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Effect of some Soil Amendments on Damping-Off and Charcoal-Rot as well as on Sunflower Growth Characteristics and Yield

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Rhizoctonia *solani* (Kuhn), *Macrophomina phaseolina* (Tassi) Ashby, *Sclerotium rolfsii* (Sacc.) and *Fusarium* spp. were found to be the most associated fungi with damping-off and charcoal-rot of sunflower plants in Behera Governorate. *Rhizoctonia solani* was the most pathogenic fungus, causing pre-emergence damping-off on sunflower (cv. Vedok). Meanwhile, *M. phaseolina* caused less disease incidence 15 days after sowing. In contrast, *S. rolfsii* was the most pathogenic fungus, causing post-emergence damping-off, 15 to 45 days after sowing. However, *M. phaseolina* was most effective to cause charcoal-rot , 45 to 90 days after sowing. Soil amendments with sulphur, compost and gypsum, either alone or in combination, recorded the highest reduction of pre-, post-emergence damping-off and charcoal-rot when compared with check (control) treatment in field experiments. Also, results indicated that the soil amendments improved growth characteristics and increased yield as well as oil contents of sunflower.

Keywords: Charcoal-rot, compost, damping-off, gypsum, sulphur and sunflower.

Sunflower (*Helianthus annus* L.) is one of the most important oil crops all over the world. In Egypt, there are great efforts for increasing the area for its cultivation to increase the local production of edible oil. Sunflower is a short duration crop (90 - 100 days) and could be grown twice a year. It is well known that sunflower has different uses of human food, livestock feed and other industrial products. Its seeds contain a high percentage of oil which ranged between 30-50% (Krizmanic *et al.*, 2004). Also, sunflower oil quality is hygienically superior comparing with the other edible ones. Moreover, sunflower oil is quite palatable free of impurities, easy to refine and contains fat soluble vitamins (A, D, E and K) and good for heart patients (Event *et al.*, 1987 and Gossal *et al.*, 1988). Besides, there is an urgent need to increase the oil production in Egypt to cover about 90% of the annually oil importation for the local market. Unfortunately, sunflower is negatively affected by several diseases in Egypt, especially the damping-off in early stages and charcoal-rot in late stages of growth (Ahmed *et al.*, 1994).

Soilborne diseases are considered as the major problem to sunflower cultivation, either in Egypt or all over the world, because of the wide host range of the causal pathogens and their strong ability of survival in the soil (Mousa *et al*., 2006).

Chemical control was massively applied, however, for the increasing public concern over the fungicide usage, alternative control methods are strongly desired for sustainable agriculture where organic amendments play an important role in this concern (Workneh and Van Bruggen, 1994; Polararapu, 2000 and Lazarouits, 2001).

This research was designed to determine the influence of sulphur, compost and gypsum, either individually or in combinations, on the incidence of damping-off and charcoal-rot of sunflower. Also, yield and oil contents were taken in consideration.

Materials and Methods

Isolation, purification and identification of the causal organisms:

Sunflower (*Helianthus annus* L.) samples, *i.e.* seedlings, roots and stems of plants, showing typical symptoms of damping-off and/or charcoal-rot diseases were collected, during 2010 growing season, from different fields located at Behera Governorate and subjected to isolation trials for the associated pathogens on PDA medium. Fungal isolates were purified using the hyphal tip technique and identified according to their cultural and microscopical characters as described by Gilman (1971), Booth (1977) and Barnett and Hunter (1987).

Pathogenicity test:

Pathogenic ability of isolated fungi, to induce damping-off and/or charcoal-rot diseases on sunflower plants, was tested under greenhouse conditions at Etay El-Baroud Agricultural Station, Behera Governorate. Sunflower seeds (cv. Vedok), obtained from Crop Dept., Agric. Res. Centre, Giza, Egypt, were surface disinfested in 2% sodium hypochlorite, rinsed in sterile distilled water and sown in plastic pots (5 seeds/pot 30-cm-diameter) filled with autoclaved clay and sand soil (1:1, v/v) that individually infested, or not, at the rate of (5% w:w) with the inoculum of each tested isolate previously grown on sand barley medium (1:1, w:w and 40% water) at 25±1°C for two weeks. Five replicated pots were used for each tested isolate. Plants were watered as needed and received the normal agricultural practices. Isolation from infected germinated seeds at pre-emergence stage as well as infected plants at post-emergence stage was carried out. Obtained fungi were compared with that used in soil infestation.

