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EFFECT OF CALCIUM SULFATE ON INCIDENCE OF SESAME AND SUNFLOWER CHARCOAL ROT DISEASES AND SEED YIELD PRODUCTION

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ABSTRACT

Greenhouse and field experiments were conducted in 2008 and 2009 to study the effect of calcium sulfate ($CaCO_4$) concentrations at 0.0., 200, 400, and 800 ppm by using two applied methods or/and seed soaking and plant sprayer on incidence of sesame and sunflower charcoal rot disease caused by *Macrophomina phaseolina* Tassi (Goid). Treated with $CaCO_4$ showed a significant reduction on the percentage of linar growth and number of sclerotial formation of *M. phaseolina* isolates which isolated from sesame and sunflower plants. The highest effect on the reduction of *M. phaseolina* linar growth and number of sclerotial formation was recorded with $CaSO_4$ at 400 ppm**.** Using both of soaking and sprayer with $CaSO_4$ whether separately or together gave a significant decrease of charcoal rot and incidence of survival plants compared with control, while the effect of $CaSO_4$ on reducing of diseases incidence increased with increasing of their concentration under greenhouse and field conditions. Use of $CaSO_4$ at 800 ppm as soaking and sprayer method together recorded the highest effect on decreasing of charcoal rot as well as increasing total seed yield of sesame and sunflower plants during the two growing seasons 2008 and 2009.

Keywords: charcoal rot, *Macrophomina phaseolina*, sunflower, sesame, calcium sulfate, linar growth, sclerotial formation

INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the most important summer oil crops in the tropical and subtropical regions, all over the world. It is grown in many locations of Egypt. *Macrophomina phaseolina* Tassi (Goid), the agent of charcoal is considered the main destructive pathogen on sesame plants especially in Upper Egypt however, it is cause the main destructive diseases of this crop and attacks sesame plants at all stages of growth (Al-Ani *et al*., 1970, Abdou *et al*., 2001 and Khalefa, 2003). In this respect, Murugeson *et al*., (1978) found that, 1% increased in incidence of *M. phaseolina* reduced yield by 180 kg/ha.

Charcoal rot disease, caused by *Macrophomina phaseolina* (Tassi) Goid. also is a serious problem of sunflower it is one of the most important diseases of field crops in arid regions of the world (Hoes, 1985 and Ibrahim, 2006). In Egypt, *M. phaseolina* has been reported more frequently from sunflower and cause charcoal rot disease (El-Deeb *et al.,* 1985, Sadik and Fayzalla, 1989 and Ibrahim, 2006). The early-infected of sunflower with *M. phaseolina* led to reducing the yield by 50% (Hilal, 1981). The infection of sunflower by charcoal rot disease caused also negative effects on seed quality in terms of the oil content, fat, protein and ash (El-Deeb *et al.,* 1985).

With regard to the effect of calcium on pathogenic fungi and plant infection many investigators reported that, calcium in high concentration can suppress the growth of many of fungi in PDA medium and it plays an important role in reducing of many plant diseases (Biggs *et al.,* 1997, Messenger *et al.,* 2000, Biggs, 2004 and Sugimoto *et al.,* 2008). Generally, calcium has a critical metabolic role in carbohydrates removal, cell wall deposition and formation of pectates in the middle lamella (Engelhard, 1993, Osman *et al.,* 2002 and El-Neshawy *et al.,* 2004).

This work was carried out to study the effect of $CaCO_4$ in reducing sesame and sunflower charcoal rot disease under greenhouse and field conditions.

MATERIALS AND METHODS

1. Isolation of causal organisms:

The fungal isolates which were used throughout this study were previously isolated by the authors from diseased sesame and sunflower roots and their pathogenic capabilities were also confirmed (Ibrahim *et al.,* 2006 and Khalefa *et al.,* 2007).

2. Preparation of fungal inoculum:

Inoculum of *Macrophomina phaseolina*, were prepared using sorghum - coarse sand - water (2:1:2 v/v) medium according to Filonow *et al.,* (1988).

