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INCIDENCE AND CHEMICAL CONTROL OF OKRA LEAF SPOT DISEASE

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Abstract

Studies were carried out in Sindh (Pakistan) for survey, identification, incidence and chemical control of leaf spot disease in okra. The disease incidence percent ranged between 53.5- 61.5% at different locations. The maximum incidence (61.5%) was recorded at Hyderabad, followed by Tando Muhammad Khan (58.4%), Tando Allah Yar (55.1%) and Matyari (53.5%). The fungus, *Alternaria alternata* was isolated and identified as cause of okra leaf spot disease. Severity of leaf spot was significantly reduced in plants sprayed with Ridomil MZ, Dolomile, Topsin-M, Diesomil, protest and Tahfuz as compare to control plants sprayed with water. The results also showed that the mean infected leaves ranged from 4.7-5.9 when the data was recorded before application of fungicides while it was between 2.0-6.4 after two application of tested fungicides. However, significant maximum reduction % in severity of leaf spot disease was recorded in case of Ridomil MZ (2.8), followed by Dolomile (1.9), Topsin-M (1.7), Diesomil (1.6), protest and Tahfuz (1.5). Okra plants infected with leaf spot and sprayed with water (control) showed highly significant reduction in plant height (61.53cm) whereas, the tall plants (95.10) were recorded from plot sprayed with Ridomil M Z followed by Dolomile (91.25 cm), Topsin-M (86.60 cm), Diesomil (78.40 cm), Protest (72.75 cm) and Tahfuz (68.38 cm). Significantly lowest number of fruits per plant of okra (10.50) was recorded in control (sprayed with water), while, highest number of fruits per plant (14.90) were obtained from plot treated with Ridomil M Z, followed by Dolomile (13.90), Topsin -M (13.20), Diesomil (12.70), Protest (12.20), and Tahfuz (11.50). The data also showed highly significant difference between treatments for fruit yield of okra infected with leaf spot. The maximum fruit yield (955 kg per acre) was observed in plot treated with Ridomil MZ, followed by Dolomile (845 kg), Topsin-M (790 kg), Diesomil (738 kg), Tahfuz (594 kg) and Protest (681 kg); whereas minimum fruit yield (495 kg) per acre was recorded in plants not sprayed with fungicides.

Introduction

Okra, (*Abelmoschus esculentus* (L). Moench belongs to the family Malvaceae, originated in Abyssinia than it was taken to North Africa, the eastern Mediterranean Arabia and India (Anon., 2008). It is one of the important vegetable, mainly grown for its tender fruits in many countries of the world. Okra seeds are good source of protein, vegetable oil and also rich in vitamin A and B, phosphorus and iodine, which play significant role in human diet (Baloch, 1994; Yadav & Dhankhar, 2001; Khushk *et al.*, 2003). Okra is a power house of valuable nutrients, soluble and insoluble fiber, which helps to lower serum cholesterol, risk of heart disease, keeps the intestinal tract healthy and decrease colorectal cancer (Anon., 2007; Broek *et al.*, 2007).

Okra crop is suffering from number of biotic and abiotic factors, including insect pests and diseases. Among them, leaf spot is a serious fungal disease, may be found on okra growing areas (Hafiz, 1986; Jha & Dubey, 2000; Kochhar, 2005; Jiskani, 2006). Maheshwari *et al*., (2000) have grown *Alternaria alternata* at eleven different temperatures and sixteen pH levels ranging from 5ºC to 40ºC and 3.0 to 10.5 pH. The optimum temperature and pH for the growth of the fungus were found to be 28ºC and pH 6.5. Minimum growth of the fungus was recorded at the temperature of 5ºC and pH 10.5. Excellent sporulation was observed at 25ºC -30ºC and pH levels of 5.5 to 6.5. While some fungi belonging to genus *Alternaria* are plant parasites that easily can change when exposed under different conditions (Slavov *et al*., 2004). Diversity in color of colonies appeared even in a single spore isolate. Numerous spores were observed when the pathogen was grown under near UV light at 25ºC. The spores germinated on leaf surface in 24 hr in high humidity and infection hyphae of germinating spores penetrated epidermal cells directly.

