

## Effect of Certain Microelements and Sowing Date on Incidence of Safflower Root Rot and Wilt Diseases

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### Abstract:

Influence of five microelements namely Boron , Cobalt, Copper, Nickel and Zinc as seed treatments on incidence of safflower root rot and wilt diseases caused by *Fusarium verticillioides* were tested under greenhouse conditions in 2010/2011 and 2011/2012 growing seasons as well as growth of the fungus *in vitro*. Effect of four sowing dates ( 15 October , 1 November , 15 November and 1 December ) on incidence of such diseases and characters of plant growth, yield and oil percentage in the two previous growing seasons under field conditions were also investigated .

All tested microelements showed a toxic effect on growth of the tested fungus *in vitro* at all tested concentrations , except Ni at concentration of 25 p.p.m. it enhanced the fungal growth . Cobalt was the most effective one , whereas Ni was the least one in this respect .

All tested microelements decreased the percentage of infected plants . Cobalt, B and Cu were the most effective microelements s in reducing percentage of infected plants , followed by Zn and Ni . Cobalt was superior in reducing the disease incidence .

Sowing date affected the incidence of the diseases . Sowing date of 15 November significantly decreased percenteges of infected plants with either root-rot or wilt followed by Sowing date of 1 November . Fifteen October and 1 December, the early and late sowing dates , respectively increased percentages of infected plants . Fifteen November sowing date was superior in decreasing the diseases incidence , whereas the late sowing date of 1 December was superior in increasing the diseases incidence .

The sowing date of 15 November significantly increased the height of plant , number of branches , number of heads per plant , seed yield per plant , 100 seeds weight , seeds yield per feddan and oil contents followed by the sowing date of 1 November . Sowing date 1 December caused the highest significant decrease in all tested agronomic traits followed by the early sowing date 15 October .

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**Keywords:** root rot, wilt, micronutrients, sowing date, *Fusarium verticillioides*, Safflower

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## Introduction

Safflower (*Carthamus tinctorius* L.) is an annual, broadleaf oilseed crop. It was originally grown for the flowers that used in making red and yellow dyes for clothing and food preparation and considered as a major oil seed crop . It is grown in many locations of Egypt, but it is concentrated in Assiut, Qena and Aswan Governorates.

Safflower root rot and wilt diseases caused by *Fusarium oxysporum* was very serious disease on safflower crop in Egypt and other countries (Pedgoankar and Mayee , 1989; Nasehi, 2010 and Govindappa *et al.*, 2011 ) .

Although, the role of microelements in increasing both plant resistance against certain diseases and crop yield was investigated (Abd-El-Moneem, 2005), the role of micronutrients on root rot and wilt diseases of safflower is still unknown . Several investigators reported the importance of sowing date in decreasing the infection of safflower with root-rot and wilt diseases (Özel *et al.*, 2003; Omid and Sharifmogadas, 2010 and Pasary and Noormohamadi, 2011). This work was design to study the effect of certain micronutrients and sowing dates on incidence of safflower root-rot and wilt diseases.

## Materials and Methods

### Isolation and identification of the causal pathogen

*Fusarium verticillioides* isolates used throughout this study were isolated from diseased safflower plants showing wilt symptoms. Diseased plants were collected from Assiut Governorate in 2009 / 2010 growing season.

Diseased roots were washed with tap water, cut into small pieces

and surfaces sterilized by dipping into 0.1% mercuric chloride solution for 2 minutes, then rinsed for several times in sterile Petri dishes on potato-dextrose-agar (PDA) medium, containing 40 mg streptophenicol / 100 ml medium, then incubated at 28°C for 48 hours. Pure cultures were obtained by using hyphal tip technique described by Brown (1924) and kept at 5 °C on PDA slants in test tubes for further studies. Identification of the isolates was carried out according to Gilman (1967), Booth(1977) and Domsch *et al.*(1980).