Field experiments:

Field experiments were carried out on sunflower (cv. Vedok) during the two successive growing seasons (2010 and 2011) at Etay El-Baroud, Agric. Res. Station, Behera Governorate, to study the effect of sulphur, gypsum and compost, alone or in combinations, on damping-off and charcoal-rot incidence as well as on sunflower growth characteristics and oil content. Seven treatments were arranged in a complete randomized block design with four replicated plots; each contained 4 lines (each equal 0.75m width × 5.0m length). Applied treatments were designed as follows:

1- Compost (at the rate of $20m^3$/fed).
2- Sulphur (at the rate of 500 kg/fed).
3- Gypsum (at the rate of one ton/fed).
4- Compost + sulphur.
5- Compost + gypsum.
6- Compost + sulphur + gypsum.
7- Sulphur + gypsum.

Seeds were sown in holes (one seed/hole) with approximately 20 cm space distance between holes. No fungicides were applied and growing plants received the normal agricultural practices for sunflower cultivation. The following treatments were applied:

1- Compost:

Compost (type Sakha), obtained from the farm of Sakha, Agric. Res. Station, Kafr El-Sheikh Governorate, was used in these experiments. Characteristics of the tested compost are shown in Table (1) as analyzed according to Page *et al*. (1982). Compost P^H was determined by suspending 5.0 g of tested compost in 10ml deionised water, then the P^H was measured after 1h. Tested compost was sun dried to adjust the moisture to 10-20% and applied at the rate of $20m^3$/fed to the abovementioned prepared field plots, one week before sowing (2nd week of May of each tested year) (El-Gizawy, 2005).

Table 1. Characterization of matured compost Sakha

Characteristics	Compost type of Sakha
PH	6.59
Electrical conductivity (E.C %)	3.01
C/N ratio	11.82
Organic matter (O.M) %	39.73
N %	3.36
P %	0.25
K %	2.23
Bulk density ($mg\text{-}cm^{-3}$)	0.91

2- Sulphur:

Tested sulphur was applied (at the rate of 500 kg/fed) during soil preparation.

Physical and chemical analyses, of the upper 30 cm of tested soil, were analyzed before sowing at the Soil and Water Res. Lab., Kafr El-Sheikh, Fac. of Agric., Tanta Univ. (Table 2).

3- Gypsum:

Tested gypsum, obtained from Ministry of Agric. in Behera Governorate, was applied as soil drenching (at the rate of one ton/fed), during sowing of sunflower.

Disease assessments:

Percentages of the pre- and post-emergence damping-off were assessed 15 and 45 days after planting date. Meanwhile, charcoal-rot was assessed 90 days after planting date as percentage of the diseased plants.

Growth characteristics and yield:

Plant height, number of leaves/plant and yield of the tested sunflower plants were determined 90 days after planting. Plant heads of each plot were harvested, left to dry, and then seed yields were weighted.

Table 2. Some physiochemical properties of the experimental soil before sowing

Soil character	Value	Dimension
HCo3	4	Meq/ l $^{-1}$
Cl	8	Meq/ l $^{-1}$
So_4	5.14	Meq/ l $^{-1}$
Ca	5.69	Meq/ l $^{-1}$
Mg	4.43	Meq/ l $^{-1}$
Na	7.3	Meq/ l $^{-1}$
K	0.12	Meq/ l $^{-1}$
Ec	1.3	dSm^{-1}
PH	8.29	%
OM	1.57	%
Particle size distribution:		
Clay	42.39	%
Silt	42.65	%
Sand	14.96	%

Determination of oil contents in sunflower (cv. Vedok) as affected by soil amendments in growing seasons of 2010 and 2011:

Dried mature seeds of each plot were grounded to a fine powder to determine oil content using Soxhelt with diethyl either as a solvent (A.O.A.C., 1980).

