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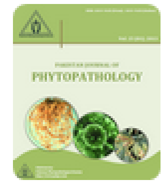

SURVEY, VARIETAL REACTION AND CHEMICAL CONTROL OF STRAWBERRY CHARCOAL ROT IN EGYPT

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A B S T R A C T

A survey was carried out to know the occurrence of charcoal rot disease of strawberry caused by *Macrophomina phaseolina* in different selected locations representing different soil types during the growing season of strawberry in Badr and Kom Hamada Districts in El-Behira governorate, Egypt. Also, the effect of five chemical fungicides at five concentrations was determined *in vitro* and *in vivo* experiments against *M. phaseolina* compared to the control. A total of 69 strawberry samples collected from farmer's fields. Disease incidence (DI) and percentage of disease incidence (PDI) and disease index were recorded. Results revealed that percentage of disease incidence varies among the surveyed locations. *Macrophomina phaseolina* isolates M3 and M4 were the most destructive isolates. Results indicate that the selected Four strawberry cultivars showed different susceptibility to charcoal rot and Festival was the high susceptible cultivar. Our findings revealed that at 100 ppm concentration the most of the fungicides used inhibited the *M. phaseolina* mycelium growth, however the higher rate was recorded with Sendo by 91.95 % followed by 91.12% in Rhizolex-T and the least rate was recorded with Ridomil gold plus by 31.67%. At 200 ppm Rhizolex-T gave the highest inhibition by 96.67%, followed by Sendo and Sandcur by 95.96 and 95.00 % respectively while Ridomil gold plus gave the least inhibition rate by 43.06%.Results illustrated that use of fungicides markedly increased the survival of strawberry plants. The highest plant survival percentage was recorded with Rhizolex-T by 80% followed Sendo by 60%, while least plants survival percentage was recorded with Ridomil gold plus by10% compared with infected untreated control.

Keywords: Distribution, *Macrophomina phaseolin*, Management, Strawberry.

INTRODUCTION

Strawberry (*Fragaria ananassa* Duch.) is an important crop that belongs to family Rosaceae. In the last five years, it put itself as one of the most important economic export crop in Egypt. In 2014, Egypt ranked as 4th globally in term of strawberry quantity and quality. Fresh strawberries are cultivated in three main growing governorates in Egypt: El-Behira, Qalubia and Ismailia. In 2016the cultivated area of strawberry was recorded as 9985 ha producing 4654958 tons (FAO, 2016). Strawberry production can be limited by several soil-borne diseases (Porras *et al*., 2007).

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In Egypt, strawberry is infected by numerous pathogens that lead to its destruction. A huge number of fungal pathogens has been found and isolated from crown and/or root of strawberry including *Cylindrocarpon destructans*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Pythium ultimum, Phytophthora cactorum*, *Phoma exigua* and *Macrophomina phaseolina*(Fang *et al*., 2011a; carter,2016).Charcoal rot in strawberry caused by *M. phaseolina*, is one the most dangerous diseases that infect strawberry is leading to their destruction and becoming problematic in Egypt and worldwide, expecting to be an emerging disease after the phase-out of methyl bromide application (Aviles *et al*., 2008). Charcoal rot was recorded in strawberry-growing farms of other countries such as:Turkey (Benlioglu *et al*., 2004),United States (Mertely *et al*., 2005), Greece (Tjamos *et al*., 2006),Spain (Aviles *et al*., 2008),Italy (Angelini and Faedi, 2010), Argentina (Baino *et al*., 2011); Iran(Sharifi and Mahdavi, 2012)and Australia (Golzar *et al*., 2007; Hutton *et al*.,2013).

Plants affected by charcoal rot suffer from wilting, stunting, drying and death of older leaves often central youngest leaves remaining green and alive. Finally, plants collapsed and die, especially if the plants are exposed to environmental stresses or were bearing a heavy load of fruit. Crowns showed that cortex and internal vascular tissues were dark brown to orange (Mertely *et al*., 2005; Aviles *et al*., 2008, Koike *et al*., 2013). There are many limitation of the charcoal rot control related to the pathogen such as survives and great longevity of the microsclerotia large number in the soil and infected plant debris which resilient resistant structures that allow the fungus to persist in fallow fields for longtime up to15 years (Baird *et al*., 2003).

Also negligence of the crop rotation in the strawberry grown area or cultivated one of the wide host range susceptible crops exceeds over 75 plant families including di-and mono-cotyledons (Dhingra and Sinclair,1978) and highly competitive saprophytic ability (Su *et al*., 2001; Zveibil *et al*., 2012). Methyl bromide (MB) was considered as the most successive option for *M. phaseolina* control but now there is variety of limitation on its use. Different methods were tested for management of charcoal rot of strawberry without MB. Solarization, soil organic amendment, compost and manures were used as eco-friendly control methods instead of chemical fungicides but this will be acceptable economically when it keeps the fungus population at low level (Chamorro *et al*., 2015). Resistant varieties were recommended for high strawberry production and there are different strawberry varieties in Egypt, but unfortunately there is a limited data on resistance of strawberry to the charcoal rot disease. Little previous research reported some differential response between several genotypes to *M. phaseolina* infection (Koike *et al*., 2013; Fang *et al*., 2011; Sanchez *et al*., 2016).In Egypt, there are few fungicides registered for charcoal rot in strawberry and for the consideration of losses and importance of the disease. Thus, it is important to evaluate commercial fungicides and their potency for controlling *M. phaseolina*.

Therefore, the present study was conducted to: 1) survey the charcoal rot disease in different locations in El-Behira governorate in Egypt. 2) Pathogenicity with different *M. phaseolina* isolates, 3) Susceptibility of some commercial strawberry varieties and 4) evaluate the efficacy of five commercial chemical fungicides for inhibiting and control *M. phaseolina in-vitro* and under greenhouse conditions.

