were picked using glass needle and plated on tap water agar. The plates were then incubated at 25°C for 7 days. Hyphal tips from the advancing colony margins were then transferred onto okra extract dextrose agar medium as part of the culture purification process. On the other hand, another isolation method was conducted to isolate other associated fungi. Infected leaves were cut into bits and surface sterilized using 1% sodium hypochlorite for 1 min. After washing with sterilized distilled water the infected bits were cultured on okra extract dextrose agar medium and incubated as mentioned above.

Identification of the pathogen: Morphological examination was made on host material for *Cercospora* sp. according to Hashem and Farrag (2005). Naturally infected okra leaves were cut into about 1 cm disks and washed several times with sterilized distilled water. Disks were put in a humid chamber at 25°C for 24 h then examined using light microscope (Zeiss, Axiostar Plus) provided with digital camera (Canon-G6) at x 1000 magnification for each structure. Fifteen measurements were obtained and the mean was calculated for observed conidia spores. The obtained data was compared with keys given by Ellis (1976) and Moubasher (1993) to determine this isolate *Cercospora* like organism or no. Other isolated fungi were identified according to description given by Ellis (1976), Domsch *et al.* (1980) and Moubasher (1993) and then confirmed in mycological Centre, Assiut University, Egypt.

The fungi were subcultured on host dextrose agar slants and allowed to grow at 25°C for 15 days and such slants were preserved in a refrigerator at 4°C and subcultured once in 30 days.

Pathogenicity test: Pathogenicity study was conducted on okra plants cv. Eskandarani. Seedlings were raised by sowing the seeds in pots (30 cm in diameter) and watering was done twice a week. Thirty-five days old plants were pin prick and sprayed with the suspension containing mycelia of *Cercospora* sp. or conidi spores of *A. alternata* and *A. niger* [1×10^3 cfu mL^{-1}] prepared in sterilized distilled water. Such inoculated plants were covered with polythene bags and kept in dark for 12 h and then maintained for 48 h at 25°C and 100% relative humidity. At the end of 48 h, the pots were kept in greenhouse under natural humidity. Regular observations were made for the appearance and development of symptoms. Control plants were sprayed with distilled water. The fungus was re-isolated from the leaves exhibited symptoms and the cultures obtained were compared with the original to confirm the identity according to Koch's postulates.

Chemical materials

Lemongrass oil: Oil was extracted from lemongrass *via* water distillation. The method started with 200 g of fresh leaves cut into small pieces with 470 mL of water in flask placed on electrical mantel. The steam and extracted essential oil pass through a water condenser, allowing the volatile oil fraction to float on top of the water. The oil was collected by drawing out the water.

Fungicide (Topsin M-70WP): Topsin M-70WP (Thiophanate) Diethyl 4-4 (O-phenylene) bis 3-thiollophanate, manufacture by Nippa Soda, Japan.

***In vitro* evaluation of fungicide and lemongrass oil:** The efficacy of Topsin M-70WP (at concentrations 0.05, 0.1 and 0.15%) and lemongrass oil (at concentrations 0.05, 0.1, 0.15 and 0.20%) against *Cercospora* sp. was analyzed by using usual methods of fungal growth *in vitro* (Smith and Onions, 1983). Lemongrass oil was emulsified by adding Tween X-100 (30 μL mL^{-1} media). Plates were incubated at 25°C for 20 days. The efficacy of fungicide or lemongrass oil was expressed as percent inhibition of mycelium growth over control according to following formula:

$$I = \frac{C\text{-}T}{C} \times 100$$

where, I = Percent inhibition; C = Radial growth in control; T = Radial growth in treatment. The effect of Tween X-100 on *Cercospora* sp. *in vitro* was tested to attest if this detergent has potentials or not to reduce fungal growth.