3. Soil infestation with *M. phaseolina***:**

Inocula of *M. phaseolina* were mixed thoroughly with soil surface of each pot at the rate of 2% w/w for studying charcoal rot disease in sesame and sunflower. The inocula were covered with a thin layer of sterilized soil. Infested pots were irrigated and kept for 7 days until sowing.

4. Treatments with calcium sulfate

Solutions of calcium sulfate at 0, 200, 400 and 800 ppm were used as seed soaking before sowing and a foliar spray after 20, 40 and 60 days from sowing.

5. Disease assessment

Disease assessment was performed 15 and 45 days after planting for pre- and post-emergence damping-off, respectively, while, the percentage of charcoal rot and healthy plants were estimated at harvest (90 days after sowing).

6. Laboratory experiments:

Effect of different concentrations of calcium sulfate (0.0,50,100,200 and 400 ppm.) on linear growth and sclerotial formation of *M. phaseolina* isolates were tested on the *in vitro* experiment. Tested concentrations of calcium sulfate were added to a known amount (10ml) of sterilized PDA medium aseptically and immediately before pouring in plates. After solidification the plates were inoculated with *M. phaseolina* by placing 5 mm disks for the center of culture plate and incubated at 27 ^{0}C. Five plates for each particular treatment were used as replicates. Linear growth was observed daily and diameters of fungal colonies in “mm” were recorded when plates of any treatment were filled with the fungal growth. Percentage of reduction in mycelial growth of *M. phaseolina* was calculated according to Maurhofer *et al.,* (1995) using the following formula: $X = 100-[G_2/G_1 x100]$

Where: X: % of reduction in growth.
G1: growth of pathogenic fungus in control plates.
G2: growth of pathogenic fungus in treated plates.

Sclerotial formation was determined after 10 days by counting average number of sclerotia in at least 4 microscopic fields (X 10).

7. Greenhouse experiment:

Ten seeds were planted in 50 cm diameter-disinfested plastic pots, which containing sterile sandy clay soil 2:1 previously autoclaved for 2 hours. Four replications were used for each treatment and allocated in the greenhouse following a complete randomized block design.

Disinfested seeds of sesame cv. Toushka 1 and strain of Sakha 53 sunflower were sown in potted soil containing inoculum (2% w/w) of *M. phaseolina* to determinate the effect of calcium sulfate on charcoal rot disease.

8. Field experiments:

Field experiments were carried out during 2008 and 2009 seasons, in naturally infested field soil, at Beni-suef governorate. The soil type was sandy clay loam (67 % sand, 9 % silt, 24 % clay; pH 7.44 and Ec 2.8). To assess the effect of calcium sulfate as seed soaking and foliar treatments on the incidence of charcoal rot under field conditions. Disinfested seeds of sesame cv. Toushka 1 and strain of Sakha 53 sunflower were used for sowing throughout this study. Seeds were sown on the first week of May with 20 cm spacing between plants for sesame and sunflower. The experimental unit area was 10.5 m2 (1/400 fed.). Each plot included six rows; 3.5m in length and 50 cm widths. The experiment was arranged in a completely randomized block design with five replicates. Cultural practices such as fertilization, irrigation and pest control were carried out as usually. Plants in individual plots were harvested based on an optimum maturity index .

Disease assessment was measured as percentages of mature plants showing charcoal rot symptoms as well as the apparent healthy plants 90 days after planting (harvest date). Seed yield was determined 20 days after harvest.

9. Statistical analysis:

The data were statistically analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS Institute, Inc, 1996). Means were separated by least significant difference (L.S.D.) at $P < 0.05$ level.

