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Resistance of Sunflower to Damping-off and Charcoal-Rot Diseases Caused by *Fusarium oxysporum* and *Macrophomina phaseolina* in Egypt

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GIZA-1 and Miak cultivars were resistant to *M. phaseolina* (Tassi) Gold whereas Import 61 was the most resistant cultivar to *F, oxysporum* Schlecht. However, Hybrid 894 and Hybrid 8941 were susceptible to both fungi.

Disease incidence increased by the increase in both depth of sowing and intervals of irrigation especially with H. 894 and H. 8941 as regards *M. phaseolina* and with shortening irrigation intervals with *F. oxysporum*. On the other hand Giza-1 was the least affected cultivar. As regards yield and yield components, Miak together with Giza-1 could be recommended for cultivation in A.R.E. for oil and other purposes.

The whole plant extract media of H. 8941 and H. 894 were the best media for the linear growth of *M. phaseolina* while that of Giza-1 was more favourable for growth of *F. oxysporum*.

Fungal filtrates reduced the germination of seeds of all tested sunflower cultivars especially by agening of filtrates However, Giza-1 was less affected in contrast with H.894. Similar results were obtained with seedlings.

Six sunflower cultivars were tested for selecting the cultivars resistant to damping-off and charcoal-rot together with the highest yield and yield components. The success in selecting a variety or more which give high yields together with resisting the disease is very important.

The aim of this investigation also included the effect of depth of sowing and irrigation intervals on sunflower susceptibility to infection. Also the effect of filtrates of both fungi on seed germination and wilt of seedlings of all the tested cultivars was studied.

Materials and Methods

Experiments were carried out in the laboratory and greenhouse of the Faculty of Agriculture, Moshtohor, Zagazig University, Kalubia.

Field experiments were carried out in a field naturally infested with *M. phaseolina* at the Experimental Farm of the Faculty of Agriculture, Assiut University, A.R.E. in the seasons of 1978 and 1979.

The pathogenic fungi causing damping-off and charcoal - rot were isolated from diseased sunflower seedlings or stems of different varieties by hyphal tip or single spore technique. Isolates were identified by the Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey, England or by the Plant Pathology Department, Pennsylvania State University, U.S.A.

Different sunflower cultivars, *i.e.* Giza-1, Miak, Import 61, aswell as Hybrids 894 (H. 894), Hybrid 8941 (H. 8941) and Hybrid 896 (H. 896) were tested to study their susceptibility to infection with *M. phaseolina* and *F. oxysporum.*

Greenhouse experiments

Soil was infested with each fungus grown on sand sorghum grain medium (Whitehead, 1957), at the rate of 5% of soil weight. The infested soil was potted in clay pots, 25 cm in diameter at the rate of 5 kg soil per pot. Ten surface sterilized healthy seeds of each variety were sown per pot. Five pots were used for each particular treatment. A set of five pots filled with uninfested soil was used for each variety as control. Determination of disease severity was carried out 2 to 4 weeks from sowing by estimating the percentages of both pre-and post-emergence damping-off. The number of healthy plants was also recorded 6 or 8 weeks after sowing for *F. oxysporum* and *M. phaseolina* treatments, respectively.

Irrigation and depth sowing

A split plot experiment was carried out using pots filled with soil infested with either *F. oxysporum* or *M. phaseolina* or uninfested. The main plots concerned the irrigation intervals, *i.e.* one day, two and four days. The split plots represented the depth of sowing of 2, 4 and 6 cm from surface. The numbers of pre-and post-emergence dead seedlings and healthy survived plants were recorded in all treatments as before.

Field experiments

These experiments were carried out in a field heavily infested with *M. phaseolina* in Assiut. Acomplete randomized block design experiment was performed to study both the susceptibility to infection and yield components

of the aforementioned six cultivars sunflower. Five replicated plots, 1/400 fed each with four 4-m long rows, represented each treatment. Five healthy sterilized seeds were sown in hills 35 cm apart. Plants were thinned to one plant per hill one month after sowing.

Fertilizers were applied at the rate of 136 kg ammonium sulphate, 129 kg calcium superphosphate and 31kg potassium sulphate per feddan as base fertilizers. Half the amounts was added after sowing and the other half was added 30 days later, after thinning and before the second irrigation. Plants were irrigated at 15 days intervals.