Control of CLS on okra: A field experiment was laid out at El-Abbasia village, Kafr El-Sheikh Governorate during 2010 and 2011 summer seasons. The efficacy of Topsin M-70WP and lemongrass oil (at 0.15 and 0.2%) were evaluated. The experiment was conducted in randomized block design with three replications and Eskandarani as okra cultivar. Plot size of 2×2 m was used per treatment. Seeds of okra were sowed on second half of May. First spray started 40 Days after Sowing (DAS) immediately after disease appearance followed by another two sprays at 15 days interval. The observation on percent disease incidence was visually assessed every 14 days starting from 39th DAS. Disease incidence was determined using

the following rating scale where 1 = no disease, 2 = light spotting in the lower plant canopy, 3 = light spotting in the lower and upper plant canopy, 4 = some spotting with light defoliation (<10%), 5 = noticeable spotting with some defoliation (<25%), 6 = spotting heavy with significant defoliation (<50%), 7 = very heavy leaf spotting with severe defoliation (<75%), 8 = numerous spots on few remaining leaves and very heavy defoliation (<90%), 9 = very few remaining leaves covered with spots and nearly complete defoliation (<95%) and 10 = plants defoliated. Percent Disease Incidence (PDI) was calculated by using the following formula (Wheeler, 1969):

$$PDI = \frac{\text{Sum of numerical rating}}{\text{Total number of leaves examined x Maximum grade value}} \times 100$$

Increases in disease incidence determined as percentage between 39 DAS and 67 DAS.

The data was statistically analyzed by procedures of Sukhatme and Amble (1985).

RESULTS

Survey of CLS in Kafr El-Sheikh area: The mean incidence ranged from 0.0 to 73.06% and from 0.0 to 44.82% for plants and leaves infected, respectively irrespective of location surveyed (Table 1). The disease was initially in the second half of June and later decreased in September. Maximum incidence (infected plants) of 92.5% was recorded in El-Abassia village followed by 88.7, 74.1, 58.2 and 51.8% in El-Dabaa, Om-Sen, El-Abaadia and El-Rasif villages in second half July. In the first half of September the disease incidence showed decreasing trend (from 1.3 to 4.8%), whereas the disease incidence not showed in the second half of December.

Isolation of the pathogen: The causal organism was isolated from diseased okra leaves showing typical symptoms of leaf spot by the conidial spore isolation technique (Fig. 1b, c). Colonies on okra extract dextrose agar medium reaching maximum diameter after 3 wks with sparse aerial mycelium, margins irregular and surface grey. On the other hand, conidia spores not detected on culture media formulation.

Pathogenithty test and symptoms: Artificial inoculation in okra plants with *Cercospora* sp., *A. alternate* and *A. niger* isolates was carried out under greenhouse conditions. Only *Cercospora* sp. induced typical symptoms which started developing on inoculated plants 7 to 9 days after their removal from humidity conditions (Fig. 1a). The lower and matured leaves are first one to be often affected by fungal inoculums. The spots are purple with ash grey centers. Later the centers turned white and dried up. The

Table 1: Survey of cercospora leaf spot disease on okra plants at Kafr El-Sheikh Governorate

	Disease incidence (%)/15 days															
	June				July				August				September			
	First half		Second half		First half		Second half		First half		Second half		First half		Second half	
Origin	I*	II**	I	II	I	II	I	II	I	II	I	II	I	II	I	II
El-Abassia	0	0	16.9a	7.4a	18.1a	6.8a	92.5a	61.8a	81.9a	44.3a	21.8a	9.5a	4.8a	1.8a	0	0
Om-Sen	0	0	15.6a	6.2a	17.5a	7.4a	74.1b	39.7b	60.7a	28.1b	23.7a	11.5a	1.3a	0.6a	0	0
El-Rasif	0	0	10.5b	4.8b	12.3b	5.2a	51.8b	22.8b	39.5c	11.8c	21.5a	6.2b	0	0	0	0
El-Abaadia	0	0	7.1c	2.5c	10.5c	3.2c	58.2b	36.1b	41.2b	13.4c	12.8b	4.3c	0	0	0	0
El-Dabaa	0	0	10.2b	5.1ab	10.8c	4.9ab	88.7a	63.7a	48.7b	16.8c	16.5b	7.8ab	0	0	0	0
Monthly mean	0	0	12.06	5.2	13.84	5.5	73.06	44.82	54.4	22.88	19.26	7.86	1.2	0.48	0	0

*I = Plants infected, **II = Leaves infected. Values with different superscripts in the same column are significantly different at $p \leq 0.05$