RESULTS

1. Effect of calcium sulfate on the percentage of linar growth reduction and number of sclerotial formation of *M. phaseolina*;

Data in Table (1) show a significant effect of $CaSO_4$ on the percentage of linar growth reduction and number of sclerotial formation of *M. phaseolina* which was isolated from sesame and sunflower plants. Generally the effect of $CaSO_4$ on the sunflower *M. phaseolina* isolate was more than sesame *M. phaseolina* isolate. Data also showed that, there was a positive relationship between the increase of $CaSO_4$ concentrations and the reduction of *M. phaseolina* linar growth and number of sclerotial formation. Treatment with $CaSO_4$ at 400 ppm recorded the highest effect on the reduction of *M. phaseolina* linar growth and number of sclerotial formation in the both isolates

Table (1): Effect of calcium sulfate on the percentage of linar growth reduction and number of sclerotial formation of *M. phaseolina* which were isolated from sesame and sunflower plants.

$CaSO_4$ (ppm)	Sesame		Sunflower	
	% Growth reduction	No. of sclerotia	% Growth reduction	No. of sclerotia
0.0	0.75	42.00	0.50	38.00
50	15.00	34.50	18.50	30.50
100	28.5	30.75	33.25	26.25
200	43.25	23.50	50.25	20.75
400	59.75	16.25	63.64	10.25
L.S.D. 5%	**2.11**	**2.64**	**1.98**	**2.32**

2. Effect of calcium sulfate on damping-off and charcoal rot diseases incidence on sesame and sunflower seedlings and plants, under greenhouse conditions:

All tested concentrations of $CaSO_4$ (Table,2) reduced the disease incidence and significantly increased survived plants compared to the controls. The presented results also show that, there is a positive relationship between the increase of $CaSO_4$ concentrations and their effect on diseases incidence and clearly show that the efficacy of $CaSO_4$ treatment increased when used as seed soaking and plant sprayer together than used any of them alone.

Use of $CaSO_4$ as seed soaking was more effective on decreasing pre-and post-emergence damping-off than use it as sprayer on seedling plant while, using both methods together gave the best effect in decreasing damping-off and charcoal rot as well as increasing the survival plant. Use of $CaSO_4$ at 800 ppm as soaking and spraying method together recorded the highest effect on decreasing damping-off and charcoal rot as well as increasing survival plant.

3. Effect of calcium sulfate on charcoal rot incidence on sesame and sunflower under field conditions during seasons 2008 and 2009.

Using both of soaking and/or spraying with $CaSO_4$ gave a significant decrease of charcoal rot and incidence of survival plant compared to untreated under field conditions during the two seasons 2008 and 2009 (Table 3 and 4).

Data also show that, the effect of $CaSO_4$ on sunflower was more than sesame and that effect increased with increasing the concentration of $CaSO_4$. In this respect, use of $CaSO_4$ at 800 ppm as soaking and spraying method together recorded the highest effect on decreasing of charcoal rot as well as increasing survival plant compared to other treatments under field conditions during the two seasons 2008 and 2009.

Table (2): Effect of calcium sulfate on damping-off and charcoal rot diseases incidence on sesame and sunflower seedlings and plants, under greenhouse conditions.