Statistic analysis:

The obtained data were statistically analyzed, whenever needed, using the American SAS/STAT Software, Version 6. Combined analysis was conducted for the two growing seasons and means were compared by the Least Significant Difference test (L.S.D).

Results

Fungi associated with damping-off and charcoal-rot:

Diseased samples of sunflower seedlings and plants showing typical symptoms of damping-off and/or charcoal-rot, were collected from sunflower fields located in Behera Governorate. Associated fungi were found to belonging to eight Genera, *i.e. Aspergillus niger*, *Fusarium* spp., *Macrophomina phaseolina*, *Pythium* sp., *Rhizoctonia solani*, *Rhizopus* sp. and *Sclerotium rolfsii* (Table 3). Also, data show that *R. solani* followed by *M. phaseolina* and *S. rolfsii*, were the most frequent isolated fungi reached 28, 26 and 22%, respectively. Meanwhile, the rest of isolated fungi recorded frequencies ranged between 3 to 12%.

Pathogenicity test:

Pathogenicity trials of the most frequent fungi (Table 4) revealed that *R. solani* followed by *S. rolfsii* and *M. phaseolina* incited 36, 28 and 8% pre-emergence damping-off, respectively, 15 days after sowing. Meanwhile, in case of post-emergence damping-off, *S. rolfsii* incited more disease incidence (32%) than *R. solani* and *M. phaseolina* (being 22 and 20%, respectively), 45 days after sowing. Concerning charcoal-rot, *M. phaseolina* recorded disease incidence reached 44%, 90 days after sowing, as evident by the presence of sclerotia in plant base. While, the rest of tested fungi did not incite any disease in this stage (Table 4).

Table 3. Frequency of fungi associated with sunflower plants, showed damping-off and charcoal-rot symptoms collected from different fields at Behera Governorate during 2010 growing seasons

Fungus	Fungal frequency (%)	No. of samples
Macrophomina phaseolina (Tassi Goid)	26	104
Sclerotium rolfsii (Sacc.)	22	88
Rhizoctonia solani (Kuhn)	28	112
Fusarium spp.	12	48
Pythium sp.	3	12
Rhizopus sp.	6	24
Aspergillus niger (Van Tieghem)	3	12

Table 4. Pathogenicity of the most frequent fungi on sunflower (cv. Vedok) conducted in a pot experiment and monitored for damping-off and charcoal-rot symptoms, 15, 45 and 90 days from sowing

Tested fungus	Monitored diseases and days after sowing		
	Damping-off		Charcoal-rot (90 days)
	Pre-emergence (15 days)	Post-emergence (45 days)	
R. solani	36	22	0
M. phaseolina	8	20	44
S. rolfsii	28	32	0
Control	0	0	0
L.S.D at 5%	14.55	11.32	

Effect of some soil amendments on pre- and post-emergence damping-off as well as charcoal-rot of sunflower cv. Vedok under field conditions in 2010 and 2011 growing seasons:

1- Effect on pre-and post emergence damping-off:

Data in Table (5) indicate that percentages of pre- and post-emergence damping-off differed according to different soil amendments. Generally, all applied treatments, either alone or in combinations, significantly decreased both disease incidence percentages, when compared with the control (check). Moreover, most treatments averaged lesser percentages of damping-off in the second growing season (2011) comparing with those of the first one (2010). Data also revealed that, on the average, combination of sulphur + compost + gypsum recorded the highest reduction in pre- and post emergence damping-off (being 4.88 and 2.63%, respectively), in comparison with those recorded in control (check) treatments (being 30.88% and 12.00%, respectively). On the other hand, the highest percentages of pre- and post-emergence damping-off (reached 13% and 7.25%, respectively), were recorded when soil treated with compost only.

