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CONTROL OF POTATO STEM CANKER AND BLACK SCURF USING SOME AGRICULTURAL TREATMENTS(*)

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ABSTRACT

Rhizoctonia solani is considered as an important soilborne fungus which survive in soil for long time due to formation sclerotia on newly formed potato tuber causing black scurf symptoms. In addition to infection of sprouts and stems causing death for sprouts and stem canker of potato plants. It is considered as one of the devastating disease infecting potato plants and tubers in field. The disease is distributed in all tested Governorates, where the highest percentages of infection recorded in summer plantation season than Nili and winter seasons, and predominant in El-Kaloubia and El-Minia than other governorates. Fifteen isolates of *R. solani* were isolated from naturally infected stems and tubers. Ten of them were belonging to AG3 anastomosis group which severely attack potato plants. Lady-Rousita, Aova and Herms cultivars were the most susceptible whereas Arezona and Areka were resistant. The disease incidence and severity decreased when tuber seeds were kept for sprouting 21 days than 7 or10 days, and when sowed tuber seeds in silty soil than in sandy-clay which gave the highest percentage of infection. The percentages of infection decreased when tuber seeds were sown at 4 cm from the soil surface, whereas it was high when seeds were planted at 20 cm.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is considered one of the world's most important staple food and more strategic crop that producing more dry matter and protein per hectare than major cereal crops. It comes in the fourth order after wheat, corn and rice. Potato plantation increased fast due to its economic importance in Egypt.

Rhizoctonia solani Kühn [the imperfect stage of the teleomorph; *Thanatephorus cucumeris* (A.B.Frank) Donk] is an important fungal pathogen that causes both stem canker and black scurf of potato (Baker, 1970), which lead to tuber yield reduction and losses in tuber quality. It is a soilborne pathogen that comprises several groups which are pathogenic to different host species. It causes cankers symptoms on stems and stolons as well as forming sclerotia (black scurf) on tubers (Hooker, 1981). Soil-borne diseases are still a major threat to vegetable plants due to its wide host range of their pathogens and their strong survival ability in the soil.

The present studies aimed to a- survey potato stem canker and black scurf disease in different governorates during three potato growing seasons, b- Isolate, identify the causal pathogen and its anastomosis groups (AGs) test and pathogenic capability on Lady Rousita cv., c- Test potato varieties response to *R. solani* infection and d- Detect effect of some agricultural practices on disease incidence.

MATERIALS AND METHODS

1- Occurrences of potato stem canker and black scurf at different surveyed governorates during three potato growing seasons:

Occurrences of stem canker and black scurf disease of potato was followed during growing season 2013 in 6 different locations, i.e. Ismailia, Gharbia, Kaloubia, Kafer El-sheghh, Giza and Minia Governorates. Three fields were examined in each locality. Randomized samples each consisted of 100 plants, were examined. Infection percentage was determined according to Carling and Leiner, (1990). The survey was carried out on different varieties of potato. Infection percentage of disease severity was estimated in three growing seasons of cultivation, namely summer (January - March), Nili (September-October) and Early winter (November-December) growing seasons. Disease incidence was determined on the foliage growth and stem base. On the other hand, ten potato plants (90 days-old) from each inspected field were brought for the laboratory examination.

2- Isolation and purification of the causal pathogen:

Isolation of the causal pathogen was carried out from both stem cankered plants and infected tubers

2-1- Isolation of *R. solani* from stem cankers of potato plants:

Potato plants showing typical symptoms of *Rhizoctonia* disease were collected from eight commercial fields in major potato growing Governorates,

i.e. Ismailia (El-Kassasen), Gharbia (Kafer El-zayat), Kaloubia (El-kanater El khiria and Tokh), Kafer El-sheghh (Baltem), Giza **(**El-Badrashen**)** and Minia (Behdal and El-Borgia), Egypt, during growing season 2013. Pieces of tissues from the margins of lesions on infected stems and stolons were cut into small (1cm long) pieces, surface sterilized by immersing in sodium hypochlorite (1%) for three minutes, rinsed several times in sterilized distilled water, and dried between two sterilized filter papers. Three pieces were arranged onto potato dextrose agar (PDA) plates supplemented with streptomycin sulfate (40 mg/100 ml). Petri dishes were incubated at 25^{o}C**,** examined daily and the fungal growth originating from each segment was cultured to new PDA plates to obtain a pure culture.

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2-2- Isolation of *R. solani* from sclerotia on tubers:

Samples of tubers (Lady Rosuita cultivar) harvested at 2013 season, showing symptoms of black scurf, were collected from the previously mentioned governorates. Tubers were thoroughly washed under running water to remove adhering soil particles. Small pieces of tubers with sclerotia were surface- disinfected in sodium hypochlorite solution (1%) for 3 minutes, sclerotia were removed from each tuber, then rinsed in sterile tap water, and allowed to air-dried in a laminar-flow bench (Malik *et al*., 2014). A minimum of four sclerotia were plated on PDA medium supplemented with streptomycin sulfate (40 mg/100 ml medium). The plates were incubated in the dark at 25^{o}C for 3-7 days. Hyphal tip of the developing fungus was transferred to PDA plates to obtain pure cultures and examined microscopically.