The symptoms of infected okra leaves firstly start as light brown spot, then turn to concentric dark brown spots, varying in size, become necrotic. These spots spread to cover large areas of infected leaves. In case of severe infection, infected leaves become brown and die (Cho & Moon, 1980; Werner, 1987; Tohyama *et al*., 1995; Canihos *et al*., 1995; Amenduini *et al*., 2003; Antonijevic *et al*., 2007). In some cases, heavy infection has led to total loss of yield (Rizzolli & Acter, 2006). Alternaria leaf spot of okra was previously observed on okra plants under field conditions in USA. According to the available literature, *Alternaria* leaf spot on okra in Egypt under natural infection in the field is the first record (Atia & Tohamy, 2004).

Yoon & Lee (1987), Yoon *et al*., (1989), Sutruedee *et al*., (1991), Baniqued (1991), Bhutta *et al*., (1995), Lagopodi & Thanassoulopoulous (1998), Corazza *et al*., (1999), Maltoni *et al*., (2000), Corazza *et al*., (2000) Corato *et al*., (2003), Timmer (2003), Urbanszki *et al*., (2003), Fischl & Dongo (2004), Minuto (2005), Bulajic *et al*., (2005), Sulviliene *et al*., (2006), Rizzolli & Acter (2006), Antonijevic *et al*., (2007), Bulajic *et al*., (2007) and Jeong *et al*., (2008) reported Apple, Sunflower, Cut flowers, Hayward and Bruno, Strawberry, Kiwifruit, Citrus, Cucumber, Water melon, Marsh, Water weed plants, Pot plants, Spices, Carrot, Rape seed, Tomato as alternate/major hosts of leaf spot disease caused by *Alternaria alternata*, therefore the disease could be very serious problem where ever okra is planted.

The leaf spot disease is commonly controlled by different fungicides, viz., Duter, Benlate-T, Dithane M-45, Topsin-M, Rovral, Mancozeb, Iprodion, Tridemorph, Ziram, Bavistin, Pencozeb, Derosol, Signum 334 WG,

Amistar 250 AC, Boscalid, Pyraclostrobin (Mondal *et al*., 1986; Orozco & Mexico, 1991; Masirevic, 1993; Ghosh *et al*., 2002; Khalequzzaman *et al*., 2003; Ayoub & Qureshi, 2004; Surviliene *et al*., 2006; Rizzolli & Acter, 2006).

The literature available indicates that a very little work has been done on this severe outbreak in world and no systematic studies were undertaken in Sindh. Keeping in view the importance, present studies were conducted in Sindh to survey the crop, for identification of the disease and its causal agent, to estimate the incidence and evaluate different fungicides for effective control of leaf spots of okra.

Materials and Methods

The tested fungicides were sprayed two times with an interval of 15 days and final data was recorded 15 days after last (2^{nd}) spray.

Survey: The survey was carried out to record and estimate incidence of leaf spot in okra during kharif season-2009. The okra crop cultivated in the surrounding of Matyari (Khawaja boundary), Tando Allah Yar (Taj Pur), Hyderabad (Bhawal Zhour) and Tando Muhammad Khan (Muhammad Luqman Thahim) was surveyed, to see the disease incidence in field. One hundred plants were randomly selected and the plants showing leaf spot symptoms were counted to estimate disease incidence percentage.

Collection of diseased samples: The diseased samples of okra plants were brought to the laboratory for isolation, identification of disease causing agent.

Isolation of *Alternaria alternata* from infected leaves: The infected leaves of several okra plants were collected from different locations. These were washed under running tap water for five minutes to remove mud and completely dried over turn papers. The leaves were cut into 0.2-0.5 cm pieces, which were surface disinfested in 0.01% $HgCl_2$ for one minute and washed thrice in sterilized distilled water, and again dried completely to avoid surface contamination and remove chemical traces, respectively. After that 5 pieces were transferred on PDA medium in Petri plates. The whole process was carried out with great care in aseptic conditions. The dishes were incubated at 30±1°C for colonizations of leaf spot causing fungi. The pure culture was developed through sub-culture technique and was multiplied for pure culture.

Multiplication for pure culture: Six mm disk of mycelial growth of fungus was cut from the margin of actively growing culture of the fungus with sterilize cork borer and then placed in the center of each Petri dish of medium. The plates were incubated for 6-7 days at 30±°C temperature.

Identification of *Alternaria alternate*: The fungus *Alternaria alternata* was identified by its colony characteristics and taxonomical keys.