Determination of charcoal-rot disease severity was carried out 8 weeks after sowing, at the maturity stage as described by Kraeva (1960) and Korshiunova *et al.* (1966). Yield components were recorded at the end of the experiment as follows :

1. Seed yield per plant, weight of 1000 seeds and number of seeds in 100g.

2. Percentage of oil content was determined after extraction according to the method recommended by the A.O.A.C. (1950)and oil yield per plant was calculated by multiplying the seed yield per plant by it's oil content.

Extracted media from plant parts

Extracts of plant parts at different vegetative stages, *i.e.* 15,30 and 45 days old, were prepared by boiling 200 g of roots, shoots or whole plant in enough water for 20 min, filtered through muslin and then water was added to the extract to make up one litre then 15g agar were added. This medium was first placed in test tubes (12 ml each), autoclaved, poured in a sterilized Petri dish and used for determining the rate of growth of any of the pathogens.

Fungal filtrates

The effect of fungal filtrates on seed germination and the appearance of disease symptoms on the seedlings were studied, as follows :

(*A*) *Effect on seed germination*

F. oxysporum and *M. phaseolina* were grown on Czapek's liquid medium at 27C for 15, 30 and 45 days. The filtrates of each were obtained by filtering through Seitz-filter. Sunflower seeds were germinated in Petri dishes, ten seeds/dish, which was lined with a filter paper and 10 ml of the filtrate of each fungus were poured per dish (Ashour *et al.* 1978). For control, the same procedure was used except that 10 ml of sterilized Czapek's liquid medium were used. Ten replicated dishes were used for each treatment. The number of germinated seeds was recorded in all treatments after five days.

(B) Effect on seedings

The filtrates were obtained as before and the technique recommended by Mathur (1968) was used. Ten healthy seedlings, 15 days old, of each cultivar were immersed in 20 ml of each filtrate in a sample glass tube, 4.5 x 20 cm. Five tubes were used for each treatment. Sterilized medium was used as control. Symptoms expression as a result of the above treatments was recorded after incubation at 27C for 2 to 7 days.

Results and Discussion

Disease resistance

(A) Greenhouse experiments

1. *Screening of sunflower cultivars*

It is clearly shown from data illustrated in Table 1 that the tested cultivars greatly differed in their susceptibility to infection with both fungi. Giza-1 and Miak were the least susceptible to *M. phaseolina* whereas Import 61 was more resistant to *F. oxysporum*. On the other hand, the two cultivars H. 894 and H. 8941 were highly susceptible to both fungi.

TABLE 1. Resistance of six sunflower cultivars to *M. phaseolina* and *F. oxysporum* infection

Cultivar	*M. phaseolina*						*F. oxysporum*					
	Damping-off				Survival healthy plants. (1)		Damping - off				Survival healthy plants. (2)	
	Pre		Post				Pre		Post			
	1978	1979	1978	1979	1978	1979	1978	1979	1978	1979	1978	1979
Giza-1	10.0	7.5	22.2	21.7	67.5	72.5	22.5	25.0	38.9	39.8	42.5	45.0
Miak	12.5	7.5	20.1	19.3	70.0	75.0	20.0	22.5	43.8	42.0	42.5	45.0
Import 61	15.0	5.0	23.6	21.8	62.5	72.5	20.0	22.5	31.3	32.2	50.0	52.5
H- 896	20.0	15.0	25.0	23.6	60.0	65.0	27.5	27.5	31.3	31.3	50.0	50.0
H- 894	25.0	25.0	34.0	34.0	50.0	50.0	35.0	37.5	50.6	48.2	30.0	32.5
H- 8941	25.0	12.5	26.8	23.4	55.0	67.5	32.5	37.5	45.3	48.8	37.5	32.5
L S.D. 05	4.3	N.S.	4.5	5.6	4.9	N.S.	4.1	5.2	7.9	N.S	7.0	7.4

(1) = Survival healthy plants after 8 weeks
(2) = Survival healthy plants after 6 weeks

2. *Effect of watering intervals and depth of sowing on disease incidence*

Data on the effect of irrigation intervals and depth of sowing on disease incidence indicate the following :

A. As regards *M. phaseolina* , data clearly show that both pre-and post-emergence damping-off increased by increasing the depth of sowing and the intervals of irrigation. H. 894 and H. 8941 were much affected by increasing the depth of sowing and irrigation intervals, whereas Giza-1 was less affected.