### Pathogenicity test :

Pathogenicity experiments were carried out on Giza 1 safflower cultivar .Surface sterilized seeds were sown in sterilized pots (30 cm in diameter), containing sterilized soil previously infested with isolated fungi. Seed disinfection was carried out by dipping the seeds in 0.1 % mercuric chloride solution for 3 minutes . Soil infestation was conducted 7 days before sowing by adding 30 ml of fungal suspension (contain approximately 10<sup>6</sup> propagules / ml). Tested isolate were mixed thoroughly with soil to each pot . Each pot was seeded with 6 seeds and 3 pots were used as replicates . Pots containing non infested soil served as control . Percentage of root rot and wilted plants were recorded after 30 and 90 days from sowing , respectively . The following formulas were used to determine the disease criteria.

$$\text{Root-rot} = \frac{\text{Number of diseased seedlings}}{\text{Total number of seedlings}} \times 100$$

$$\text{Wilt \%} = \frac{\text{Number of wilted plants}}{\text{Total number of plants}} \times 100$$

**Effect of certain microelements on growth of *Fusarium verticillioides* in vitro:**

The highly pathogenic isolate (No. 1) was used in this study. The tested micronutrients were added to Czapek's liquid medium to give a final concentrations of 0, 25, 50, 100 and 200 p.p.m. and distributed in 100 ml conical flasks, each containing 20 ml of the liquid medium (pH 7). Flasks were inoculated with equal disks (5mm in diameter) of the tested fungus taken from 3 days old cultures. Untreated medium was used as control. Four replicates were used for each treatment. After 8 days incubation period at 28 °C, dry weight of growth of the tested fungus was determined. The work was arranged in factorial experiment design with four replicates.

**Effect of safflower seed treatment with certain microelements on incidence of root rot and wilt diseases:**

Microelements namely Copper as  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ , Cobalt as  $\text{CoSO}_4 \cdot 7 \text{H}_2\text{O}$ , Zinc as  $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$ , Nickel as  $\text{NiCl}_2 \cdot 6 \text{H}_2\text{O}$  and Boron as  $\text{H}_3\text{BO}_3$  were tested for their effect on incidence of root rot and wilt diseases under greenhouse conditions in 2010 / 2011 and 2011 / 2012 seasons. Sterilized seeds of Giza 1 safflower cultivar were treated with microelements 2 days before sowing by soaking seeds in 0.1 % solution of microelements separately for ten hours, then treated seeds were dried under room temperature (Ancpok, 1990). Sterilized treated seeds with each of the previous microelements were sown in sterilized pots (30 cm in diameter) containing infested soil with the highly pathogenic isolate of *F. verticillioides* (No. 1). Each pot was seeded with 5 seeds. Four pots

were used as 4 replicates. Pots containing non treated seeds with microelements were used as control. Percentage of root rot and wilt were recorded after 30 and 90 days from sowing, respectively.

**Effect of different sowing dates of safflower on incidence of root rot and wilt diseases :**

Field trials were conducted in 2010/2011 and 2011/2012 winter growth seasons at the Res. Station, Agric. Res. Center, Assiut, Egypt.

Air temperature concerning the research region are given in Table (1) in both growing seasons.

In this experiment 4 sowing dates (15 October, 1 November, 15 November and 1 December) were tested for their effect on incidence of root rot and wilt diseases. Sterilized treated seeds were sown in rows in plots (3x2.1 m) contained 4 rows (60 cm apart). Each row contained 6 hills spaced 30 cm. Every hill was sown with 3 seeds. A complete randomized block design with three replicates was adopted. Inocula were prepared by inoculating sterilized milk bottles 0.5 L. containing barley medium (75g barley + 25g clean sand + 2g sucrose + 0.1g yeast extract + 100ml water) with tested fungus and incubated at 28 °C for two weeks.

Equal amounts of inoculum were placed in each hill after sowing, then the hills were covered with soil and irrigated at the same time. Plants were thinned to one plant per hill after 20 days from sowing. The recommended cultural practices for safflower production were adopted throughout the growth season. Percentage of infected plants in every plot was recorded in seedling stage (30 days old) and mature stage (90 days old).

**Effect of different sowing dates of safflower on growth , yield and seed oil percentage of plants**

At harvest time, plant samples (5 healthy plants each) were taken at random from each plot to determine the following traits 1) Height of plant (cm), 2) Number of branches / plant, 3) Number of heads / plant, 4) Seed yield/ plant(g), 5) 100-Seed weight (g), 6) Seed yield/ Feddan (kg) and 7) percentage of oil content which estimated by Soxhlet apparatus using petroleum ether (BP 40-60 °C) as solvent according to the Official Method (A.O.A.C.,1995).

All data were statistically analysed according to Gomez and Gomez (1984)

**Results and Discussions**

**The causal pathogen of root-rot and wilt of safflower :**

Seven isolates of fungi belonging to the genus *Fusarium* were isolated from diseased safflower plants collected from Assiut Governorate. Isolate No.(1) was identified as *Fusarium verticillioides* (Saccardo) Nirenberg, isolates No.2, 3 and 4 were identified as *F.oxysporum f.sp. carthami* (Kisiewicz and Houston) and isolates No.5,6 and 7 were identified as *F.solani* (Mart.) Sacc.