MATERIALS AND METHODS

Survey of charcoal rot disease on strawberry in different locations in El-Behira Governorate: A survey was carried out to test the occurrence of charcoal rot diseaseon strawberry plantation in different locations with a different soil types in Badr and Kom Hamada Districts in El-Behira governorate, Egypt. Sixty-Nine samples were collected from different locations during growing season of strawberry. Disease incidence (DI) recorded according to the presence or absence of symptoms on the aerial part and crown of strawberry plants. The percentage of disease incidence (PDI) was calculated according to Reznikov *et al*.,(2018) using the following formula:

$$\text{Percentage of Disease Incidence (PDI)} = \frac{\text{Number of diseased plants}}{\text{Total Number of observed plants}} \times 100$$

Data regarding the soil type in which the crop is grown and the variety of strawberry cultivated was also recorded in the respective surveyed fields.

Isolation, purification and Identification of the causal organism: Naturally infected strawberry plants showing charcoal rot symptoms were collected from different areas of strawberry growing in El-Behira governorate. Plants samples were carefully brought to the laboratory and processed immediately or kept in a refrigerator for further work. Strawberry roots and crown of diseased plants were cut to small pieces, washed thoroughly with running tap water to remove any adhering soil particles, surface sterilized by immersing in 0.25% sodium hypochlorite for 4 minutes, followed by 2 minutes in 70% ethanol then washed several times by sterilized distilled water then blotted between two sterilized filter papers to removing excess water. Sterilized samples were transferred to Petri dishes containing Potato Dextrose Agar medium (PDA) containing penicillin (50 units/ml), 20 ppm Terramycin, and 40 ppm streptomycin sulfate to avoid bacterial contamination. Plates were incubated in darkness at 28 ± 2ºC for 5 days and examined daily for the occurrence of fungal growth. The growing fungi were microscopically examined and then purified using the single spore and hyphal tip-technique (Dhingra and Sinclair 1977) and transferred to new PDA plates.

Identification of *M. phaseolina* Isolates: The pure cultures of the isolated fungi were examined microscopically and identified at the Agricultural Botany Department, Faculty of Agriculture, Menoufia University according to their features described by Dhingra and Sinclair (1978) and Watanabe (2010). Pure cultures of the isolates were multiplied and maintained on fresh PDA slants and kept at 5°c for further studies.

Pathogenicity test: Seven representative isolates of *M. phaseolina* of different localities were evaluated for their pathogenicity to the commercially grown susceptible strawberry cultivar Festival. Inoculum were prepared using barley grains medium (500 ml glass bottles each containing 75 gm barley grains along with 25 gm sand and 100 ml water were autoclaved for 20 minutes at 121°C). Bottles were inoculated and incubated for 15 days at 25°C. Inoculum at 3% of soil weight were mixed thoroughly with sterilized sandy soil, and then potted in sterilized clay pots (30 cm in diameter).Two strawberry plants were transplanted in 30cm dim pots. Pots containing sterilized sandy soil only were used as a control. Five replicates used for each treatment. Plants watered, and agricultural practices done as needed (Gammon, 1972). Thirty days after transplanting, disease incidence was recorded as numbers and percentages of plants initially showing signs of water stress and subsequently collapse and number of survived plants. At the end of the experiment re-isolation of the fungus was done to confirm the association of the fungi with current disease symptoms.

Susceptibility of some strawberry cultivars to *M. phaseolina:* The susceptibility of Four of the most common wide grown cultivars: Festival, winter star, Fortuna and 029 to the most virulent isolate of *M. phaseolina* were evaluated by artificial inoculation under greenhouse conditions. The inoculum were prepared using barley grains medium as above. Plants transplanted in pots filled with soil free of inoculum served as control. Ten replicates were used for each particular treatment. Plants were watered and fertilized with a nutrient solution (Gammon, 1972) as needed. Thirty days after transplanting, disease incidence, collapse and number of survival plants was recorded.

Chemical control: *In-vitro* The experiment was conducted at the Modern Agriculture Company (PICO) lab, Beheria governorate, Egypt. The potency of five different fungicides belonging to diverse chemical groups as illustrated in (Table 1) were used to determine *M. phaseolina* mycelial growth in vitro using PDA medium following the technique described by Tonin *et al*., (2013). The sensitivity of *M. phaseolina* mycelium growth to the five fungicides conducted using one strain isolated from strawberry plants crown and roots collected previously in the survey from Beheria governorate, Egypt. The concentrations of 5 ppm, 10 ppm, 50 ppm, 100 ppm and 200 ppm of each fungicide active ingredient were used in the bioassay while, control treatments were substituted with distilled water. One gm of each fungicide was transferred, to a flask containing 1000 mL final volume of distilled sterile water (stock suspension). For dilution, volumes from the stock suspension according to the needed concentrations adds to 100 mL PDA supplemented with 150 mg of streptomycin sulphate to avoid bacterial contamination growth. Concentrations of each active ingredient were prepared from the stock suspension based on the formula as fallow:

$C1 \times V1 = C2 \times V2$

C1 = stock solution concentration.

V1 = volume needed from stock solution.

C2 = desired final concentration.

V2 = desired volume for the final solution.