Table 5. Effect of some soil amendments on sunflower pre- and post-emergence damping-off under field conditions in 2010 and 2011growing seasons

Amendment	Pre-emergence (%)		Mean	Post-emergence (%)		Mean
	2010	2011		2010	2011	
Sulphur (Su)	8.0	8.0	8.00	5.0	4.8	4.87
Compost (Co)	14.3	11.8	13.00	8.3	6.3	7.25
Gypsum (Gy)	8.0	6.3	7.13	4.5	4.0	4.25
Su + Co	10. 8	10.0	10.38	6.5	6.5	6.25
Su + Gy	9.0	6.0	7.50	3.3	2.8	4.00
Gy + Co	9.0	7.5	8.25	5.8	4.8	5.25
Su + Gy + Co	5.3	4.5	4.88	2.8	2.5	2.63
Control	27.5	34.3	30.88	11.8	12.3	12.00
L.S.D. at 0.05%	0.4	0.4		0.3	0.4	

2- Effect on charcoal-rot:

Data in Table (6) indicate that percentages of charcoal-rot (90 days after sowing) differed according to different soil amendments. Generally, applying sulphur, compost and/or gypsum, either alone or in combinations, significantly decreased percentages of charcoal-rot when compared with that of untreated (check) soil. Moreover, most treatments averaged lesser percentages of charcoal-rot in the second growing season (2011) comparing with those of the first one (2010). Also, data show that application of soil with combination of sulphur + gypsum + compost caused the highest decrements in charcoal-rot percentage, when reached as low as7.75% and 7.25%, followed by treatment of sulphur and gypsum (being 10.25% and 9.25%) in the two tested 2010 and 2011growing seasons, respectively. Meanwhile, in the control treatment, charcoal-rot reached as high as 26.25% and 31.75% in the two tested growing seasons, respectively. On the other hand, application of compost alone averaged the highest percentage of charcoal-rot (reached 16.00%) when compared with the other treatments.

Table 6. Effect of some soil amendments on charcoal-rot disease incidence of sunflower plants cv. Vedok

Amendment	charcoal-rot incidence (%)		Mean
	Season		
	2010	2011	
Sulphur (Su)	13.57	13.0	13.25
Compost (Co)	17.75	13.25	16.00
Gypsum (Gy)	15.0	10.25	12.625
Su + Co	14.25	14.0	14.125
Su + Gy	10.25	9.25	9.75
Gy + Co	13.75	12.25	13.00
Su + Gy + Co	7.75	7.25	7.50
Control	26.25	31.75	29.00
L.S.D at 0.05	3.44	3.53	

Effect of soil amendments on morphological characteristics and yield of sunflower:

After 90 days of sowing, the response of sunflower plant growth to the soil amendments, either individually or in combinations, was determined by means of measuring height, leaves number per plant and weight of seed yield (kg/plot). Data in Table (7) show significant increments, with different extents, in all sunflower growth parameters, comparing with those of control treatment. In this respect, application of combined treatment (sulphur + compost + gypsum) recorded, on the average, the highest increments in plant height (168.70 cm) and leaves number per plant (26.36) as well as seed yield (5.05 kg/plot). However, the lowest measurements in plant height, leaves per plant and seed yield (being 155.55cm, 22.15 and 4.40 kg/plot, respectively), were recorded when sulphur applied alone. Moreover, most treatments averaged more plant growth measurements in the second growing season (2011) comparing with those of the first one (2010).

Table 7. Effect of soil amendments on growth characteristics and seed yield of sunflower in fields naturally affected with damping-off and charcoal-rot diseases

Amendment	Pant height (cm)		Mean	No. of leaves/plant		Mean	Seed yield (kg/plot)		Mean
	2010	2011		2010	2011		2010	2011	
Sulphur (Su)	154.4	156.7	155.55	21.0	23.3	22.15	4.3	4.5	4.40
Compost (Co)	164.2	164.0	164.10	23.8	24.8	24.30	4.6	4.6	4.60
Gypsum (Gy)	159.5	159.3	159.40	23.2	24.7	23.95	4.7	4.7	4.70
Su + Co	162.6	163.6	163.10	23.0	25.8	24.40	4.7	4.7	4.70
S u + Gy	157.1	159.1	158.10	22.4	23.3	22.85	4.6	4.7	4.65
Co + Gy	165.6	160.9	163.25	23.9	25.7	24.80	5.0	4.9	4.95
Su + Co + Gy	167.8	169.6	168.70	25.2	27.5	26.36	5.0	5.1	5.05
Control	150.8	155.1	152.95	20.7	23.4	22.05	4.0	4.1	4.05
L.S.D. at 0.05%	4.51	4.56		1.96	2.30		0.49	0.35	