Testing the pathogenic capability of the isolated fungi (Table 2) indicated that all tested isolates were pathogenic to safflower plants. Such results are in accordance with those reported by Ghosal, *et al.* (1977), Chokrabarti(1979) and Govindappa, *et al.*(2011).

Results also indicate that isolate of *F. verticillioides* caused the highest percentage of root rot and wilt followed by *F.solani* isolates No.7, 5, 6 and then *F.oxysporum f.sp. carthami* isolate No.4, whereas

*F.oxysporum f.sp. carthami* isolates No.2 and 3 caused the lowest percentage of root rot and wilt. This is the first report on occurrence of the disease in upper Egypt.

**Effect of certain microelements on growth of *Fusarium verticillioides* in vitro :**

Results in Table (3) reveal that most of the tested microelements showed toxic effect on growth of the tested fungus *in vitro*. Toxicity of microelements to the tested fungus was increased by increasing of the concentration. All tested microelements at all tested concentrations reduced dry weight of fungal growth except Ni at concentration of 25 p.p.m.,it enhanced the fungal growth compared with the control. Cobalt exhibited the highest toxic effect on the tested fungus at all concentrations followed by B, Cu and Zn , Nickel exhibited the least toxic effect on the tested fungus. These results are in agreement with those reported by Abd-El-Moneem (1996), Abd-El-Moneem *et al.* (2005) and Govindappa *et al.*(2011) who worked on effect of microelements on growth of *Fusarium* root rot of sesame, *Fusarium* basal rot of garlic and *Fusarium* wilt of safflower *in vitro* respectively.

**Effect of safflower seed treatment with certain microelements on incidence of root rot and wilt diseases :**

Results in Table (4) indicate that seeds treated by 0.1 solution of Co and B decreased the percentage of infected plants with root rot, while Cu, Zn and Ni had no effect in this respect in 2010/2011 season. In season 2011/2012, treated seeds by Co, B, Cu and Zinc reduced the percentage of infected plants with

root rot, whereas Ni showed no effect in this respect. Results also indicate that treated seeds by all tested microelements reduced the percentage of infected plants with wilt in the two tested seasons (2010/2011 and 2011/2102) as compared with the control. Generally, Co was superior in reducing the disease incidence. Such findings are similar to those reported by Jackson *et al.*, (1989), Abd-El-Moneem (1996) and Abd-El-Moneem *et al.* (2005), who worked on fusariosis of other hosts.

**Effect of different sowing dates of safflower on incidence of root rot and wilt diseases in 2010/2011 and 2011/2012 seasons.**

Results in Table (5) show that sowing date affected the incidence of root rot and wilt diseases. Sowing date of 15 November significantly decreased percentages of the infected plants with either root rot or wilt followed by sowing date of 1 November in the two growth seasons. On the other hand, 15 October and 1 December, the early and late sowing dates, respectively had increased percentages of infected plants with either root-rot or wilt in the two growth seasons. Fifteen November sowing date was superior in decreasing the diseases incidence, whereas the late sowing date of 1 December was superior in increasing the disease incidence. These results are in agreement with those reported by Navas- Cortes and Jimenes -Diaz (1998), Landa *et al.* (2004) Matheron *et al* (2005), who worked on *Fusarium* wilt of chekpea, *Fusarium* wilt of chekpea and *Fusarium* wilt of lettuce. Increment of the disease incidence in early and late sowing dates (15 October and 1 December, respectively) due to the warm

climate through October and December months as compared with that respect in November. Results in Table (1) indicate that the maximum temperatures in October was 29 °C and 31,5 °C in 2010 /2011 and 2011 /2012 seasons, respectively, whereas it was 20 °C and 24,5 °C in November in 2010/2011 and 2011/2012 seasons, respectively. Results also in Table (1) indicate that the minimum temperature in December recorded the lowest degree (10 °C). Generally, November was at mid position in this respect. The warm climate seemed to be favorite for the growth of *Fusarium verticillioides* and consequently that increased the diseases incidence. These results are in harmony with those reported by Abd-El-Moneem (1990), who worked on root-rot disease of wheat, caused by *Fusarium graminearum*.