Pathogenicity test: The pathogenicity test was carried out by making a suspension of a fungus. One Petri dish of well developed pure culture of *Alternaria alternata* was thoroughly mixed in 500ml sterilized water and mixed that suspension in knapsack spray machine in 4 liter water and sprayed on okra plant. The typical symptoms of leaf spot appeared after 15 days of spray.

Re-isolation of the fungus: The fungus was re-isolated from artificially inoculated plants to confirm the cause of disease.

Collection of seed: Seed of Subzpari okra variety was collected from Hyderabad market and cultivated on 4^{th} April 2009 in the experimental field at village Mir Ali Murad Talpur, Sakhiabad, Hyderabad. During vegetative phase of the crop field was regularly monitored for natural infection of leaf spot of okra. Fungicides with their recommended doses given in Table 1 were sprayed with first start of the symptoms. Two sprays of each fungicide were done with an interval of 15 days, untreated plants were considered as control. After fungicide application, data was recorded for disease incidence percentage and other following parameters.

Plant height: The height of treated and untreated plants was recorded in cm.

Number of fruits per plant: Total number of fruits from each plant was recorded during the pickings.

Yield: The yield of each replication per treatment was recorded.

Chemical control of okra leaf spot disease: The experiment was conducted on leaf spot disease, using the available fungicides in market, with recommended dozes (Table 1).

Lay out plan: The experiment was conducted on complete randomized block design (Table 2).

Table 1. List of fungicides used, doses and their chemical composition.

Fungicides	Dose	Active Ingredient	Source
Ridomil MZ	4 grams in 4 liter water	Metalyxl- Mancozeb	Syngenta
Topsin-M	8 grams in 4 liter water	Thiophanate-methyl	Arista Agro Pakistan Pvt. Ltd.
Dolomile	4 grams in 4 liter water	Metalyxl- Mancozeb	Target
Diesomil	4 grams in 4 liter water	Cymoxil- Mancozeb	Target
Protest	6 grams in 4 liter water	Propinib	Target
Tahfuz	4 grams in 4 liter water	Fostel Allumonium	Target
No Fungicide	Only water 4 liter	--	--

Table 2. Layout for chemical control of okra leaf spot disease.

R*$_1$	T**$_7$	T_6	T_5	T_4	T_3	T_1	T_2
R_2	T_2	T_7	T_4	T_5	T_6	T_3	T_1
R_3	T_1	T_3	T_5	T_6	T_4	T_7	T_2

R= Replication and T= Treatment, whereas, T_1= Ridomil MZ, T_2= Dolomile, T_3= Topsin-M, T_4= Diesomil, T_5= Tahfuz, T_6= Protest and T_7= No Fungicide

Disease incidence: The disease incidence percentage was calculated by using the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased leaves/treatment}}{\text{Total number of leaves/treatment}} \text{ X } 100$$

Statistical analysis: The collected data were statistically analyzed by using "Student edition of statistix, version 1.0" computer software to test the superiority of treatment means.

Results

Survey: In case of leaf spot disease in okra, light brown spot appeared on the infected leaves as initial symptoms, then turned to concentric dark brown spots varying in size. These spots also spread and cover large areas of infected leaves, which became brown and died. The symptoms of disease are shown in Plate 1.

The results showed in Fig. 1 that the disease incidence percent ranged between 53.5-61.5% at different locations. The maximum incidence (61.5%) was recorded at Hyderabad, followed by Tando Muhammad Khan (58.4%), Tando Allah Yar (55.1%) and Matyari (53.5%).

Okra plants showing symptoms of leaf spot disease were collected from different locations of okra crop during survey and brought to the laboratory for isolation and identification of causal fungus.

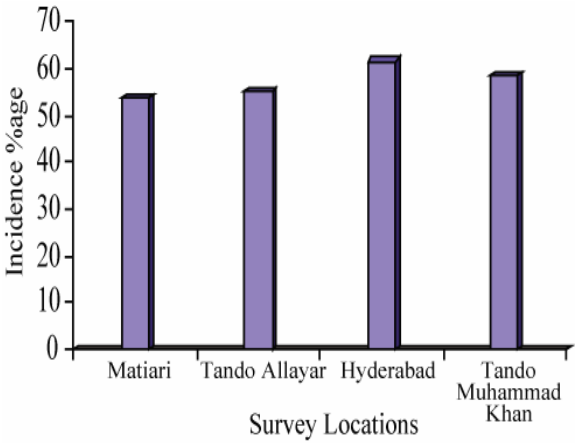

Fig. 1. Survey for incidence of leaf spot disease in okra at different locations for Collection of diseased samples.