Effect of soil amendments on percentage of oil content in sunflower cv. Vedok:

Data in Table (8) indicate that, on the average, all the tested soil amendments increased oil contents compared with non-amended (control) treatment. It is also clear that, in the two tested (2010 and 2011) growing seasons, the highest oil content (47.7% and 47.8%) was recorded when soil applied with a combination of sulphur, compost and gypsum, followed by the combination of gypsum and compost (47.5% and 47.6%), respectively. Application of sulphur alone recorded the lowest oil content (46.2% and 46.0%) in the two growing seasons, respectively. However, control treatments recorded the lowest oil contents (46.0% and 45.7%) compared to all soil amendments.

Table 8. Effect of soil amendments on percentage of oil content in sunflower seeds cv. Vedok

Amendment	Oil content (%) in season		Mean
	2010	2011	
Sulphur (Su)	46.2	46.0	46.10
Compost (Co)	47.3	47.4	47.35
Gypsum (Gy)	47.0	47.1	47.05
Su + Co	46.6	46.9	46.75
Su + Gy	47.0	47.3	47.15
Gy + Co	47.5	47.6	47.55
Su + Gy + Co	47.7	47.8	47.75
Control (non-treated)	46.0	45.7	45.85
L.S.D at 0.05%	0.54	0.55	

Discussion

Diseased samples of sunflower plants, showing typical symptoms of damping-off and/or charcoal-rot, were collected from different fields located at Behera Governorate and subjected to isolation and identification of the causal pathogens. Associated fungi were found to belonging to *Aspergillus niger*, *Fusarium* spp., *Macrophomina phaseolina*, *Pythium* sp., *Rhizoctonia solani*, *Rhizopus* sp. and *Sclerotium rolfsii*. Also, it was found that *R. solani* followed by *M. phaseolina* and *S. rolfsii*, were the most frequent isolated fungi. Moreover, obtained results of pathogenicity trials showed *R. solani* to cause the heist percentage of pre-emergence damping-off followed by *S. rolfsii* and *M. phaseolina*. Meanwhile, *S. rolfsii* incited the heist percentage of post-emergence damping-off followed by *R. solani* and *M. phaseolina*. Concerning charcoal-rot, *M. phaseolina* caused the highest disease incidence, as evident by the presence of sclerotia in plant base. These results are in harmony with those recorded by many researchers (El-Zarka, 1976; Ahmed *et al.*, 1994; Liebig *et al*., 2004 and Mousa *et al*., 2006).

The effect of different soil amendments, *i.e.* compost, gypsum and/or sulphur, on controlling soilborne pathogens and on the improving of crop characters and yield, was extensively studied by many researchers (Nadph and Reddy, 1996; Hussein *et al.*, 2000; Sahu *et al.*, 2001; Thakuria *et al.*, 2004; Scheuerellss *et al*., 2005; Morsy, 2005; Ibrahim, 2006; Mousa *et al*., 2006 and 2009).

In the present research, results cleared that application of such amendments, either individually or in combinations, to the tested soil before or during sowing of sunflower seeds caused significant decrements, to different degrees, in the infection percentages of damping-off and/or charcoal-rot diseases, in comparison with the untreated (control) treatments. Also, comparing with the control treatments, significant increments in seed yield and oil contents were recorded. In this concern, Morsy *et al.* (2009) found that the irrigation of sunflower at 20 days intervals and application of different sources of fertilizers recorded the highest oil content when compared with other tested treatments. Also, several composts were reported to be successful, as soil amendments, in protecting different plants against soilborne pathogens (Scheuerellss *et al*., 2005). Such composts were found to reduce the propagule density of many pathogens, *i.e. R. solani*, *M. phaseolina*, *Fusarium* spp., *Verticillium* spp., *Pythium* spp. and *Streptomyce scabies* (Mousa *et al*., 2006). On the other hand, composts were found to increase the antagonistic microbial activity and, consequently, suppressed root-rot and damping-off pathogens (Scheuerellss *et al*., 2005).