Then flasks containing PDA gently shaken and the medium poured into sterilized 90 mm Petri dishes. Then 5.0 mm diameter disks of seven days mycelial growth *M. phaseolina* were cultured in the center of each poured poisoned PDA dish. Each treatment repeated four times. Then plates incubated at 27 ± 2ºC and growth was observed on daily basis. When the control plates covered by fungal mycelium, the mycelium growth of *M. phaseolina* was measured and Inhibition of mycelial growth was calculated using the following formula:

$$\text{Inhibition of Mycelium Growth (IMG) \%} = \frac{A - T}{A} \times 100$$

A= Mycelium growth in control, T= Mycelium growth in treatment.

Table 1. Trade name, active ingredients and chemical group of used Fungicides.

Trade name	Active Ingredient (AI)	Chemical group	Used dose
Sendo	Carbendazim 50%	Benzimidazol	1.0 g/L
Moncut	Flutolanil 25%	Carboxamide	2.5g/L
Sand Cure	Mancozeb 64% + Metalaxyl 8%	Dithiocarbamate + Phenylamideacylalanine	1.5 g/L
Rhizolex-T	Tollofes-methyl	Chlorlnitro phenyl + Dimethyl dithiocarbamate	2.0 g/L
Ridomilgold Plus	Mefenoxam 2.5%	Acylalanine	2.0 g/L

Under greenhouse conditions: Five fungicides, i.e., Sendo, Moncut, sand cure, Rhizolex-T and Ridomil gold pluswere evaluated for controlling the *M. phaseolina* in strawberry cv. Festival. All these fungicides were added as soil drench at dose shown in (Table 1). Ten potted strawberry transplanting were soil drenched with the selected five fungicides concentrations. Plants inoculated by *M. phaseolina* served as a negative control. The positive control treatment was simply watered. Plants kept in the greenhouse and treatments repeated two weeks in intervals. Plants were irrigated and fertilized regularly. Disease initial symptom, collapse, survival plants and growth parameters were calculated at 30 day after transplant.

STATISTICAL ANALYSIS

Data was statistically analyzed using a computer costat 6.3 version program. Analysis of variance (ANOVA) was carried out and comparisons of means at the 5% level of significance.

RESULTS

Survey of charcoal rot disease in different locations in El-Behira Governorate: Strawberry plants are infected by the charcoal rot when showing the initial symptoms of charcoal rot which usually occur after the plants are well established and begin to produce fruits: the older leaves wilt, turn grayish green in color, and begin to dry up as in (Figure 1A) compared with the healthy plants. As disease progresses, virtually all of the foliage collapsed and dried up with the exception of the central youngest leaves. Histological symptoms of the infected plants showed that plant crowns internal tissues, vascular and cortical tissues were dark brown to orange brown in colour, (Figure 1B).

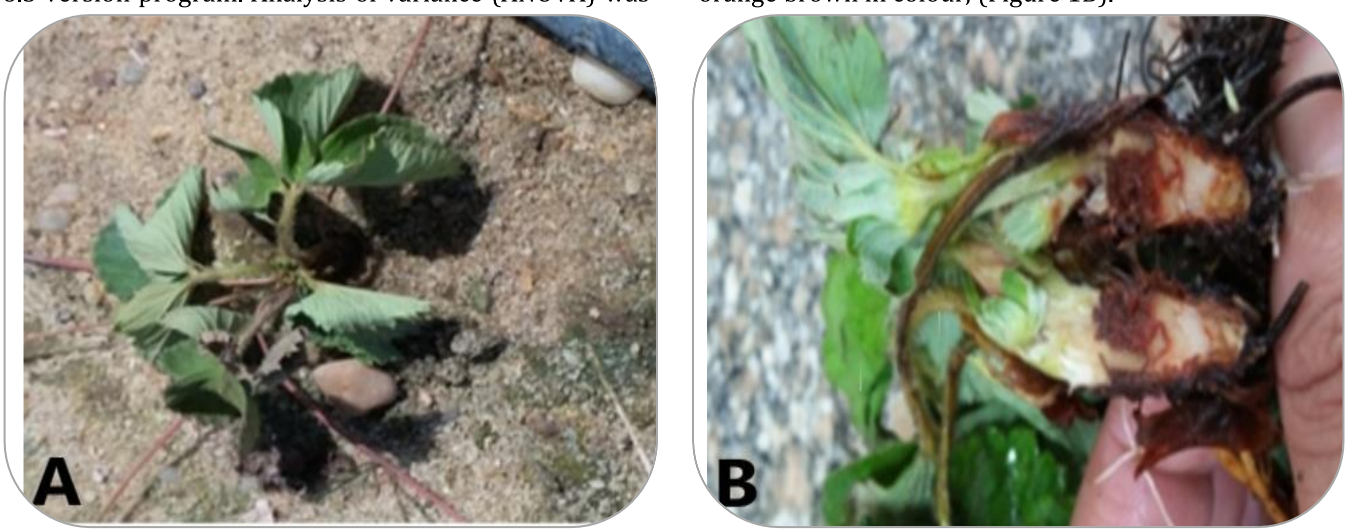

Figure 1. Symptoms of charcoal rot in strawberry infected with *M. phaseolina* .A) Morphological and Initial symptoms, B) Strawberry crown tissue show a dark to orange brown discoloration.

The survey was carried out in the selected locations to record the occurrence of charcoal rot disease during the growing season of strawberry. Results revealed that the charcoal rot was recorded in 38 of 69 samples collected from the survey locations in El-Behira Governorate as presented in (Figure 2). The PDI varied up to 30% from different locations. Disease incidence was recorded in all samples of Festival cultivar in all surveyed locations in both of clay and sandy soil. The most affected locations in Badr was Airport Area and Abdel hamed zedan by 30 % of disease occurrence followed by El Magd location by 28% while the disease was not recorded in samples from Ali Ebn AbyTaleb and El-Fateeh as shown in (Table 2).The highest PDI were recorded Kom Sherek and Abo El Khawey by 30% followed by 22% in Kafr Zyada location in Kom Hamada District as illustrated in (Table 3).