B. As regards *F. oxysporum*, results clearly indicate that the percentages of pre-and post-emergence damping-off increased by increasing the depth of sowing. On the other hand, the disease percentage decreased with shortening the intervals of irrigation. These results are in agreement with those of Fahmy (1928), Fikry (1932) , Dastur *et al.* (1960) and Chakrabarti and Basuchandhary (1978) reported that Fusarium wilt of many host plants developed more rapidly in moist weather. On the other hand, the results as regards *M. phaseolina* agree with Crandall (1954),who concluded that charcoal-rot of soybean was most serious in seasons when the rainfall was below normal.

The results of pre-and post-emergence phases as regards the depth of sowing are in agreement with those of Secker (1946), Enrico and Krag (1971) and Crossan. (1965).

(*B*) *Field experiment*

The aforementioned six cultivars of sunflower were sown in a naturally infested field with *M. phaseolina.* The data of varietal resistance in Table 2a prove that H. 896 was the most susceptible cultivar followed by H. 8941 in both seasons. Whereas Giza-1 was more resistant to infection with *M. phaseolina* in both seasons. Miak came next to it in season 1978. These results are in agreement with those obtained in greenhouse experiments. Generally disease incidence was lesser in 1978 season than in 1979 season which may be due to climatic factors.

Data of yield components in Table 2b were obtained during 1979 season only. Data clearly show that Giza - 1 is the cultivar most resistant to the disease which also gave the best results as the yield of seeds, the weight of 1000 seeds , the number of seeds in 100 g and head diameter, however, it gave the lowest percentage of oil in seeds.

The best yield of oil per plant was obtained by Miak which ranks the second to Giza-1 in seed yield per plant , weight of 1000 seeds number of seeds in 100g and head diameter. Thus, Miak together with Giza-1 could be recommended for cultivation in A.R.E. for oil and other purposes.

TABLE 2-a. Percentages of pre-emergence damping-off and disease severity of different sunflower cultivars in 1978 and 1979 seasons

Cultivar	Percentage of pre-damping-off after 15 days of sowing		Percentage of diseased plants at maturity	
	78	79	78	79
Giza 1	4.0	8.3	11.0	17.6
Miak	4.3	9.7	12.0	46.0
Import 61	5.0	12.0	15.6	44.6
H. 896	7.4	27.6	27.6	67.6
H. 894	5.8	14.8	21.9	23.5
H. 8941	6.3	18.1	26.1	43.5
L.S.D. at 5 %	1.1	2.0	4.6	3.3

Table 2-b. Mean values of yield components of different sunflower cultivars in 1979.

Cultivars	Seed yield/ pl. (g)	Oil %	Oil yield/ pl. (g)	Wt. of 1000 seed (g)	No. of seeds in 100 g	Head diameter (cm)
Giza 1 . . .	91.9	29.12	26.77	84.0	1192.2	21.0
Miak . . .	83.6	39.78	33.27	82.8	1209.4	18.8
Import 61. .	76.3	39.48	30.11	58.5	1712.7	17.8
H. 896 . . .	55.0	45.88	25.19	53.1	1885.8	15.5
H. 894 . . .	60.5	47.86	28.92	57.0	1773.0	18.0
H. 8941 . .	59.0	47.87	28.20	54.6	1837.5	18.0
L.S.D. at 5 %	4.6	3.9	2.4	6.3	179.1	0.8

B- Physiological studies

1. *Effect of plant parts extract media on linear growth of M. phaseolina and F. oxysporum*

Stem extract medium of H. 894, was the best medium for the linear growth of *M. phaseolina*, whereas root and stem extracts media of Giza-1 were the best for *F. oxysporum*.

Extracts of parts of 45-day-old plants induced the highest growth of *M. phaseolina*, whereas extracts of parts of 15-day-old plants gave the highest linear growth of *F. oxysporum*.

The best whole plant extract media were that of H. 8941 and 894 for *M. phaseolina* and that of Giza-1 for *F. oxysporum*.