**Effect of different sowing dates of safflower on growth, yield and seed oil percentage of plants:**

Data presented in Table (6) indicate that the tested sowing dates affected the height of plant, number of branches per plant, number of heads per plant, seed yield per plant, 100- seeds weight, seed yield per feddan and percentage of oil content in the two tested growth seasons. The sowing date 15 November significantly resulted in the highest height of plant (116.3 and 121.4 cm), number of branches (6.56 and 7.56), number of heads per plant (14,11 and 16, 8) seed yield per plant (22,01 and 26.85 g), 100- seeds weight (4,69 and 5,7 g), seed yield per feddan (647,1 and 729,6 Kg) and oil content (31. 24 and 32.16 %) followed by the sowing date 1 November in 2010 /2011 and 2011/2012 growth seasons, respectively. Data also indicate that

the late sowing date 1 December caused the highest significant decrease in all tested agronomic traits followed by the early sowing date 15 October in 2010/2011 and 2011/2012 growing seasons. Superiority of 15 November sowing date in increasing all agronomic traits may be due to the increase of vegetable growth period,

consequently that lead to develop well canopy and biomass and increased its capacity to absorb enough of water and nutrients and consequently possessed more effective productive organs. These findings are similar to those obtained by Konopinsk (2002 ) and Strasil and Vorliceck ( 2002 ) .

**Table (1): Monthly means of air temperatures for Assuit in 2010/2011 and 2011/2012 seasons.**

Month	2010/2011			2011/2012		
	Temperature °C			*Temperature °C		
	Max.	Min.	Mean	Max.	Min.	Mean
October	29	16	22.5	31.5	18	24.8
November	20	11	15.5	24.5	10	17.3
December	24	10	17	17	11	14
January	18	13	15.5	17	9	13
February	16	16	16	20	10	15
March	25	9	17	27	10	18.5
April	31	22	26.5	31	19	25
May	33	20	26.5	31	23	27

\*Air temperature ( data from Metereological Station at Assiut).

**Table(2): Pathogenic capability of *F. solani*, *F. oxysporum f. sp. carthami* and *F. verticillioides* isoltes on Giza 1 safflower cultivar.**

Isolate number	Fungal isolates	% of infected plants*	
		Root rot	Wilt
1	<i>Fusarium verticillioides</i> (Saccardo) Nirenberg	44.44	66.66
2	<i>F.oxysporum f.sp. carthami</i> (Kisiewicz and Houston)	33.33	33.33
3	<i>F.oxysporum f.sp. carthami</i> (Kisiewicz and Houston)	22.22	33.33
4	<i>F.oxysporum f.sp. carthami</i> (Kisiewicz and Houston)	22.22	38.88
5	<i>F.solani</i> (Mart.) Sacc.	33.33	44.44
6	<i>F.solani</i> (Mart.) Sacc.	33.33	44.44
7	<i>F.solani</i> (Mart.) Sacc.	44.44	49.99
Control		5.55	5.55
L.S.D. at 5%		23.02	28.51

\* Data recorded after 30 days and 90 days from sowing date for root-rot and Wilt , respectively.

**Table (3): Effect of certain micronutrients on growth of *Fusarium verticillioides* in vitro:**

Micronutrients (Micro.)	<i>F. verticillioides</i> Dry weight (mg)					
	0	25*	50	100	200	X
None	434.5					
Copper		339**	259	205.5	130.5	233.5
Coblat		305.25	206.5	164.5	99.75	194
Zinc		351	325	268	154.5	274.6
Nickel		464	421	420	402.75	426.9
Boron		320	227	147.5	102.25	199.18
$\bar{x}$	434.5	355.85	287.7	241.1	177.95	

L.S.D. for micronutrients at 5% = 2.1

L.S.D. for concentrations at 5% = 2.0

L.S.D. for Micro. x Conc. at 5% = 5.5

\*Concentration p.p.m.

\*\*Average of four replicates.

**Table(4):Effect of safflower seed treatment with certain micronutrients on incidence of root rot and wilt disease under greenhouse conditions in 2010/2011 and 2011/2012 seasons.**

Micronutrients	Percentage of infected plants			
	Root rot		wilt	
	2010/2011	2011/2012	2010/2011	2011/2012
Copper	25	20	10	5
Coblat	15	15	10	5
Zinc	30	25	15	10
Nickel	35	30	20	15
Boron	15	20	15	10
Control	35	40	55	50
L.S.D. at 5%	16.10	13.08	15.14	15.14

**Table (5): Effect of different sowing data of safflower on incidence of root rot and wilt disease in 2010/2011 and 2011/2012 seasons.**