Table 2. Survey of charcoal rot disease in Badr District, El-Behira Governorate.

Location	No	Cultivar	Soil Type	DI	PDI
Ezz El-din	1	Festival	Clay	+	6
	2	Fortuna	Clay	-	0
El-Khofog	1	Festival	Clay	+	3
Om Saber	1	029	Sandy	+	9
	2	Fortuna	Sandy	+	5
	3	Winter Star	Sandy	+	11
	4	Festival	Sandy	+	14.5
	5	Fortuna	Sandy	-	0
Manshetnaser	1	Festival	Sandy	+	8
	2	Fortuna	Sandy	-	0
Omar Shahen	1	Festival	Sandy	+	13.5
	2	Winter Star	Sandy	+	7
	3	Fortuna	Sandy	-	0
Omar Makram	1	Florida	Sandy	+	11
	2	029	Sandy	-	0
	3	Fortuna	Sandy	-	0
Salah El-din	1	Festival	Sandy	+	13
	2	Fortuna	Sandy	-	0
	3	Florida	Sandy	-	0
AbdelmegedMoorsy	1	Festival	Sandy	+	12
	2	Fortuna	Sandy	+	11
	3	Florida	Sandy	-	0
	4	Winter Star	Sandy	+	5
Badr	1	Florida	Sandy	+	22
	2	Fortuna	Sandy	-	0
Ali EbnAbyTaleb	1	029	Sandy	-	0
AdelsalaamAref	1	Festival	Sandy	+	17.5
El-Fateeh	1	Fortuna	Sandy	-	0
Baghdad	1	Winter Star	Sandy	+	15
Abo Bakr el Sedek	1	Festival	Sandy	+	18
	2	029	Sandy	-	0
Center 2	1	Festival	Sandy	+	20
	2	Winter Star	Sandy	-	0
Osman EbnAfan	1	029	Sandy	+	8.7
El khartom	1	Winter Star	Sandy	+	20
El khartom	1	Winter Star	Sandy	+	20
El magd	1	Festival	Sandy	+	28
	2	Fortuna	Sandy	-	0
Airport Area	1	Festival	Sandy	+	30
	2	Festival	Sandy	-	0
	3	Florida	Sandy	-	0
Abdel hamedzedan	1	Festival	Sandy	+	30
	2	Winter Star	Sandy	-	0
Ahmed Orabe	1	Winter Star	Sandy	+	25
Nabil El Waqad	1	Fortuna	Sandy	+	15
	2	029	Sandy	-	0
Khaled EbnElwaled	1	Fortuna	Sandy	+	15
	2	029	Sandy	-	0
	3	Florida	Sandy	-	0
Mobarak	1	Florida	Sandy	+	10

DI=Disease incidence. + = Symptoms present and positive infection, - = Symptoms absent and Negative infection. PDI = Percentage of Disease Incidence.

Table 3. Survey of charcoal rot disease in Kom Hamada District, El-Behira Governorate.

Location	No	Cultivar	Soil Type	DI	PDI
Alkam	1	Festival	Clay	+	15
	2	Fortuna	Clay	-	0
Maghnen	1	Festival	Clay	+	25
Komsherek	1	Festival	Clay	+	30
	2	Fortuna	Clay	-	0
Abo el khawey	1	Festival	Clay	+	30
	2	Winter Star	Clay	-	0
	3	Fortuna	Sandy	-	0
	4	Florida	Sandy	-	0
El Tayarya	1	029	Sandy	+	10
	2	Fortuna	Sandy	-	0
El Tayary El balad	1	Festival	Sandy	-	15
	2	Winter Star	Sandy	-	0
	3	Florida	Sandy	-	0
KafrZyada	1	Festival	Clay	+	22
EL Zafrn	1	Festival	Clay	+	17
	2	Winter Star	Clay	-	0
Waqed	1	Winter Star	Clay	+	22
Manshit Abo Raya	1	Festival	Sandy	+	20

DI=Disease incidence. + = Symptoms present and positive infection, - = Symptoms absent and Negative infection. PDI = Percentage of Disease Incidence.