Isolation of *Alternaria alternata* from infected leaves: The leaf spot causing fungus, *Alternaria alternata* was isolated from infected leaves of several okra plants, collected from different locations.

Multiplication for pure culture: The isolated leaf spot causing fungus, *Alternaria alternata* was multiplied as pure culture on PDA medium with standard method for further studies.

Identification of *Alternaria alternate*: The isolated leaf spot causing fungus, *Alternaria alternata* was identified by its morphological characteristics as described by Logrieco *et al*., (1990). The mycelium and conidia were typically darker in colour. The condia were large, multicellular, transversely and latitudinaly septate and arranged acropatally in chain.

Pathogenicity test: The pathogenicity test was carried out for the confirmation of disease causing fungus. The typical symptoms of leaf spot appeared after 15 days of spray of artificially multiplied fungal culture of disease causing fungus and were confirmed after re-isolation from leaf spots.

Re-isolation of the fungus: The fungus *Alternaria alternata* was re-isolated from artificially inoculated plants after the pathogenecity test.

Effect of different fungicides on severity of leaf spot of okra: Disease severity of leaf spot of okra was also estimated during experiment. Severity of leaf spot was significantly reduced in plants sprayed with Ridomil MZ, Dolomile, Topsin-M, Diesomil, protest and Tahfuz as compared to control, plants sprayed with water (Table 3).

The results showed that the mean infected leaves ranged from 4.7-5.9 when the data was recorded before application of fungicides (Table 3). While it was between 2.0-6.4 after two application of tested fungicides (Table 3). However, significantly maximum reduction % in severity of leaf spot disease was recorded in case of Ridomil MZ (2.8), followed by Dolomile (1.9), Topsin-M (1.7), Diesomil (1.6) and protest and Tahfuz (1.5).

Effect of different fungicides on plant height of okra infected with leaf spot: Okra plants infected with leaf spot and sprayed with water (control) showed highly significant reduction in plant height (61.53cm) whereas, the tall plants (95.10) were recorded from plot sprayed with Ridomil M Z followed by Dolomile (91.25 cm), Topsin-M (86.60 cm), Diesomil (78.40 cm), Protest (72.75 cm), and Tahfuz (68.38 cm) as shown in (Fig. 2).

Table 3. Effect of different fungicides on severity of leaf spot of okra.

Fungicides	Mean pre application	Mean post application	Mean reduction %
Tahfuz	5.7b	4.2b	1.5
Protest	5.3c	3.8c	1.5
Diesomil	5.0d	3.4d	1.6
Topsin-M	5.2c	3.5d	1.7
Dolomile	4.7e	2.8e	1.9
Ridomil MZ	4.8e	2.0f	2.8
Control	5.9a	6.4a	-0.5
LSD (p<0.05)	0.1624	0.2476	-

All the values followed by similar letters are not significantly different from one another at 0.050 percent level of probability

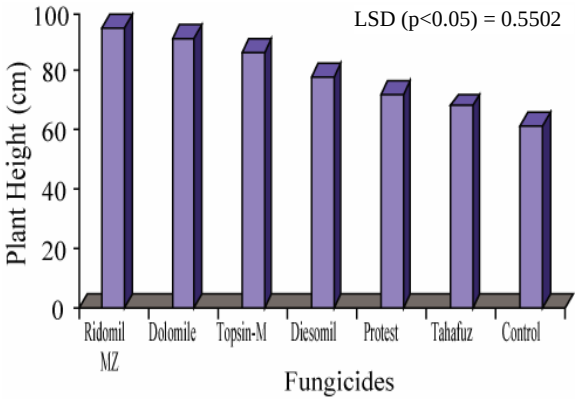

Fig. 2. Effect of different fungicides on plant height of okra infected with leaf spot.