3- Identification of the pathogen*:*

The typical growth of the hyphae includes branching near the distal septum of the cells, constriction of hyphae and formation of septa in a short distance from the origin of the hyphal branching as described by Sneh *et al*. (1991) was isolated in pure culture. The identification of the isolated fungus was carried out using characteristics of the mycelia as described by Gilman (1957), Barnett and Hunter (1972) and using the key adopted by Sneh *et al* (1991).The identified *Rhizoctonia solani* isolates were confirmed by Mycological Center (AUMC), Faculty of Science, Assiut University. Stock cultures were maintained on PDA slants and stored in refrigerator at 5^{o}C for further studies.

4- Identification anastomosis groups of R. solani isolates:

Fifteen isolates of *R. solani* selected for the anastomosis group test that varied in their virulence on the potato Lady Rousita cultivar were assigned to an anastomosis group by pairing the isolates with a tester strain (AG3) and observing the hyphae for fusion. The clean slide technique was used in this study. The tester isolate (AG3) was kindly taken from Vegetable Disease Department, Plant Pathology Research Institute, ARC,

Giza, Egypt. Each isolate of *R. solani* was paired with the tester isolate on 2% water agar coated slides with two replicates in Petri dishes **(**Windels and Nabben, 1989). Discs of mycelium from the margins of unknown isolate and an appropriate tester strain were placed on the slide approximately 2-3 cm apart in the 9-cm Petri dishes. After that the plates were incubated at 25^{o}C in the dark until advancing hyphae made contact and slightly overlapped. About 2-3 cm^{2} in the area of contact with 0.001% (w/v) cotton blue in diluted lactophenol stain and scanned for hyphal fusion at 400x by microscopic inspection (Carling and Leiner, 1986). Individual hyphae were often traced back to ensure that anastomosis was made between the paired isolates and not between branches of the same isolate.

5 - Pathogenicity test:

The ability of ten *R. solani* isolates to cause symptoms of stem canker and black scurf on potato (Lady Rousita cv.) was tested, during summer growing season 2013, under greenhouse conditions. Inocula of *R solani* were prepared on autoclaved barley medium in 500 ml glass bottles (75g washed dried barley grains, 100g washed dried coarse sand and 75 ml tap water). Each bottle was inoculated with a disc (5mm in diam.), taken from the margin of a one week-old culture of the isolates grown on PDA medium. The bottles were incubated at 25^{o}C in the dark for two weeks. Autoclaved clay-sand soil (4:1 w/w), were filled in formaldehyde solution (5%) disinfected plastic pots (50 cm in diam.), each pot containing 20 kg of soil. The content of bottles was thoroughly mixed in a sterilized plastic container and used as a source of inoculum. Inoculum of each isolate was added to the soil in pots at a rate of 1% (w/w), mixed well and irrigated. Two sets of pots were used, in the first set inoculation was carried one week pre-planting, but in the another set, pots were inoculated by adding and incorporating inoculum, 30 days after planting.

Potato tubers were placed in the dark at room temperature until sprouted (13-15 days), then were cut into quarters or halves and were left for 48 hrs before planting at 30^{o}C. In each pot, two pieces of potato tubers having 3 eyes of almost same size were arranged in the pot 5-7 cm below the soil surface and covered with soil and watered weekly as required (Naz *et al.*, 2008). Pots containing soil mixed with 1% (w/w) barley grain medium free of the fungus were used as control.

There were two sets with four replications of the trial. The data regarding eyes germination (eyes germination inhibition; EGI) and sprouts killing (SK) were taken one month after sowing by harvesting one set of experiment, whereas, the percentages of stem canker (SCI) and black scurf severity (BSS) were recorded when plants showed signs of maturity, 90 days after sowing by harvesting the second set of experiment as described by Rauf *et al.* (2007-c). All pots were kept under careful observations in greenhouse in

natural light of Mallawy Metrological Station, ARC. Percentages of EGI and SK were calculated as mentioned by Naz *et al*. (2008).

Stem canker severity was expressed as stem canker index (SCI). Severity was assessed on a 0-4 visual disease rating scale as described by Carling and Leiner, (1990) using the following formula:

$$\text{Stem canker severity \%} = \frac{\text{Number of stems in each rating x rating}}{\text{Total number of stems x4}} \text{ x100}$$

Whereas:

0= no damage, no lesions.

1= minor damage, one to several lesions less than 5mm long.

2=intermediate damage, lesions longer than 5mm, girdling of some tissue.

3= major damage, large, lesions, girdling and death of most tissue.