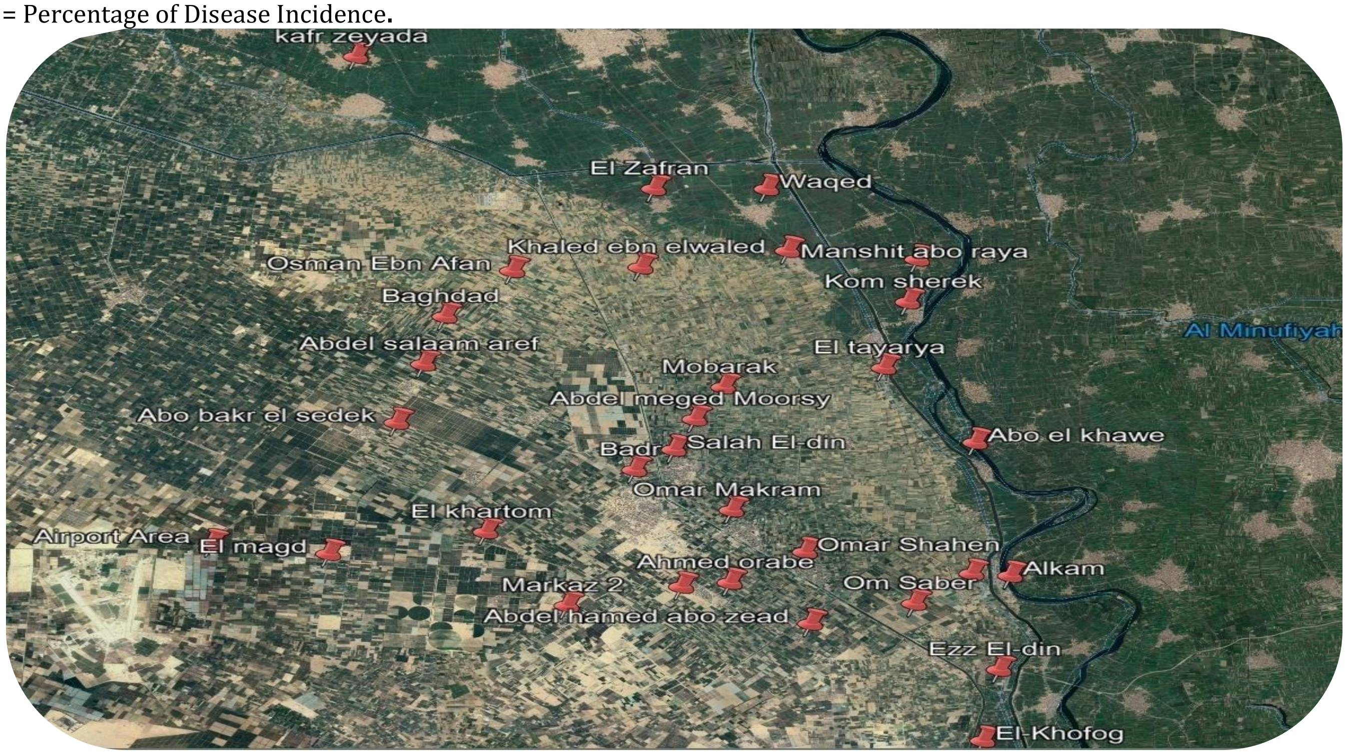

Figure 2. Occurrence of *M. phaseolina* in strawberry production locations in different regions in El-Beheira Governorate

Isolation of *M. phaseolina* from infected strawberry plants: Data presented in (Table 4) indicated that *M. phaseolina* was the main isolated pathogen from charcoal rot infected plant materials from strawberry plants of different growing areas. Other isolated pathogenic and non-pathogenic fungi were discarded according to the microscopic examination. Seven isolates of *M.phaseolina* coded as M1-M7 were isolated from seven grown locaties in El-Behira governorate. The fungus was isolated from different strawberry cultivars,

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Festival cultivar was the most dominant one followed by Winter star, Florida and 029 cultivars.

Table 4. Isolates of *M. phaseolina* isolated from different area in El-Behira governorate from different strawberry cultivars.

Isolate	code	District	Location	Cultivar
1	M 1	Badr	Om saber	Festival
2	M 2	Kom Hamada	KomSherek	Festival
3	M 3	Badr	AbdelmegedMorsy	Festival
4	M 4	Kom Hamada	Waqed	Winter star
5	M 5	Badr	Center 2	Festival
6	M 6	Badr	Airport Area	029
7	M7	Badr	Mubarak	Florida

Pathogenicity test: Data presented indicate that, significant differences were recorded between all tested isolates in disease parameters and in their ability to cause plant death on the tested cultivar as compared to control treatment.

The virulence of the tested *M. phaseolina* isolates was significantly varied in inducing charcoal rot disease severity. The most pathogenic Isolates were M3 and M4 as they produced the highest percentage of infected plants (100 %)followed by isolates M2 and M6 by91.6 %,while the isolate M5 was the least pathogenicas illustrated in (Figure 3). Control plants remained symptomless. According to pointed out the results the selected isolates could be separated in different groups concerning to its pathogenicity i.e.:Moderately pathogenic isolates (M5) highly pathogenic isolates (M1and M7), aggressive isolates (M2and M3) and destructive isolates (M3 and M4). *Macrophomina phaseolina* was re-isolated from all plants showing symptoms.