Concerning other amendments, Chen and Hung (1992) reported that application of gypsum reduced pod-rots and improved the appearance and quality of peanut pods. Moreover, Nadph and Reddy (1996) reported that application of gypsum reduced the incidence of groundnut dry root-rot caused by *M. phaseolina,* especially when combined with NPK and Zn. Also, Mahmoud (2004) found that application of gypsum at 500 kg/fed reduced peanut pod rots, caused by *R. solani, M. Phaseolina, Fusarium* spp*., S. rolfsii* and *Aspergillus* spp., as well as the colonization of pods by aflatoxigenic fungi. These findings are in harmony with those obtained in the present study and, accordingly, could be attributed to the role of calcium in building cell walls of the plant tissues through the formation of calcium pectate, which is more resistant to pectic enzymes that play an important role in the pathogenesis (Nadph and Reddy, 1996).

In fact, it is well known that sulphur playing an important role in reducing soil pH, which occur when sulphur is oxidized and that causing suppression of many soilborne pathogens (Adams, 1975; Ries *et al*., 1982 and Ibrahim, 2006). Moreover, sulphur is considered as one of the most important nutrients for plant especially oil seed crops. Sahu *et al*. (2001) found that application of sulphur at 40 or 50 k/ha through phosphor-gypsum significantly increased pod yield and oil contents in peanut.

In conclusion, application of compost, gypsum and sulphur, either individually or in combinations, could be recommended as soil amendments to decrease pre-, post-damping-off and charcoal-rot diseases as well as to improve the oil contents and other plant characteristics of sunflower.

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تأثير بعض الاضافات للتربه على الموت المفاجئ والعفن الفحمى وصفات النمو والمحصـول لنبات عبــاد الشمـس

صابر محمد على مرسى
معهد بحوث امراض النباتات- مركز البحوث الزراعيه- الجيزة.

تم العزل من نباتات عباد الشمس تبدو عليها علامات المرض وكانت اكثر الفطريات تواجدا الريزوكتونيا سولانى والمكروفومينا فاصولينا والاسكلروشيم رولفيسياى وأنواع من الفيوزاريوم بالاضافه الى الريزوبس والاسبرجلس نيجر وانواع من البيثيوم.

اظهرت تجارب العدوى ان الفطر *S. rolfsii* و *R. solani* اكثر الفطريات شدة فى المراحل الأولى حتى ٤٥ يوم من نمو النباتات بينما كان الفطر *M. phaseolina* اكثر شدة فى المراحل المتأخرة من النمو (٤٥-٩٠ يوم).

وقد أظهرت التجارب الحقلية أن معامله التربه قبل الزراعة بالكبريت والكمبوست والجبس تؤدى الى خفض نسبة الموت قبل الظهور فوق سطح التربة ما بين (٤,٩ - ١٣%) بينما كانت النسبة بعد الظهور فوق سطح التربة ما بين (٢,٦ - ٧,٣%) وذلك بالمقارنه بغير المعامل (٣٠,٩ - ١٢%) على التوالى.

كما أدت معامله التربة بالمركبات السابقة الى خفض بنسة الاصابه بالعفن الفحمى حيث تراوحت ما بين (٧,٥ الى ١٦%) مقارنة بغير المعامل (٢٩%). كما ادت المعاملة بالكبريت والكمبوست والجبس الزراعى الى تحسن فى صفات النمو من حيث ارتفاع النباتات وعدد الاوراق وايضا المحصول مقارنة بغير المعامل. كما كانت لمعاملات التربة تأثير ايجابى على نسبة الزيت المستخلص من البذور مقارنة بالكنترول غير المعامل.

ولذلك يمكن التوصية بإضافة المركبات السابقة من الكبريت والجبس والكمبوست ، سواء منفردة أو فى خليط ، إلى التربة لتقليل أمراض عباد الشمس وزيادة انتاجيته.