4= all tissue dead

Black scurf severity was determined by using 0-5 disease severity grades based on percent tuber surface showing disease symptoms **(Malik *et al*., 2014).**

$$\text{Black scurf severity\% (BSI,\%)} = \frac{0(n_1)+1(n_2)+2(n_3)+3(n_4)+4(n_5)+5(n_6)}{\text{N (Total number of tubers) x6}} \text{ x100}$$

Wheare, n_1 to n_6 = number of tubers in 0 - 5 grades, 0 = no symptoms on potato tubers; 1= less than 1% tuber area affected; 2=1-10% tuber area affected; 3=11-20% tuber area affected; 4=21- 50% tuber area affected and 5=51% or more of tuber area affected.

6 - Effect of some agricultural factors on the percentage of stem canker and black scurf disease.

Pot experiments were carried out under greenhouse conditions, at Plant Pathology Department, Malawy Station, ARC, during summer seasons of 2014 and 2015. Sterilization of pots (50 cm in diam.) and soil, as well as potato seed disinfestation, were made as described before in pathogenicity test. The highest aggressive isolate (isolate No 1 obtained from El-Kanater El-Khairia) was used in all experiments. Inoculum (1%) was similar to that used in pathogenicity test, was thoroughly mixed with the soil in each pot, 7 days before planting.

Disinfected potato tubers (Lady Rousita cv.) were used in all experiments. Except when soil type was investigated, clay-loamy soil was used. Also, in all experiments, except in that of sowing depth, the tuber seeds were sown at 15 cm depth. Irrigation was done as needed. Unless specified otherwise, in all experiments, four replicates (each contains 2 pots). Two pieces of seed tubers (3 eyes/each) were sown in each pot. Pots were fertilized as recommended. Two sets of pots were used. Half of pots was harvested, 30 days after sowing to determine EGI% and SK%, while the second set were harvested after 90 days (when plants showed signs of maturity) to calculate the SCI% and BSS% as mentioned before.

6-1- Reaction of potato cultivars to attack with plant pathogen infection.

Eight potato cultivars (namely, Aova, Herms, Lady Rosuita, Lady Blfor, Kara, Mont Carlo, Arezona and Areka) were tested for their response to infection by the most pathogenic isolate of *R. solani,* under greenhouse conditions. Potato cultivars were kindly taken from Vegetable Disease Research Department, Plant Pathology Research Institute, ARC, Giza,

6-2- Seed tuber sprouting:

This experiment was designed to study the effect of potato tuber seeds pre germination (sprouting) on potato stem canker and black scurf disease incidence. Seed tubers were surface sterilized, kept in sterilized bags in dark for 7, 10, 15 and 21 days before sowing as described before.

6-3- Effect of soil type on disease incidence

Four soil types (i.e. sandy–clay, sandy, clay and silt soils) were kindly taken from Department of Soil and Water, Malawy Station, ARC., were tested to study their effect on disease incidence and severity.

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6-4- Sowing depth (cm):

Four sowing depth levels, *i.e.* 4,8, 15 and 20 cm from the soil surface, were applied to study their effect on potato stem canker disease.

Statistical analysis:

All trials used a randomized complete block design with four replicates. The obtained data were subjected to statistical analysis using MSTATC computer program (Michigan Statistical Program Version C). Least significant difference (L.S.D., p = 0.05) for comparison between means of treatments was used as mentioned by Gomez and Gomez (1984).

EXPERIMENTAL RESULTS

1- Occurrences of potato stem canker and black scurf at the different surveyed Governorates during three potato growing seasons:

Data presented in Table (1) show that the estimated percentages of the disease incidence resembling the occurrence of stem canker and black scurf disease on potato. The highest percentages of stem canker and black scurf disease were recorded in summer plantation season, ranging between 48.86% and 15.91 in El-Kaloubia and El-Gharbia governorates, respectively, for stem canker, and between 30.68% in El-Kaloubia and 4.55% in both El-Gharbia and Ismailia governorates, for black scurf. Disease severity was recorded middle infection at winter plantation season (ranged between 37.5 and 6.82% in El-Kaloubia and El-Gharbia for stem canker and between 27.27 and 4.55% for black scurf in the same locations, respectively. The lowest infection percentage was obtained for either stem canker or black scurf in Nili season, which ranged between 22.73 and 0.0% in El-Kaloubia and El-Gharbia for stem canker and between 13.64 and 0.0% for black scurf in the same locations. It must be noted that, no infection was recorded in El-Gharbia governorate at Nili cultivated plantation season.

Table (1): Occurrence of potato stem canker and black scurf at six different surveyed governorates, during three potato growing seasons at 2013 season.