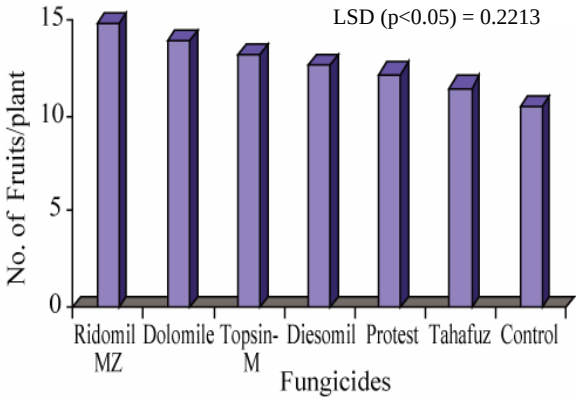

Fig. 3. Effect of different fungicides on number of fruits per Plant of okra infected with leaf spot.

Effect of different fungicides on number of fruits per plant of okra infected with leaf spot: The data in (Fig. 3) showed that the significantly lowest number of fruits per plant of okra (10.50) was recorded in control (sprayed with water), while, highest number of fruits per plant (14.90) were obtained from plot treated with Ridomil M Z, followed by Dolomile (13.90), Topsin -M (13.20), Diesomil (12.70), Protest (12.20), and Tahfuz (11.50).

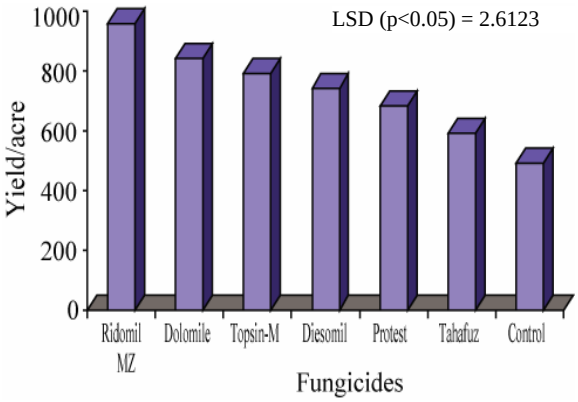

Fig. 4. Effect of different fungicides on fruit yield of okra infected with leaf spot.

Effect of different fungicides on fruit yield of okra infected with leaf spot: The data showed highly significant difference between treatments for fruit yield of okra infected with leaf spot (Fig. 4). The maximum fruit yield (955 kg per acre) was observed in plot treated with Ridomil MZ, followed by Dolomile (845 kg), Topsin-M (790 kg), Diesomil (738 kg), and Protest (681 kg) Tahfuz (594 kg); whereas minimum fruit yield (495 kg) per acre was recorded in plants not sprayed with fungicides.

Discussion

In case of leaf spot disease in okra, light brown spot appeared on the infected leaves as initial symptoms, then turned to concentric dark brown spots varying in size. These spots also spread and cover large areas of infected leaves, which become brown and died. Similar symptoms of disease are reported by Cho & Moon (1980), Werner (1987), Masirevic (1993), Tohyama *et al*., (1995), Canihos *et al*., (1999), Amenduini *et al*., (2003), Urbanszki *et al*., (2003), Minuto (2005), Antonijevic *et al*., (2007) and Jeong *et al*., (2008).

The disease incidence percent ranged between 53.5-61.5% at different locations. The maximum incidence (61.5%) was recorded at Hyderabad, followed by Tando Muhammad Khan (58.4%), Tando Allah Yar (55.1%) and Matyari (53.5%). Similar results are reported by Yoon & Lee (1987), Yoon *et al*., (1989), Baniqued (1991) and Maltoni *et al*., (2000).

The fungus, *Alternaria alternata* was isolated and identified by its morphological characteristics as described by Logrieco *et al*., (1990), it was confirmed as cause of okra leaf spot disease. The findings are in conformity with Cho & Moon (1980), Sutruedee *et al*., (1991); Corazza *et al*., (1999); Corazza *et al*., (2000); Slavov *et al*., (2004); Fischal & Dongo (2004). They

also reported similar fungus as causal agent of okra leaf spot disease.