Soaking (ppm)	Spraying (ppm)	Sesame				Sunflwer			
		Damping-off		Charcoal rot	Healthy	Damping-off		Charcoal rot	Healthy
		Pre-emergence	Post-emergence			Pre-emergence	Post-emergence		
0.0	0.0	15.0	15.0	47.5	22.5	20.0	10.0	37.5	32.5
	200	15.0	15.0	37.5	32.5	15.0	10.0	30.0	45.0
	400	15.0	10.0	32.5	42.5	15.0	10.0	25.0	50.0
	800	10.0	10.0	25.0	55.0	10.0	10.0	17.5	62.5
200	0.0	10.0	10.0	35.0	45.0	10.0	10.0	27.5	52.5
	200	7.5	5.0	32.5	55.0	10.0	10.0	25.0	55.0
	400	7.5	5.0	27.5	60.0	5.0	7.5	20.0	67.5
	800	5.0	5.0	22.5	67.5	5.0	5.0	17.5	72.5
400	0.0	7.5	5.0	32.5	55.0	7.5	10.0	22.5	60.0
	200	7.5	5.0	25.0	62.5	7.5	7.5	20.0	65.0
	400	5.0	2.5	22.5	70.0	5.0	5.0	17.5	72.5
	800	5.0	2.5	17.5	75.0	5.0	2.5	15.0	77.5
800	0.0	5.0	5.0	27.5	62.5	7.5	5.0	20.0	67.5
	200	5.0	2.5	25.0	67.5	5.0	5.0	17.5	72.5
	400	2.5	2.5	20.0	75.0	5.0	2.5	15.0	77.5
	800	2.5	2.5	17.5	77.5	5.0	2.5	12.5	80.0
L.S.D. 5 %:	**Soaking (A):**	**5.23**	**4.72**	**3.87**	**14.85**	**4.89**	**4.20**	**3.11**	**13.69**
	Spraying (B):	**2.35**	**2.15**	**4.10**	**10.63**	**2.06**	**1.85**	**3.98**	**9.83**
	(A) x (B):	**7.85**	**6.73**	**8.21**	**24.68**	**6.26**	**5.93**	**7.63**	**23.11**

Table (3): Effect of calcium sulfate on charcoal rot incidence on sesame and sunflower under field conditions during season 2008.

Soaking (ppm)	Spraying (ppm)	Sesame		Sunflower	
		Charcoal rot	Healthy	Charcoal rot	Healthy
0.0	0.0	33.61	66.39	29.63	70.37
	200	29.12	70.88	25.21	74.79
	400	25.96	74.05	22.16	77.84
	800	21.95	78.05	20.29	79.71
200	0.0	31.10	68.90	24.60	75.40
	200	29.35	70.65	21.30	78.70
	400	24.73	75.27	18.39	81.61
	800	20.71	79.29	16.87	83.13
400	0.0	28.33	71.67	20.25	79.75
	200	24.96	75.04	16.04	83.96
	400	20.65	79.35	15.99	84.01
	800	17.71	82.29	13.92	86.08
800	0.0	21.95	78.05	18.68	81.32
	200	19.23	80.77	15.54	84.46
	400	15.97	84.03	12.87	87.13
	800	13.18	86.82	11.03	88.97
L.S.D. 5 %	**Soaking (A):**	**1.23**	**5.18**	**1.05**	**4.87**
	Spraying (B):	**0.73**	**4.98**	**0.69**	**3.89**
	(A) x (B):	**1.87**	**10.26**	**1.63**	**9.02**

Table (4): Effect of calcium sulfate on charcoal rot incidence on sesame and sunflower under field conditions during season 2009.

Soaking (ppm)	Spraying (ppm)	Sesame		Sunflower	
		Charcoal rot	Healthy	Charcoal rot	Healthy
0.0	0.0	37.83	62.17	32.25	67.75
	200	33.30	66.70	28.35	71.65
	400	27.90	72.10	25.69	74.31
	800	23.76	76.24	23.44	76.56
200	0.0	33.00	67.00	29.99	70.01
	200	29.08	70.92	24.83	75.17
	400	25.22	74.78	20.14	79.86
	800	21.26	78.74	18.31	81.69
400	0.0	28.06	71.94	25.45	74.55
	200	23.95	76.05	21.79	78.21
	400	20.06	79.94	18.13	81.87
	800	18.70	81.30	15.85	84.15
800	0.0	23.45	76.55	20.11	79.89
	200	20.06	79.94	18.54	81.46
	400	18.06	81.94	15.34	84.66
	800	16.76	83.24	12.65	87.35
L.S.D. 5 %	**Soaking (A):**	**1.58**	**5.47**	**1.22**	**4.86**
	Spraying (B):	**1.48**	**4.96**	**1.13**	**3.59**
	(A) x (B):	**2.98**	**10.79**	**1.98**	**9.14**

4. Effect of calcium sulfate on total seed yield of sesame and sunflower under field conditions.

Concerning to the effect of $CaSO_4$ on total seed yield of sesame and sunflower during the two seasons 2008 and 2009, the data presented in Table (5) demonstrated that, most of tested concentrations of $CaSO_4$ caused significant increase in total seed yield of sesame and sunflower compared to the control during the two growing seasons.