Governorate	Season	Disease incidence, %	
		Stem canker %	Black scurf %
El-Kaloubia	Summer	48.86	30.68
	Nili	22.73	13.64
	Winter	37.50	27.27
Minia	Summer	34.09	27.27
	Nili	14.78	7.96
	Winter	Not recorded	Not recorded
Giza	Summer	29.55	18.18
	Nili	7.96	6.82
	Winter	20.45	13.64
El-Ismailia	Summer	23.86	11.36
	Nili	5.69	11.36
	Winter	15.91	4.55
El-Gharbia	Summer	15.91	5.69
	Nili	0.00	0.00
	Winter	6.82	4.55
El-Kafer El-sheghh	Summer	19.32	7.86
	Nili	2.28	1.14
	Winter	14.78	7.96
L.S.D at 5%		A (locaton)= 0.63 B (Pl. period)= 0.45	A (locaton) = 0.57 B (Pl. period)= 0.40

2- **Isolation, purification and identification of the causal pathogen:**

Fifteen isolates (Table 2) of a fungus were isolated from diseased plants, tubers and rhizosphere of potato plants. The culture microscopic studies revealed that all isolates fitted well as *Rhizoctonia solani*. The typical growth of the hyphae includes branching near the distal septum of the cells, constriction of hyphae and formation of septa in a short distance from the origin of the hyphal branching.

3- Identification of the causal pathogen and determination of the anastomosis group:

All fifteen isolates of *R. solani* were tested for their ability to anastomosis with a tester isolate (AG-3, previously isolated from El-Kanater El-Khairia); by using the clean slide technique. Data presented in Table (2) demonstrate that ten isolates of *R. solani* which were isolated from Ismailia, Gharbia, Kaloubia, Kafer El-sheghh, Giza, and Minia fused perfectly with the tester isolate (AG-3).

When hyphae of two strains were opposed, they fuse freely and completely (Figures 1). Isolates of *R. solani* which were isolated from Monofya (Sheben El-koam, Berket El-sabaa and El-khatatba) and EL Behera (Housh Essa) no anastomosis reaction was observed between them and El-kanater El-khiria; a tester isolate (AG-3).

4- Pathogenicity test:

Pathogenic capability of ten isolates of *R. solani*, obtained from Ismailia, Gharbia, Kaloubia, Kafer El-sheghh, Giza and Minia governorates was tested. Data presented in Tables (3) indicate that all *R. solani* isolates were able to infect Lady-Rousita cv., causing typical symptoms of stem canker and black scurf disease. The ten tested isolates deeply varied in their ability to cause stem canker and black scurf disease. The isolate obtained from El-kanater El-khiria (No 1) was showed the highest aggressive one causing the highest percentage of stem canker and black scurf being, (80.44, 71.58%), followed by isolates of Tokh, Behdal, El-borgia. These isolates are aggressive showing more than 50% infection. Isolates obtained from El-badrashen, El-kassasen and Kafer El-zayat were moderate aggressive causing between 25 and 49% of infection. However, Baltem isolate is less virulent than the others, causing the lowest percentage of stem canker and black scurf (13.23, 17.11%).

Table (2): Identification of the anastomosis groups of *R. solani* isolated from potato collected from 8 governorates in Egypt.(*)

Fungal source	No. of isolate	Isolate locality	Anastomosis group AG-3
El-Kaloubia	1	El-kanater El khiria	+(*)
	2	Tokh	+
Minia	3	Behdal	+
	4	El-borgia	+
Giza	5	El- badrashen	+
El-Ismailia	6	El-kassasen	+
El-Gharbia	7	Kafer El-zayat	+
Kafer El-shegh	8	Baltem	+
Monofya	9	Sheben El-koam	-
	10	Berket El-sabaa	-
	11	El-khatatba	-
EL Behera	12	Housh Essa	-

(+) This test was carried out between El-kanater El-khiria (a tester isolate AG-3) and hyphae of the same isolates AGs collected from different governorates and locations.

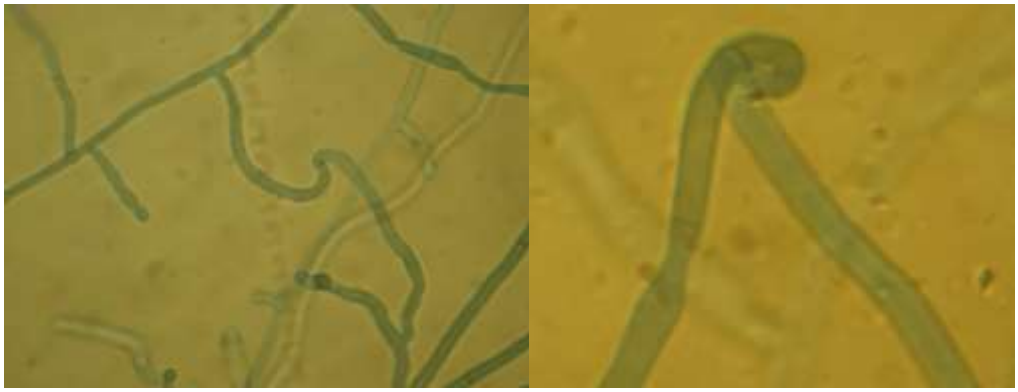

Figure (1): Hyphae of two isolates of *Rhizoctonia solani* (a tester isolate (AG-3) and hyphae of Tokh isolate) attracted to each other, forming perfect fusion

Data in Table (3) also indicate that all *R. solani* isolates are capable to inhibit the germination of eyes and developed sprouts with different degrees of infection. The isolate obtained from El-kanater El-khiria caused the highest percentage of nongerminated eyes and death of emerging sprouts (85.71, 87.50%), followed by isolates of Tokh, Behdal, El-borgia. While the isolates obtained from El-badrashen, El-kassasen and Kafer El-zayat gave moderate percentages of infection. The lowest infection was caused by Baltem (25.00, 12.50%) isolate.