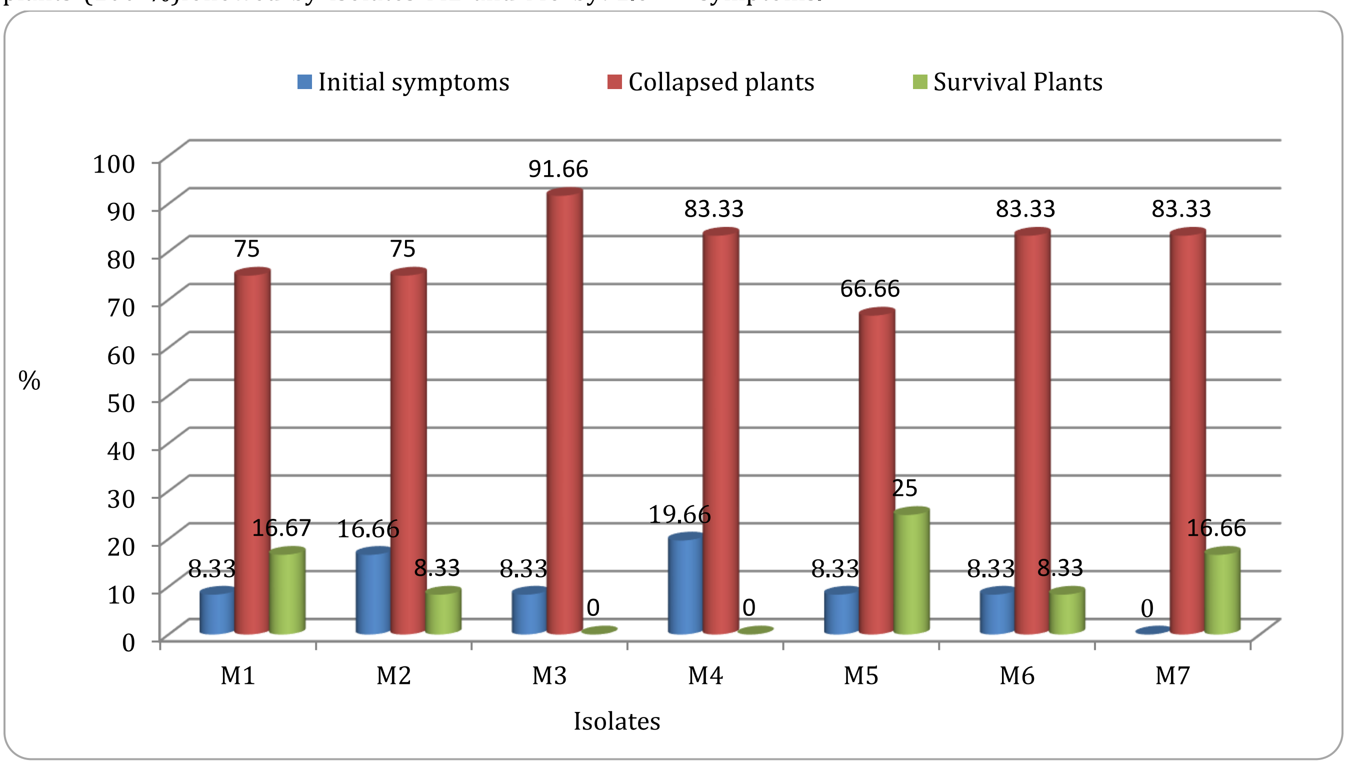

Figure 3. Pathogenicity test of *M. phaseolina* fungi on strawberry plant CV. Festival under greenhouse conditions.

Susceptibility of some strawberry cultivars to *M. phaseolina*.: *Macrophomina phaseolina* isolate M3 in relation to strawberry cultivars i.e. festival, winter star, 029 and Fortuna was evaluated. Results were concluded that there was a significant difference between the different cultivars. The most susceptible cultivar with 95%of disease severity followed by Winter Star by 80% and Fortuna by 65%. The least susceptible one to charcoal rot was 029 (30%) as showed in (Figure 4).

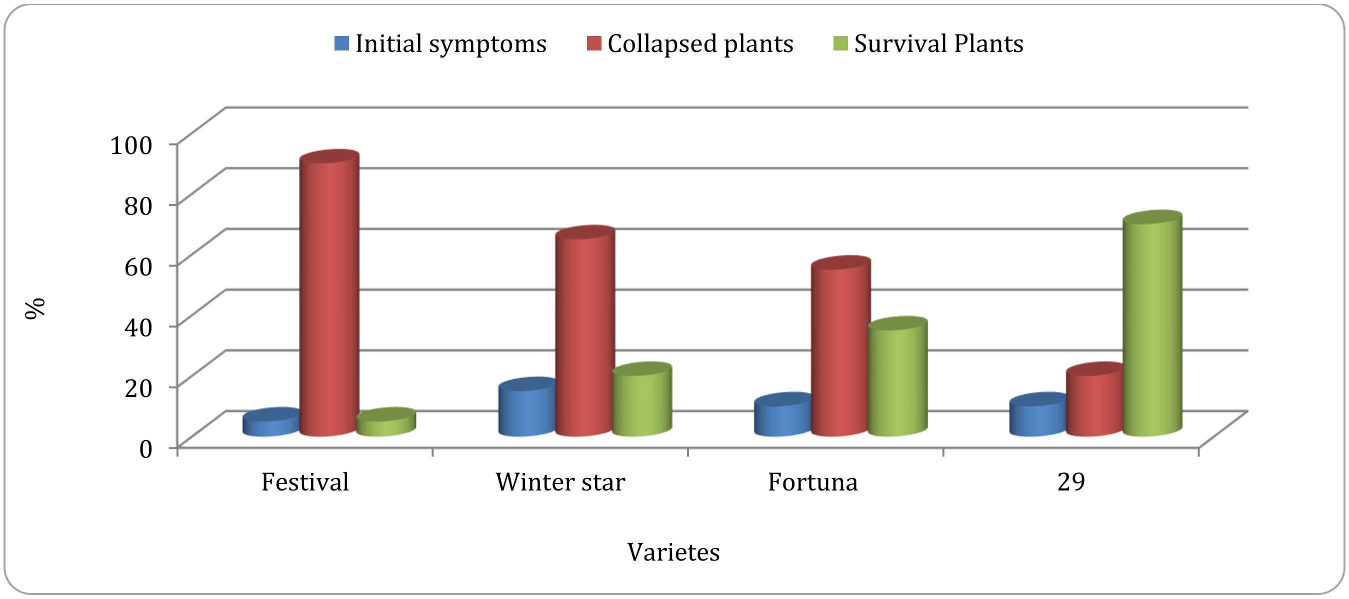

Figure 4. Susceptibility of strawberry varieties to *M. phaseolina* under greenhouse conditions.