Sowing date	Percentage of infected plants			
	Root rot		wilt	
	2010/2011	2011/2012	2010/2011	2011/2012
15 October	34.72	33.33	9.25	8.32
1 November	17.12	12.95	4.63	1.85
15 November	11.11	10.18	2.77	1.39
1 December	40.27	39.34	11.11	9.25
L.S.D. at 5%	13.37	10.5		4.91

Table ( 6) Effect of different sowing date of safflower on growth , yield and seed oil percentage of plants

Characteristic	Height of plant(cm)		Number of heads/plant		Seed yield / plant (g)		100 seeds weight (g)		Seed yield / feddan (kg)		Oil content (%)	
	2010/2011	2011/2012	2010/2011	2011/2012	2010/2011	2011/2012	2010/2011	2011/2012	2010/2011	2011/2012	2010/2011	2011/2012
<b>15 October</b>	103.1	107.8	10.60	12.69	16.72	21.51	3.87	4.91	501.6	589.3	29.19	30.20
<b>1 November</b>	108.7	113.6	10.57	12.64	19.08	24.21	4.23	5.03	566.8	653.8	30.65	31.69
<b>15 November</b>	116.3	121.4	14.11	16.08	22.01	26.85	4.69	5.70	647.1	729.6	31.24	32.16
<b>1 December</b>	104.1	109.6	12.11	14.13	18.81	23.79	4.46	5.11	619.9	721.1	30.39	31.39
<b>L.S.D.at 5 %</b>	2.6	2.3	0.5	0.6	0.6	0.7	0.3	0.2	26.4	22.8	0.3	0.2

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تأثير المغذيات الصغرى وميعاد الزراعة على حدوث مرض  
عفن الجذور والذبول فى القرطم  
هدى عبد الفتاح مصطفى أحمد - أعادة بدر عبد العزيز  
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اجرى هذا البحث لدراسة تأثير معاملة بذور القرطم خمسة مغذيات صغرى ( البورون والكوبالت والنحاس والنيكل والزنك ) على حدوث أمراض عفن الجذور والذبول فى القرطم المتسبب عن الفطر فيوزاريوم فيرتسلويدس تحت ظروف الصوبة فى موسمى ( ٢٠١١/٢٠١٠ و ٢٠١٢/٢٠١١ )، كما درس تأثير هذه العناصر على نمو المسبب المرضى ، كما تم دراسة تأثير أربعة مواعيد للزراعة ( ١٥ أكتوبر ، ١ نوفمبر ، ١٥ نوفمبر و ١ ديسمبر ) تحت ظروف الحقل فى موسمى ( ٢٠١١/٢٠١٠ و ٢٠١٢/٢٠١١ ) ، كما درس تأثير هذه المواعيد على صفات النمو والمحصول ونسب الزيت فى النباتات الناتجة .  
أوضحت النتائج أن كل المغذيات المختبرة عند كل التركيزات ذات تأثير سام على نمو المسبب المرضى ما عدا النيكل عند التركيز ٢٥ جزء فى المليون حيث أنه شجع نمو المسبب المرضى .

كما أوضحت النتائج أن كل هذه المغذيات قللت نسبة النباتات المصابة بالمرض ، الكوبالت والبورون والنحاس كانوا من أكثر المغذيات تأثيرا فى تقليل نسبة النباتات المصابة متبوعا بالزنك والنيكل ، كان الكوبالت من أكثر هذه المغذيات تأثيرا فى تقليل نسبة النباتات المصابة .

أوضحت النتائج أن مواعيد الزراعة المختبرة أثرت على حدوث المرض . ميعاد الزراعة ١٥ نوفمبر قلل نسبة النباتات المصابة متبوعا بميعاد الزراعة ١ نوفمبر ، ميعادى الزراعة المبكر ١٥ أكتوبر والمتأخر ١ ديسمبر زادا من نسبة النباتات المصابة . كان ميعاد الزراعة ١٥ نوفمبر الأكثر تأثيرا فى تقليل نسبة النباتات المصابة بينما كان ميعاد الزراعة ١ ديسمبر الأكثر تأثيرا فى زيادة نسبة النباتات المصابة .

أدى ميعاد الزراعة ١٥ نوفمبر الى زيادة فى طول النبات وعدد الافرع وعدد الرؤوس والمحصول لكل نبات والمحصول لكل فدان كما أدى أيضا الى زيادة محتوى الزيت فى البذور متبوعا بالميعاد ١ نوفمبر . أدى ميعاد الزراعة ١ ديسمبر الى تقليل كل الصفات المحصولية السابقة متبوعا بالميعاد ١٥ أكتوبر .