6- Agricultural factors affecting stem canker and black scurf incidence.

6-1-Reaction of potato cultivars to *R. solani* infection

Eight potato cultivars were screened for their reaction to the most pathogenic isolate of *R. solani*, El-kanater El- khiraia (Number 1) causing stem canker and black scurf disease. Data in Tables (4) declared that the cultivars Aova, Lady-Rousita, Herms, Lady-Blfor, Kara followed by Mont Carlo, Arezona and Areka were markedly affected through the two successive seasons. The cultivars Lady-Rousita, Aova and Herms recorded the highest percentages of both stem canker and black scurf symptoms at 2014 (ranged between 80.4-51.5% and 71.6- 50.2%), respectively. Ledy-blfor, Kara and Mont-Carlo are moderate susceptible (the infection percentages are ranged between 41.18 -29.41% for stem canker and 39.38 and 23.69% for black scurf). However, the cultivars Arezona and Areka are the less affected ones. Data also indicate that the cultivars Lady-Rousita, Aova, Herms and Lady-Blfor gave the highest percentages of eyes nongerminated and death of emerging sprouts, through the two experimental seasons, followed with Kara, Mont Carlo and Arezona, while, Areka cv. gave the least percentage of disease incidence. The data in the second season was showed as the same trend of the 1st one.

Table (3): Pathogenicity test of *R. solani* isolates on potato (Lady-Rosuita cv.) during summer 2013, under greenhouse conditions.

Fungal source	Isolate Number	Isolate locality	Disease index%			
			Non germinated eyes %	Dead sprouts %	Stem canker %	Black scurf %
El-Kaloubia	1	El-kanater El-khiria	85.7*(	87.5	80.4	71.6
	2	Tokh	60.7	75.0	73.5	68.4
El-Minia	3	Behdal	50.8	65.4	58.8	63.2
	4	El-borgia	46.4	59.2	47.1	52.6
El-Giza	5	El- badrashen	39.3	44.6	45.6	42.1
El-Ismailia	6	El-kassasen	32.2	33.3	35.3	34.2
El-Gharbia	7	Kafer El-zayat	28.6	27.1	25.0	31.6
Kafer El-shegh	8	Baltem	25.0	12.5	13.2	17.1
Control			0.0	0.0	0.0	0.0
L.S.D at 0.05%			1.7	0.8	0.9	0.8

*) Each reading is an average of 4 replicates.

Table (4): Susceptibility of different potato cultivars to infection with the most pathogenic isolate of *R. solani*, under greenhouse conditions, during 2014 and 2015 seasons.

Cultivars	Disease index%, at 2014 season				Disease index%, at 2015 season			
	Non germinated eyes, %	Dead sprouts %	Stem canker %	Black scurf %	Nongerminated eyes %	Dead sprouts %	Stem canker %	Black scurf %
Aova	82.14*)	87.50	69.12	57.87	75.00	72.92	75.00	60.51
Lady Rousita	85.71	87.50	80.44	71.58				
Herms	78.57	79.17	51.47	50.15	60.71	62.08	67.65	48.91
Lady-Blfor	57.14	65.42	41.18	39.38	57.14	50.83	47.06	41.11
Kara	50.00	52.92	36.76	35.48	35.72	33.34	39.71	36.49
Mont Carlo	32.14	39.59	29.41	23.69	28.57	25.00	29.41	28.95
Arezona	21.43	33.33	22.06	19.74	21.43	18.75	23.53	21.05
Areka	17.86	14.58	19.12	15.79	14.29	8.33	17.65	15.79
L.S.D, 0.05	1.12	0.94	0.56	0.53	0.64	0.63	0.41	0.30

*) Each reading is average of 4 replicates (pots), each contains two tuber seed pieces contain 6 eyes.

6-2- Seed tuber sprouting:

The effect of sprouting pre-sowing on stem canker and black scurf diseases of potato (Lady-Rousita cv.) caused by *R. solani*, was studied. Four periods, i.e. 7, 10, 15 and 21 days, to germinate the eyes and development the growing sprouts before planting were applied. Data presented in Table (5) indicate that the percentages of infection decrease with prolonging the period of sprouting. The highest percentages of infection was observed when potato tuber seeds were kept 7 days for sprouting, whereas, the least infection was recorded in 21 days

treatment. Tubers kept for 10 and 15 days for sprouting before planting resulted middle infected cankered stems and tuber black scurf infection.