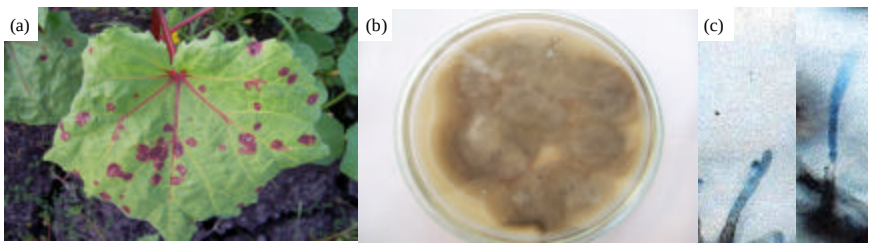

Fig. 1(a-c): Cercospora leaf spot symptom (a), Pure culture of *cercospora* sp. isolated from okra spots (b) and light micrographs of *cercospora* sp. conidiophores and conidia grown from disease lesions (c). Notice, pale brown unbranched conidiophores and multiseptate, hyaline conidia were shown

spot size varied from about 5 to 10 mm in diameter. Injured leaves will often roll and withered prematurely. *Cercospora* sp. was re-isolated from such symptoms and compared with original culture, while other tested fungi failed to induce any symptoms and can't be re-isolated.

Identification of the causal pathogen: From our studies of the fungi involved in leaf spot it appears that *Cercospora* sp. is predominant. This postulate was proved by several experiments in which typical symptoms were induced by inoculating leaves with inoculum suspension of the organism followed by successful re-isolation. *Cercospora* sp. produces, short unbranched conidiophores which are tufted, pale brown, septate and arise through disease lesion. The conidia are slender, slightly curved, thin walled, hyaline, multiseptate i.e., 3-6 septate and measure 40-75×3-5 μ in size (Fig. 1c). On the basis of these characters the pathogen was identified as *Cercospora* sp.

***In vitro* evaluation of fungicide and lemongrass oil against pathogen:** Topsin and lemongrass oil were tested for their efficacy against *Cercospora* sp. Mycelium growth results presented in Fig. 2a and b indicated that using 0.05% of Topsin M-70WP lead to 83.4% inhibition over control. Inhibition percentage increased by fungicide dose increasing until 0.15% which cause 100% inhibition. On the other hand, lemongrass oil was more effective at all the concentrations tested. Minimum inhibition of 85.3% was recorded at 0.05%. Increasing lemongrass oil concentration to 0.1, 0.15 and 0.20% lead to complete inhibition of fungal growth. Also, data obtained for radial growth in the presence Tween X-100 showed that detergent has no antifungal effect on *Cercospora* sp.

Effect of Topsin M-70WP and lemongrass oil on disease incidence of CLS: When compared with control, all treatments reduced the disease incidence of CLS, except Topsin M-70WP at 0.15% (Table 2). As indicated by disease incidence of 4.8, noticeable leaf spotting with about 50% defoliation was observed for untreated okra when determined 39 DAS. In comparison Topsin M-70WP and lemongrass oil, the later one proved more effective in controlling CLS. Topsin M-70WP at 0.2% was nearly equal to lemongrass oil at 0.15%. The dicrease of Disease incidence (DDI) between 39 and 67 DAS for plots treated with lemongrass oil at 0.15 and 0.20% and Topsin at 0.2% was higher than in control and Topsin at 0.15%. On contrary, control treatment lead to increase in disease incidence (IDS).

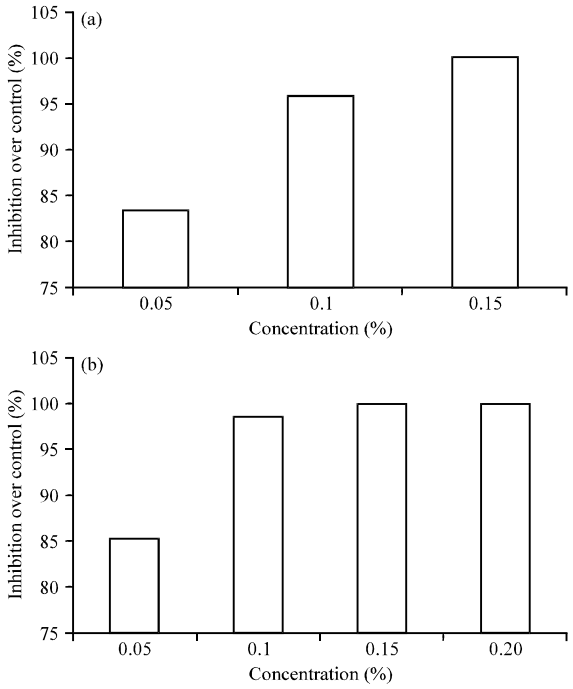

Fig. 2(a-b): Inhibition of *Cercospora* sp. by Topsin M-70WP (a) and lemongrass oil (b)