Table (5): Effect of calcium sulfate on total seed yield of sesame and sunflower under field conditions during two seasons 2008 & 2009.

Soaking (ppm)	Spraying (ppm)	2008		2009	
		Sesame (kg/plot)	Sunflower (kg/plot)	Sesame (kg/plot)	Sunflower (kg/plot)
0.0	0.0	0.30	1.92	0.33	1.74
	200	0.34	2.18	0.38	1.84
	400	0.41	2.23	0.42	1.90
	800	0.48	2.28	0.55	1.99
200	0.0	0.35	2.20	0.35	1.80
	200	0.39	2.26	0.37	1.97
	400	0.44	2.33	0.45	2.01
	800	0.50	2.38	0.55	2.07
400	0.0	0.40	2.28	0.40	1.92
	200	0.46	2.38	0.44	2.00
	400	0.59	2.40	0.58	2.08
	800	0.65	2.41	0.66	2.10
800	0.0	0.48	2.34	0.53	2.02
	200	0.60	2.40	0.64	2.06
	400	0.68	2.43	0.72	2.13
	800	0.70	2.46	0.82	2.25
L.S.D. 5 %	**Soaking (A):**	**0.083**	**0.100**	**0.079**	**0.098**
	Spraying (B):	**0.056**	**0.062**	**0.044**	**0.055**
	(A) x (B):	**0.129**	**0.178**	**0.124**	**0.158**

However, using both seed soaking and plant sprayer with $CaSO_4$ together led to increasing of total seed yield of sesame and sunflower during the two growing seasons compared with used any of them alone.

The presented results also showed that, there is a positive relationship between the increase of $CaSO_4$ concentrations and the increase of total seed yield of sesame and sunflower. In this respect, use of $CaSO_4$ at 800 ppm as soaking and spraying method together give the highest total seed yield of sesame and sunflower during the two growing seasons 2008 and 2009.

DISCUSSION

With regard to the effect of $CaCO_4$ on the linar growth and sclerotial formation of *M. phaseolina* data showed a significant effect of $CaSO_4$ on the percentage of linar growth reduction and number of sclerotial formation of *M. phaseolina* which isolated from sesame and sunflower plants moreover, this effect increase with increasing the concentration of $CaCO_4$. This is in agreement with (Biggs *et al.,* 1997, Chardonnet *et al.,* 2000, Messenger *et al.,* 2000, Biggs, 2004 and Sugimoto *et al.,* 2008) who's stated that, calcium in high concentration can suppress the growth of many fungi in PDA medium and their capability on sclerotial and spores formation This may be due to the role of calcium in inhibition of fungi enzymes which responsible on fungi nutrition (Biggs *et al.,* 1997). One hypothesis is that high external concentrations of Ca^{2+} may lead to increase concentration of Ca^{2+} in the cytosol which may be toxic to the fungus and make calcium have fungistasis effect (Conway and Sams, 1984 and Droby *et al.,* 1997). While Biggs and Peterson (1990) stated that, the effects of calcium may result partly from suppressing pathogen activity.