6-3- Soil type:

In order to study the effect of soil texture on the potency of the most pathogenic isolates of *Rhizoctonia solani*, causing stem canker and black scurf of potato, four textures (i.e. sand, clay, sand-clay and silty soils were used. Data in Table (6) clear that soil types had affected stem canker and black scurf incidence, in potato cultivar Lady-Rositta. The highest non-germinated eyes (70.8 and 71.4%), dead sprouts (78.6 and 87.5%) and infection percentage (52.6 and 69.12% of stem canker and 53.3 and 61.83% of black scurf, at 2014 and 2015 season, respectively) was obtained when potato was planted in sandy-clay soil. While, in the sandy soil, percentages of stem canker and black scurf decreased to 47.9 and 50.0% and 42.1 and 46.13%, respectively. Moderate infection was observed in clay soil (28.2 and 30.88% and 34.2 %), respectively. Whereas, the lowest disease index (i.e., 13.1 and 16.18% stem canker and 18.3 and 18.4%, black scurf) was showed when potato cultivated in silty soil.

Table (5): Effect of period green germination of potato tubers (Lady-Rositta cultivar) on infection by the most pathogenic isolate of *R. solani*, (No.1) under greenhouse conditions.

Period for green germination, days	Disease index%, at 2014 season				Disease index%, at 2015 season			
	Nongerminated eyes, %	Dead sprouts, %	Stem canker %	Black scurf %	Nongerminated eyes %	Dead sprouts %	Stem canker %	Black scurf %
7	70.8	71.4	53.4	53.3	85.7	77.1	69.1	67.1
10	45.8	52.4	46.3	38.8	50.0	46.7	44.1	44.7
15	22.9	29.7	25.0	23.8	28.6	33.3	30.9	27.6
21	12.5	9.5	13.0	14.2	17.9	10.4	17.7	18.4
L.S.D. 0.05	12.1	10.1	8.5	8.9	15.2	13.4	14.0	13.7

6-4- Sowing depth:

The effect of sowing depth on potato stem canker and black scurf disease incidence was studied. Four sowing depth 4, 8, 15 and 20 cm were applied. Data in Table (7) show that the percentages of infection, dead sprouts and non-germinated eyes were increased gradually with increasing the depth of sowing. The highest infection was observed when seed tubers of potato were sown at 20 cm depth. The least infection with improving the percentages of healthy sprouts and germinated eyes were shown when seeds of potato were cultivated at 4 cm depth, at the two successive experimental seasons**.**

Table (6): Effect of the soil types on potato stem canker and tuber scurf disease on Lady-Rousita cultivar by the most pathogenic isolate of *R. solani*, (No.1) under greenhouse conditions, at 2014 and 2015 seasons.

Soil types	Disease index%, at 2014 season				Disease index%, at 2015 season			
	Nongerminated eyes, %	Dead sprouts, %	Stem canker %	Black scurf %	Nongerminated eyes %	Dead sprouts %	Stem canker %	Black scurf %
Sandy-clay	70.8	78.6	52.6	53.3	71.4(*)	87.5	69.1	61.8
Sandy	43.8	51.9	47.9	42.1	46.4	61.3	50.0	46.1
Clay	25.0	30.6	28.2	43.2	28.6	35.4	30.9	34.2
Slity	10.4	9.3	13.1	18.3	17.9	8.3	16.2	18.4
L.S.D. 0.05	11.3	8.2	9.3	11.1	14.9	13.7	11.6	10.3

(*) Each reading is an average of 4 replicates.

Table (7): The effect of seed piece burial depths (cm) on potato (Lady-Rousita cv.) stem canker and black scurf severity caused by the most pathogenic isolate of *R. solani*, (No.1) under greenhouse conditions.

Burial depths (cm)	Disease index%, at 2014 season				Disease index%, at 2015 season			
	Nongerminated eyes, %	Dead sprouts, %	Stem canker %	Black scurf %	Nongerminated eyes %	Dead sprouts %	Stem canker %	Black scurf %
4	16.7	15.4	18.8	15.4	17.9(*)	12.5	19.1	15.8
8	20.8	31.6	25.5	28.8	28.6	37.5	26.5	30.2
15	41.7	50.0	35.9	46.3	50.0	65.4	42.7	50.1
20	50.0	62.5	50.3	50.8	60.7	83.3	69.2	64.5
L.S.D.at 0.05	8.7	11.6	10.7	9.1	10.0	14.6	8.0	8.5

(*) Each reading is an average of 4 replicates.

DISCUSSION

Rhizoctonia solani Kühn (teleomorph *Thanatephorus cucumeris* (A. B. Frank) Donk) is an important fungal pathogen (Baker, 1970) that causes both stem canker and black scurf of potato (*Solanum tuberosum* L.), which lead to tuber yield reductions and losses in tuber quality. It can be found on all underground parts of the plant at different times during the growing season. Stem canker consists of stem lesions that can reduce tuber yield by reducing the transport of nutrients throughout the plant. Black scurf is the formation of sclerotia, the long-term survival structure of the fungus, on newly formed tubers. This disease is considered as one of the devastating disease infecting plants and tubers in field. The fungus is found in most potato producing areas of the world (Banville *et al.*, 1996). Once it becomes established in a field, it remains viable there indefinitely (Agrios, 2005) and difficult to control.