The lowest linear growth of *M. phaseolina* was on Giza-1 root extract medium. As regards *F. osysporum* the lowest linear growth was on H. 896 whole plant extract medium (45 days old).

2. *Effect of fungal filtrates on seed germination and seedlings*

Fungal filtrates reduced seed germination of all tested varieties especially filtrates obtained after 45 days. This may be due to the increase of inhibitory metabolites produced by both fungi by ageing. On the other hand, Giza -1 variety, which was the most resistant variety, was less affected with the filtrates of both fungi when compared with H. 894, the most susceptible cultivar.

Similar results were noticed as regards to the effect of fungal filtrates on wilting of seedlings of different cultivars. This effect increased by ageing which indicates that *M. phaseolina* and *F. oxysporum* secreted higher amounts of inhibitory metabolites which affected greatly the seedlings of different sunflower cultivars. These results conform with cultivar resistance experiments which indicated that Giza-1 was the most resistant cultivar to both fungi in contrast with H. 896 cultivar. Also, these results hold fairly good with those on water melon reported by Ashour *et al.* (1978) which indicated that the filtrates of the different tested fungi, *i.e. M. phaseolina* and *F. oxysporum* on Czapek's medium decreased significantly the percentage of germinated seeds and their effect increased with ageing of culture filtrates and differed as regards to water melon varieties. Reid (1958) stated that *F. oxysporum* f. *melonis* produced at least three chemical fractions which may contribute to wilting, one was thermostable non-dialysable, apparently a protein and possibly an enzyme. In this respect, Cochrane (1958) mentioned that *Fusarium* sp. and *M. phaseolina* produced protopectinase. David (1967) also reported that the different species of *Fusarium* produce toxic substances incitant of wilting for different plants. Also other investigators, *i.e.* Mathur (1968), Chan and Sakston (1973) and Maklad (1978) reported that *M. phaseolina* filtrate induced symptoms on sunflower plants similar to those which appeared on inoculated plants.

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دراسات على قابلية بعض أصناف نبات عباد الشمس للاصابة بمرض موت البادرات ومرض العفن الفحمى المسببة عن الفطريات فيوزاريوم اكسيسبورم وماكروفومينا فاسيولينا فى مصر ٠

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أثبتت الدراسات أن الصنفين جيزة ١ ومياك أكثر الأصناف مقاومة للفطر ماكروفومينا فاسيولينا فى حين أن الصنف أمبورت ٦١ كان الأكثر مقاومة للفطر فيوزاريوم اكسيسبورم ٠

ووجد أن نسبة الاصابة بموت البادرات والمسببة عن الفطر ماكروفومينا فاسيولينا تزداد بزيادة عمق الزراعة وزيادة فترة الرى وخاصة الصنفين هجين ٨٩٤ وهجين ٨٩٤١ ، وفى حين كانت نسبة الاصابة بالفطر فيوزاريوم اكسيسبورم تزداد بزيادة عمق الزراعة ونقص فترة الرى وكان الصنف جيزة١ أقل الأصناف تأثرا بالمرض ٠

ووجد أن الأصناف مياك وجيزة ١ أعطت أحسن النتائج بالنسبة لمحصول البذرة ومحصول الزيت ويمكن النصح بزراعتها كمصدر لمحصول الزيت فى مصر ، وغيرها من الأغراض ٠

وأثبتت الدراسات المعملية أن بيئة مستخلص النبات الكامل لأصناف هجين ٨٩٤١ وهجين ٨٩٤ أحسن البيئات لنمو الفطر ماكروفومينا فاسيولينا فى حين كان مستخلص النبات الكامل للصنف جيزة (١) أفضل لنمو الفطر فيوزاريوم اكسيسبورم ٠

أما مرشحات الفطريات فكان تأثيرها واضح فى تقليل نسبة انبات البذور وزيادة نسبة موت البادرات للأصناف المختلفة المستعملة من نبات عباد الشمس وخاصة عندما كانت هذه المرشحات مأخوذة من مزارع عمرها ٣٠ يوم فأكثر وكانت بذور وبادرات الصنف جيزة ١ أقل تأثرا بعكس الصنف هجين ٨٩٤ الذى كان أكثر تأثرا ٠