Results of this study provided evidence that, $CaCO_4$ played an important role in reduction of charcoal rot diseases incidence on sesame and sunflower crops under greenhouse and field conditions. The presented results also show that, there is a positive relation between the increase of $CaSO_4$ concentrations and their effect on diseases incidence and the efficacy of $CaSO_4$ treatment increased when used as seed soaking and plant sprayer together than used any of them alone during the two growing seasons 2008 and 2009. This is in agreement with (Biggs *et al.,* 1997, Messenger *et al.,* 2000, Nagwa-Osman *et al.,* 2002, Biggs, 2004, El-Neshawy *et al.,* 2004 and Sugimoto *et al.,* 2008). who's reported that, calcium play an important role in reducing many plant diseases. This may be due to the role of calcium ions in reducing the incidence of fungal infection by directly inhibiting fungal growth, decreasing the production by fungi of cell-wall-degrading enzymes, and/or by being incorporated into the cell wall, thus making it less accessible to cell wall-degrading enzymes produced by the pathogens (Conway and Sams 1984, Biggs *et al.,* 1994, Biggs *et al.,* 1997, and Droby *et al.,* 1997) thought to be a key enzyme in the infection process, is inhibited by Ca (Biggs *et al.,* 1997). While, Conway and Sams (1984) described the role of Ca^{2+} in reducing the effectiveness of fungal polygalacturonase (PG) enzymes by forming cation cross bridges between pectic acids in the plant cell walls, thus making the cell walls more resistant to digestion. However Ca^{2+} ion can stimulate the synthesis of phytoalexins and/or phenols (Kohle *et al.,* 1985).

Generally, calcium has a critical metabolic role in carbohydrates removal, cell wall deposition and formation of pectates in the middle lamella (Engelhard, 1993, Osman *et al.,* 2002 and El-Neshawy *et al.,* 2004). On the other hand, Calcium/calmodulin-dependent protein kinases (CDPKs) are known to play pivotal roles in intracellular signaling during abiotic and biotic stress responses. Based on biochemical or transcriptional activation, CDPKs signaling has been implicated previously in a range of abiotic and biotic stress responses, including light, cold, salinity, or wounding, as well as symbiotic plant–microbe interactions and plant defense (Uhm *et al.,* 2003, Böhmer *et al.,* 2006; Ludwig *et al.,* 2004).

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تأثير كبريتات الكالسيوم علي إصابة نباتات السمسم وعباد الشمس بمرض العفن الفحمي ومحصول البذور الناتج

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أجريت هذه الدراسة تحت ظروف الصوبة و الحقل خلال موسمي ٢٠٠٨ و ٢٠٠٩ لدراسة مدي تأثير المعاملة بكبريتات الكالسيوم بتركيزات صفر و٢٠٠و٤٠٠و٨٠٠ جزء في المليون وباستخدام طريقتان للمعاملة نقع البذور ورش النباتات سواء معاً أو كلاً علي حدي علي إصابة نباتات السمسم وعباد الشمس بمرض العفن الفحمي الذي يسببه فطر *Macrophomina phaseolina*. أظهرت النتائج أن هناك تأثيراً معنوياً للمعاملة بكبريتات الكالسيوم علي تثبيط النمو الطولي وإنتاج الأجسام الحجرية لعزلات فطر *Macrophomina phaseolina* المعزولة من نباتات السمسم وعباد الشمس. أعطت المعاملة بكبريتات الكالسيوم عند تركيز ٤٠٠ جزء في المليون اعلي تأثير علي تثبيط النمو الطولي وإنتاج الأجسام الحجرية لعزلات فطر*Macrophomina phaseolina* .

أوضحت الدراسة أن استخدام كبريتات الكالسيوم سواء نقع البذور أو رش النباتات معاً أو كلاً علي حدي أعطت تأثيراً معنوياً في خفض الإصابة بالعفن الفحمي وكذلك زيادة النباتات السليمة بالمقارنة بالكونترول, بينما زادت قدرة كبريتات الكالسيوم في خفض نسبة الإصابة بزيادة تركيزها تحت ظروف الصوبة والحقل. كانت المعاملة بتركيز ٨٠٠ جزء في المليون باستخدام نقع البذور ورش النباتات معاً هي أفضل المعاملات تأثيراً في خفض نسب الإصابة بالعفن الفحمي وكذلك زيادة المحصول لكلاً من السمسم وعباد الشمس تحت ظروف الحقل خلال الموسمين ٢٠٠٨ و ٢٠٠٩.

قام بتحكيم البحث

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