Dry sclerotia of the pathogen are reported to survive up to six years when stored at room temperature (Kumar, 1976). The symptoms of this disease are found on both above and below ground of the plant organs. Black scurf is the most conspicuous sign of *Rhizoctonia* disease. In this phase of the disease the fungus forms

sclerotia "dark brown to black hard masses on the surface of the tuber". Sclerotia are superficial and irregularly shaped, ranging from small, flat, barely visible blotches to large, raised lumps. Although, these structures adhere tightly to the tuber skin, but they do not penetrate or damage the tuber, even in storage. However, they will perpetuate the disease consider as primary inoculum for the fungus if infected tubers are used as seed. Although, black scurf is the most noticeable sign of *Rhizoctonia*, stem canker is the most damaging of the disease as it occurs underground and often goes unnoticed. *Rhizoctonia* lesions are always dry and usually sunken. Poor stands, stunted plants, reduced tuber number and size, and misshapen tubers are characteristic of diseases caused by *R. solani*.

Under Egyptian climate conditions, several diseases attack potato crop and cause destructive losses in plants and yield. Disease distribution is differed due to the seasons of plantation time, *i.e*. summer, Nili and winter growing seasons. The most potato common and prevailing diseases of root system and vegetative growth are early and late blights, *Fusarium* and *Verticillium* wilt, *Rhizoctonia* stem canker and black scurf and leaf spots. In the present study, an attempt was conducted to throw lights on black scurf and stem canker disease caused by *Rhizoctonia solani*. This study revealed that stem canker and black scurf of potato is distributed in all areas cultivated with potato in different governorates under investigation. Except in El Gharbia governorate at Nili plantation season, disease was recorded at the three different plantation seasons; summer, Nili and winter. The highest percentages of disease index were recorded at summer, while the minimum one was recorded at Nili plantation season. Fifteen isolates of the fungus were isolated from diseased plants, tubers and rhizosphere of potatoes collected from different districts.

Rhizoctonia solani has many synonyms and is divided into subgroups called anastomosis groups (AG's), in which isolates are categorized according to the ability of their hyphae to anastomose (fuse) with one another. In this investigation, anastomoses test showed that ten isolates (66.7%) of *R. solani* belong to AG3, (The hyphal fusion dissolution of fused cell walls and the advent of hyphal protoplasm (anastomosis) were observed between the tester isolates and the examined one). Whereas the rest (5 isolates; 33.3%) observed no fused with the tester isolate (AG3). Rauf *et al.* (2007- a) reported that *R. solani* anastomosis group 3 is the primary cause of black scurf in Pakistan, like in most parts of the world (Banville *et al.*, 1996). Three AG's of *R. solani* are prevalent in Michigan (Wharton *et al.*, 2013).They mentioned that *Rhizoctonia solani* AG-3 is relatively specific to potato and sclerotia on tubers belong almost exclusively to AG-3. Other AG's of *R. solani* like AG2-2 and AG-4, may be

pathogenic to potato at some temperatures, but they generally cause little damage. Rauf *et al.* (2007-b) isolated 127 isolates of *R. solani* from infected potato in Pakestan. They found that all isolates were found multinuclulate. Anastomosis group determination revealed 81.89% isolates belonging to AG 3 followed by AG 5 (8.66%) and AG 4, presented 5.5%. They reported that occurrence of AG 3 was the highest in all the potato production zones as compared to other AGs indicating *R. solani* AG 3 isolates pre-dominance on potato. Also, in Egypt, Hassan (2013) isolated 15 isolates of *R. solani*, infected potato stems and tubers, from different governorate, and the author found that all tested isolates were belonging to AG3 group.

Pathogenicity test revealed that all ten tested isolates (AG3) of *R. solani* are capable to inhibit germination of potato eyes, killed new sprouts and induce stem canker and black scurf symptoms on Lady-Rousita cv. Isolates of El-kanater El-khiria followed by Tokh, Bedhal and El-Borgaia were highly aggressive showing infection between 80.4 and 47.1 stem canker and 71.5 and 52.6 tuber black scurf. Soil-borne inoculum of *R. solani* is the main cause of black scurf on potato tubers and also contributes to eyes germination inhibition, sprouts killing, stem, stolon and root damage (Hide *et al*.1973; Frank and Leach, 1980). Black scurf attacks potato plants causing delay in tuber initiation and reduction in tuber yield which corroborated the present results.