Chemical control: *In vitro* Results presented in (Table 5) showed significant effect of the used fungicides in reducing the mycelia radial growth of *M. phaseolina* comparing with untreated control. Data clarify that *M. phaseolina* varied in their sensitivity against the fungicide tested (Sendo, Moncut, Sandcure, Rhizolex-T, Ridomil gold plus). In general, toxicity was gradually increased by increasing in concentration of the fungicide. The toxic effect on fungal mycelial growthwas observed at 5 ppm with Rhizolex-T and then with Sendo, Moncut, Sandcure while Ridomil gold plus was not effective at this concentration. At 10 ppm a clear inhibition of mycelialradial growth was found with Sendo, Sandcure, Rhizolex-T, then with Moncut, while Ridomil gold plus did not show any toxic effect.At 50 ppm of concentration, Sendo gave the highest percentage of mycelial growth inhibition (87.23%) followed by Rhizolex-T as it inhibit the mycelium growth by 75.5% while, Ridomil gold plus did not show any inhibition effect. At a concentration of 100 ppm all the fungicides used showed inhibition of *M. phaseolina* mycelial growth but at different rates, 91.95 % was recorded with Sendo and 91.12% with Rhizolex-T but the least effect was recorded with Ridomil gold plus by 31.67%of mycelial growth inhibition.In the plates treated with 200 ppm of the tested fungicides, Rhizolex-T gave the highest inhibition rate by 96.67%,followed by Sendo and Sandcur by 95.96 and 95.00%, respectively, while Ridomil gold plus gave the least inhibition rate by 43.06%as show in (Figure 5).

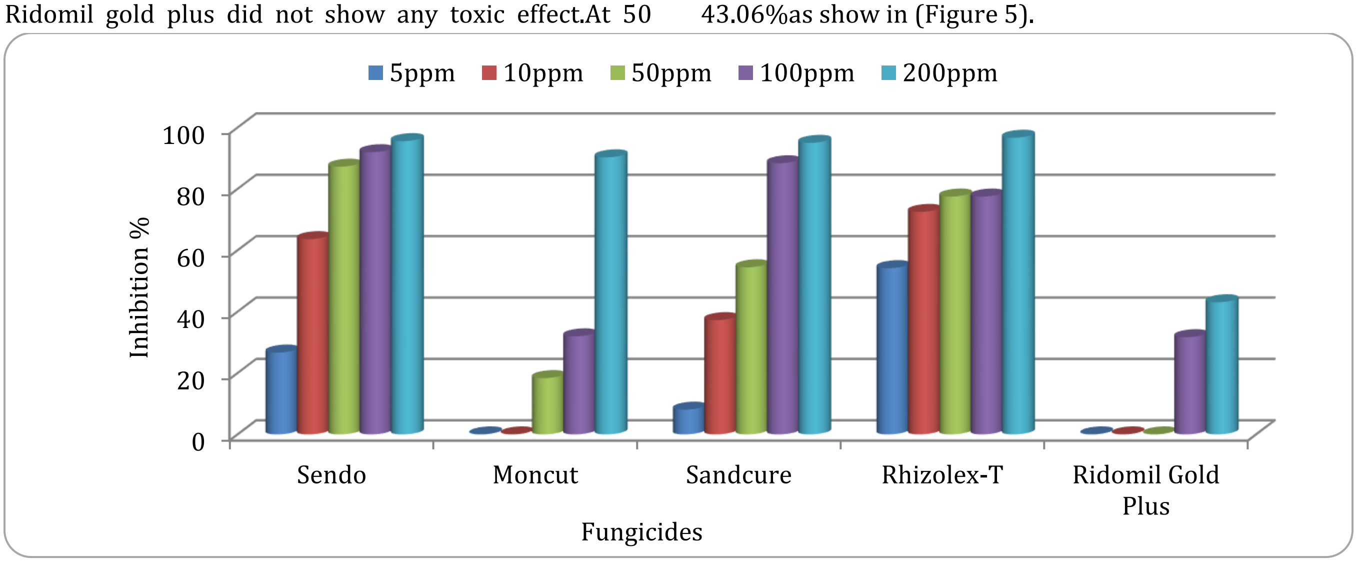

Figure 5. Inhibition percentage of *M. phaseolina* mycelium growth by different concentrations of different fungicides.

Table 5. Effect of five fungicides with different concentration on the growth of *M. phaseolinain vitro*.

Fungicide	ppm	Colony diameter(mm)	Growth inhibition (%)
Sendo	5	80.775a	26.67
	10	30.40h	63.62
	50	10.15 k	87.23
	100	00.725 i	91.95
	200	00.40m	95.56
Mon Cut	5	90.00a	00.00
	10	90.00a	00.00
	50	70.35c	18.34
	100	60.325d	31.95
	200	0.875 kl	90.28
Sand cure	5	80.275b	8.06
	10	50.875e	37.23
	50	40.10g	54.45
	100	10.05 kl	88.34
	200	00.45 m	95.00
Rhizolex-T	5	40.225g	54.17
	10	20.675 i	72.50
	50	20.025 j	77.50
	100	00.80 i	77.50
	200	00.30 m	96.67
Ridomil gold plus	5	90.00a	00.00
	10	90.00a	00.00
	50	90.00a	00.00
	100	50.125f	31.67
	200	60.15d	43.06
Control		90.00a	00.00
L.S.D 0.05		0.264	

Means followed by the same letter in each column are not significantly different.

Under greenhouse conditions: Results illustrated in (Figure 6)revealed that, fungicides, i.e. Sendo, MonCut, sand cure, Rhizolex-T and Ridomil gold plus as a drenching soil at the used dose decreased the disease parameters and increased the survival of strawberry plants in pots infested by *M. phaseolina* in comparing to control treatment. Data illustrated that using the fungicides markedly inhibited charcoal rot and increased the plant survival. Rhizolex-T was the most effective fungicide tested giving 80% plant survival, followed by sendo 60% survival plants, while the least effective was Ridomil gold plus with 10% plant survival.