Table 2: Comparison of Topsin M-70WP and lemongrass oil for control of Cercospora leaf spot disease on okra

		Disease incidence				
		Days after sowing				
Treatments	Application rate (%)	39	53	67	Decreasing rate (%)	Increasing rate (%)
Topsin	0.15	4.1b	2.9b	3.4b	17.07	0.0
M-70WP	0.20	4.3c	1.5c	1.8c	58.14	0.0
Lemongrass	0.15	4.6c	1.8c	2.1c	54.35	0.0
oil	0.20	4.2c	1.3c	1.5d	64.29	0.0
Control	0.00	4.8a	6.3a	7.2a	00.00	41.46

Values with different superscripts in the same column are significantly different at $p \leq 0.05$

DISCUSSION

Okra (*Abelmoschus esculetus* L.) is a traditional vegetable crop with considerable area under cultivation in Egypt. Okra has been considered a minor crop and no attention was paid to its improvement. The genus *Cercospora* Fresen. was described by Fuckel in 1863 (Groenewald *et al*., 2006) and currently is one of the largest and most heterogeneous genera of hyphomycetes (Crous and Braun, 2003). Species belonging to this plant pathogenic genus are distributed worldwide and cause CLS on most of the major plant families (Crous and Braun, 2003). Survey investigation in this study revealed that July and August months are favorable period for CLS.

Generally, the high relative humidity and warm weather during these two months is the major environmental factors that helped in development and spread of the disease. Similar reports of sever incidence of frog eye spot caused by *C. nicotiana* was reported (Anonymous, 2002). The symptoms of disease are seen initially on lower and more matured leaves as small spots. Often the centers may turn white and dry up. As disease progress these spots joined together and formed patches. Later, the leaves are dry and remained intact with stem of plant. Similar reports of symptom expression were recorded on sugar beet (Ziedan and Farrag, 2011). The success of *Cercospora* sp. isolation from infected tissues depends on the media used and whether the Cercospora spot is invaded by secondary organisms or not. The fungus grows satisfactorily on dextrose agar media supplemented with okra extract. The growth is more vigorous, therefore, this media considered more suitable for isolation. On the other hand, such tap water agar or okra dextrose agar medium failed to support sporulation of the fungus. This result are in contrary to earlier reports of maximum conidial production by *C. nicotiana*, by carrot leaf decoction agar and antibiotic dextrose carrot agar medium reported by Stavely and Nimmo (1968) and Schneider *et al.* (1973), respectively. Hyphae of fungus were hyaline and septate. Growth was slow taking about 20 days to reach maximum growth. Also, morphological examination for conidial measurements was made on host materials. On basis of all these characters, the isolated culture was identified as *Cercospora* sp. The specie may be *C. Abelmochi* as reported by Raid and Palmateer (2006). *In vitro* studies, Topsin M-70WP as a fungicide was found effective against *Cercospora* sp. at highly concentration (0.15%) but lemongrass oil was found effective at all concentrations tested. The use of biocides from plant origin in crop protection is an important means of promoting biopesicides in crop production. In this field study, attempts were made to control okra CLS disease. The incidence of CLS was low in plots treated with lemongrass oil and Topsin M-70WP. These findings show that lemongrass oil has a strong capacity to reduce the spread of CLS on okra plants. Much curiosity has been developed to the Graminea family, especially *Cymbopogon citrates* which, considered as one of the main sources of potential active metabolites (Edris and mahmoud, 2003). Lemongrass oil has been reported to be active against plant pathogens such as *Colletotrichum gloeasporioides* (Duamkhanmanee, 2008) and seed borne fungi of sorghum (Somda *et al*., 2007).

Finally, the application of lemongrass oil considerably reduced the progression of CLS on okra crop. Lemongrass is easy to obtain, cheap and can grow in all regions. Using it in crop protection would be economically and environmentally rewarding to many crop producers.

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