Rhizoctonia disease commence by seed or soil-borne inoculum and both inocula are equally damaging. Presently, it is not possible to entirely control this disease, but severity may be limited by following a combination of crop protection strategies for successful disease management. As the most significant measures are cultural, chemical controls should also be employed. The present study revealed that the tested eight potato cultivars differed in their susceptibility against El-Kanater El Khiria *R. solani* isolate (No 1) infection. Lady-Rousita, Aova and Herms were the most susceptible cultivars. Lady-blfor, Kara and Mont-Carlo are moderate susceptible, whereas Arezona and Areka were the most resistant ones. These results are in agreement with that obtained by Frank *et al.* (1976) and Rauf *et al.* (2007-c). Differences in disease expression of potato cultivars have been reported by Frank *et al.*, (1976). Greenhouse screening of fifteen potato breeding lines and cultivars, obtained from Potato Program (NARC), Islamabad, for host plant resistance to *R.solani* (isolate CL-58; the highly aggressive isolate in Pakistan) by soil inoculation revealed that potato cvs. Cardinal and Desiree were resistant and susceptible, respectively (Rauf *et al*., 2007-c).

Keeping tuber of potato, Lady-Rousita cv., for sprouting pre planting, for different periods, led to increase the percentages of germinated eyes and decrease the killed sprouts, stem

canker and black scurf indexes with increasing the period of sprouting. The keeping tubers for 21 days gave the best results than 7 days treatment.

Rhizoctonia solani attacks germinating sprouts underground before they emerge from the soil. The sprout may be killed outright if lesions form near the growing tip. Damage at this stage results in delayed emergence and is expressed as poor and uneven stands with weakened plants **(**Wharton *et al*., 2013). Authors also mentioned that reduction in crop vigor results from expenditure of seed energy used to produce secondary or tertiary sprouts to compensate for damage to primary sprouts.

Cultivation of potato tuber seeds in sandy clay soil led to increase the percentages of dead sprouts, stem canker and black scurf and decreased the percentage of germinated eyes. While planting potato in silty soil improved the percentage of germinated eyes and decreased the percentages of infection. The present study revealed also that the degrees of infection were related with the sowing depth; the values of disease determinations increased gradually with increasing the depth of planting. The least infection was showed when pieces of tuber seeds were planted on 4 cm, this will minimize the time between planting and sprout emergence and therefore may minimize sprout infection and reduced disease levels. However, this practice may not influence root and stolon infection and will not reduce the formation of sclerotia on tubers. While the highest percentages of infection was recorded when potato cultivated at 20 cm of the soil surface. These results are in agreement with that mentioned in U.K. by Lacy and Hammerschmidt (1994) who reported that when soil temperatures are below 8^{o}C, seed should be planted within 4-5 cm of the soil surface. They reported also that plant seed pieces at a depth of two inches or less when soil temperatures are at least 55^{o}Fto encourage rapid germination and emergence. *Rhizoctonia* diseases are initiated by seedborne or soilborne inoculum. The pathogen overwinters as sclerotia and mycelium on infected tubers, in plant residue, or in infested soils. When seed tubers are planted in deep level potatoes plants affected are characterized by a lack of vigor because much of their energy has been used to produce secondary or tertiary sprouts before a plant emerges. In the same time, the produced stolons and roots, like sprouts, can be killed by the pathogen. The root system is reduced when this occurs. The number, shape and size of tubers produced are also reduced when the roots and stolons are attacked.

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مقاومة تقرح الساق والقشرة السوداء فى البطاطس باتباع بعض العمليات الزراعية

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يعتبر الفطر ريزوكتونيا سولانى من فطريات التربة الخطيرة حيث يعيش فى التربة لفترات طويلة بسبب تكوينه للأجسام الحجرية التى تصيب درنات البطاطس المتكونه حديثا مسببا لها أعراض مرض القشرة السوداء كما يهاجم السوق تحت سطح التربة والنموات الجديدة مسببا تقرحها ينتشر هذا المرض فى زراعات البطاطس بجميع المحافظات وقد سجلت أعلى إصابة فى العروة الصيفية بالمقارنة بالعروتين النيلية والشتوية وفى محافظتى القليوبية والمنيا عن باقى المحافظات الأخرى.

تم عزل خمسة عشر عزلة لهذا الفطرمن سوق ودرنات مصابة طبيعيا. عشرة عزلات منها ثبت أنها تنتمى إلى المجموعة AG3التى تهاجم بشدة نباتات البطاطس. بينت الدراسة أن الأصناف " ليدى–روزيتا، أوفا وهيرمز" أكثر قابلية للإصابة بالمرض بينما كان الصنفان "أريزونا وأريكا" الأكثر مقاومة للإصابة. وقد نقصت كل من نسبة وشدة المرض عند انبات تقاوى البطاطس قبل زراعتها بواحد وعشرون يوما عن معاملات الأيام السبعة والعشرة، وعندما زرعت البطاطس فى تربة سلتية عن التربة الرملية – طينية أو التربة الطينية. كما قلت نسبة الإصابة بالزراعة على عمق 4 سنتيمترات من سطح التربة بينما كانت أعلى مايمكن عند الزراعة على عمق 20 سنتيمتر من سطح التربة.