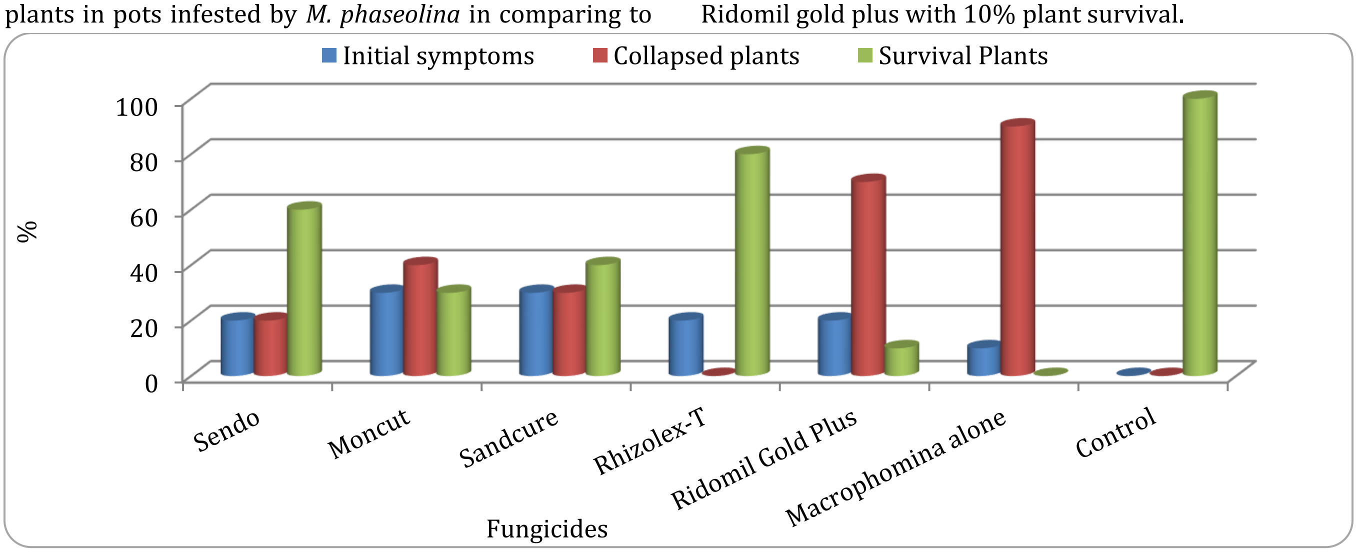

Figure 6. Effect of some fungicides on the incidence of Initial symptoms and Collapse of Plant.

DISCUSSION

Charcoal-rot disease caused by *M. phaseolina*, is one of the most destructive soil borne diseases of strawberry all over the world. Throughout the survey, incidence of charcoal rot was reported in the strawberry fields and the incidence was varies in production districts. The disease development generally may be enhanced by some combination of heat stress, irrigation water deficiency, light-textured soil, or the stress associated with host reproduction (Mihail, 1989).High temperatures seasons create a suitable condition for *M. phaseolina* development and multiplication, especially in the intensive agricultural system and this confirms the presence of M. phaseolina during late spring/early summer when the temperatures almost above 30°C (Fang et al., 2011). In the present study *M. phaseolina* was isolated from the collected strawberry samples and the aggressiveness of the fungus isolates in commercial strawberry cultivars showed significant differences among all tested isolates in disease severity. The most virulent Isolates were M3 and M4 and the last one was M5. We were grouping the isolates according to isolates pathogenicity to moderately pathogenic, highly pathogenic, severe and destructive isolates. The variation among *M. phaseolina* isolates pathogenicity can be referred to the high genetic diversity and the diversity in the genotypes can refer to a phenotypes differences between these isolates, and therefore affect their virulence (Sánchez *et al*., 2016).

Our results revealed that the tested strawberry cultivars differed in their susceptibility to the infection with, *M. phaseolina.* Festival was the most susceptibile to charcoal rot followed by Winter Star and Fortuna while 029was the least susceptible one. Similar results previously obtained by Fang *et al*., (2011), who found that cultivar Festival was susceptible to *M. phaseolina*. The varieties of strawberry show different susceptibility to crown and root rot caused by *M. phaseolina* affected by differed in their factors such as, differences in the pathogenicity of the fungus isolate and plant genotype.(Avilés, *et al*.,2008; S´anchez *et al*., 2016).

Machrophomina phaseolina showed various sensitivity to the tested chemicals fungicide. All chemical fungicides inhibit the growth of *M. phaseolina in vitro* but the rate of inhibition was different among the selected fungicides. The fungus genetics can affect its sensitivity toward the fungicides as a sensitive or insensitive to a chemical molecule. The sensitivity means fungi toxicity but insensitivity means as no fungal toxicity (Reis *et al*. 2010). Also the differences in the chemical group and the active ingredient of the used fungicides are much supported for the potential variation.

The obtained results concerning the efficacy of the tested fungicides against the fungus *M. phaseolina in vitro* and *in vivo* are in the same line with previous researchers (Khalikar *et al*., 2011; Thombre and Thombre, 2018). Our findings are in agreement with those obtained by Tonin *et al*., (2013) and Parmar *et al.,* (2017) as they found that carbendazim was the most efficient fungicide in inhibition the mycelia radial growth of *M. phaseolina,* while the fungus was insensitive to the active ingredients of Metalaxyl. It was acceptable that not all chemicals are causing toxicity to fungi and therefore there is no fungicide control all fungi (Reis *et al*., 2010).

CONCLUSION

It is urgently important to know the distribution of *M. phaseolina* in the grown strawberry areas. Moreover, knowledge about the nature of strawberry commercial varieties susceptibility for use in wide cultivation area and looking forward to find new more resistant varieties to *M. phaseolina*. Exploration of the effective fungicides chemical ingredient will provide information that can be involved in future planning programs for charcoal rot disease management.

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