Disease severity of leaf spot of okra was also estimated during experiment. Severity of leaf spot was significantly reduced in plants sprayed with Ridomil MZ, Dolomile, Topsin-M, Diesomil, protest and Tahfuz as compared to control, plants sprayed with water. The tested fungicides were sprayed two times with an interval of 15 days and final data was recorded 15 days after last (2^{nd}) spray. The results showed that the mean infected leaves ranged from 4.7-5.9 when the data was recorded before application of fungicides while it was between 2.0-6.4 after two application of tested fungicides. However, significantly maximum reduction % in severity of leaf spot disease was recorded in case of Ridomil MZ (2.8), followed by Dolomile (1.9), Topsin-M (1.7), Diesomil (1.6) and protest and Tahfuz (1.5). Okra plants infected with leaf spot and sprayed with water (control) showed highly significant reduction in plant height (61.53cm) whereas, the tall plants (95.10) were recorded from plot sprayed with Ridomil M Z followed by Dolomile (91.25 cm), Topsin -M (86.60 cm), Diesomil (78.40 cm), Protest (72.75 cm), and Tahfuz (68.38 cm). Significantly lowest number of fruits per plant of okra (10.50) was recorded in control (sprayed with water), while, highest number of fruits per plant (14.90) were obtained from plot treated with Ridomil M Z, followed by Dolomile (13.90), Topsin -M (13.20), Diesomil (12.70), Protest (12.20), and Tahfuz (11.50). The data also showed highly significant difference between treatments for fruit yield of okra infected with leaf spot. The maximum fruit yield (955 kg per acre) was observed in plot treated with Ridomil MZ, followed by Dolomile (845 kg), Topsin-M (790 kg), Diesomil (738 kg), Tahfuz (594 kg) and Protest (681 kg); whereas minimum fruit yield (495 kg) per acre was recorded in plants not sprayed with fungicides. All these findings are close to other researchers viz., Cho & Moon (1980), Werner (1987), Orozco & Mexico (1991), Tohyama *et al*., (1995), Canihos *et al*., (1995), Lagopodi & Thanassoulopoulos (1998), Ghosh *et al*., (2003), Amenduini *et al*., (2003), Khaleaquzzaman *et al*., (2003) Atia & Tohamy (2004), Ayoub & Qureshi (2004), Jiskani (2006), Surviliene *et al*., (2006), Antonijevic *et al*., (2007) and Bulajic *et al*., (2007). In some cases, heavy infection has led to total loss of yield (Rizzolli & Acter, 2006).

Conclusions

It is concluded from present studies that initially light brown spot appeared on the leaves infected with leaf spot disease of okra. These spots turn to concentric dark brown spots varying in size and can spread and cover large areas of infected leaves, which become brown and die. The disease incidence percent ranged between 53.5-61.5% at different locations. The maximum incidence (61.5%) was recorded at Hyderabad, followed by Tando Muhammad Khan (58.4%), Tando Allah Yar (55.1%) and Matyari (53.5%). The fungus, *Alternaria alternata* was isolated and identified as cause of okra leaf spot disease. Disease severity of leaf spot of okra was also estimated during experiment. Severity of leaf spot was significantly reduced in plants sprayed with Ridomil MZ, Dolomile, Topsin-M, Diesomil, protest and Tahfuz as compare to control, plants sprayed with water. The tested fungicides were sprayed two times with an interval of 15 days and final data was recorded 15 days after last (2^{nd}) spray. The results showed that the mean infected leaves ranged from 4.7-5.9 when the data was recorded before application of fungicides. While it was between 2.0-6.4 after two application of tested fungicides. However, significantly maximum reduction % in severity of leaf spot disease was recorded in case of Ridomil MZ (2.8), followed by Dolomile (1.9), Topsin-M (1.7), Diesomil (1.6) and protest and Tahfuz (1.5). Okra plants infected with leaf spot and sprayed with water (control) showed highly significant reduction in plant height (61.53cm) whereas, the tall plants (95.10) were recorded from plot sprayed with Ridomil M Z followed by Dolomile (91.25 cm), Topsin-M (86.60 cm), Diesomil (78.40 cm), Protest (72.75 cm), and Tahfuz (68.38 cm). Significantly lowest number of fruits per plant of okra (10.50) was recorded in control (sprayed with water), while, highest number of fruits per plant (14.90) were obtained from plot treated with Ridomil M Z, followed by Dolomile (13.90), Topsin-M (13.20), Diesomil (12.70), Protest (12.20), and Tahfuz (11.50). The data also showed highly significant difference between treatments for fruit yield of okra infected with leaf spot. The maximum fruit yield (955 kg per acre) was observed in plot treated with Ridomil MZ, followed by Dolomile (845 kg), Topsin-M (790 kg), Diesomil (738 kg), Tahfuz (594 kg) and Protest (681 kg); whereas minimum fruit yield (495 kg) per acre was recorded in plants not sprayed with fungicides.

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