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HISTOPATHOLOGICAL STUDIES ON BLACK SCURF DISEASE OF POTATO PLANTS

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ABSTRACT

The experiments were carried out through two successive agricultural seasons of 2010/2011 and 2011/2012 at the greenhouse of Faculty of Agriculture, Zagazig University, Sharkia Governorate, Egypt in order to study the effect of black scurf caused by *Rhizoctonia solani* on the quality and quantity of sponta potato cultivar (*Solanum tuberosum* L.) and also, select the proper control methods for the pathogen. The experimental treatments were divided into two groups; these groups namely (A) and (B) were untreated soil with *Rhizoctonia solani* and treated soil with *Rhizoctonia solani*, respectively. Each group was cultivated by the following treatments: untreated seed tubers, treated seed tubers with *Trichoderma virdi*, treated seed tubers with *Bacillus subtilis*, treated seed tubers with Rhizolex. Evaluation of the pathogen effect, different biological agents and chemical fungicide on potato plants were done taking into consideration the following indicators plant height, chlorophyll, proline, carbohydrate, starch, protein, disease ratio and anatomical studies. The experimental results reveal that the previous indicators were in the optimum region under one of the following recommended conditions: *Trichoderma virdi* as an effective biological control against the pathogen which motivates the growth of potato plants. Rhizolex as a chemical control to *Rhizoctonia solani* fungus at the recommended dose that reduces the disease ratio of potato tubers.

Key words: Potato plants, black sucrf, biological and chemical control, Morphological and physiological characters, anatomical studies.

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. The cultivated area with potato plants in Egypt amounted to be about 334509 feddans (FAO, 2010). Furthermore, in Egypt potato crop ranked first exported vegetable crop. The total value of potato crop production in Egypt is 4338430 ton (FAO, 2011).

The black scurf disease is one of which severely affects the potato production and yield. The causal agent of this disease is the soil borne fungus *Rhizoctonia solani* Kühn which considered one of an important fungal pathogen that causes both stem canker and black scurf on potato tubers world-wide. This pathogen is belonging to the anastomosis group AG3 which considered one of multinucleate *Rhizoctonia* spp. El-Kot (2008) stated that the effects were similar in most cases to those of Rhizolex fungicide, which increased emergence, chlorophyll content of potatoes leaf, potato tuber yield and reduced black scurf and dry rot severity. Mohammed *et al.* (2008) evaluated the efficacy of *Trichoderma virdi* as an integrated strategy of *Rhizoctonia* disease management in potato crop. The challenge inoculation with *T. virdi* caused a significant reduction *in vitro* in the linear growth of *Rhizoctonia solani* particularly, when it was performed closer to the

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time of pathogen inoculation. With the exception of the number of stems, yield and growth attributes of potato plants infected with *R. solani* were significantly affected. *T. virdi* application significantly increased the growth components (*i.e.,* plant height, shoot fresh and dry weights, root fresh and dry weights) and tuber yield (*i.e.,* number and weight of tubers) compared to potato plants inoculated with *R. solani* alone. Moreover, the disease incidence and severity, as stem canker or black scurf on progeny tubers, were also significantly alleviated by *T. virdi* inoculation. Lutomirska (2010) studied the influence of severity of seed tubers infestation with black scurf as related to potato plant growth, stem canker, yielding and incidence of black scurf on progeny tubers. Increasing levels of sclerotia on seed tubers caused worse plant emergence, increasing stem canker incidence and disease symptoms on stems, but their severity was dependent on weather conditions. Increasing of black scurf on mother tubers affected relatively the yield and tuber quality. The development of black scurf on progeny tubers was less dependend on seed tuber infestation with black scurf. Woodhall *et al.* (2012) stated that *Rhizoctonia solani* is a species complex of 13 related but genetically distinct anastomosis groups (AGs). In potato, *R. solani* can infect the stems, stolons and roots resulting in quantitative losses. It can also cause qualitative losses through blemishes occurring on progeny tubers, such as black scurf and elephant hide (corky cracking). Knowledge of the AG in local populations is important because they differ in host range, fungicide sensitivity, and disease severity.

So, the objectives of this work are to:

1. Study the effect of *Rhizoctonia solani* at different growth stage of potato plants.
2. Compare the effect of pathogen and different control methods on potato plants characteristics.
3. Optimize different control methods to eliminate black scurf disease of potato.
4. Evaluate the potato plants from its yield components and tubers quality.

MATERIALS AND METHODS

The experiments were carried out through two successive agricultural seasons of 2010/2011 and 2011/2012 at the greenhouse of Faculty of Agriculture, Zagazig University, Sharkia Governorate, Egypt to study the effect of black scurf disease caused by *Rhizoctonia solani* on the yield and quality of sponta potato cultivar (*Solanum tuberosum* L.) and also, select the proper control methods for *R. solani* pathogen.

Mechanical analysis of the experimental sandy loam soil was 66.22% sand, 13.90% silt and 19.88% clay, while chemical analysis was 3.45 mg/l Ca^{+}, 0.35 mg/l Mg^{+}, 2.20 mg/l Na^{+}, 0.55 mg/l K^{+}, 0.50 mg/l Hco_3^{-}, 2.30 mg/l Cl^{-}, 3.75 mg/l So_4^{-}, 0.71 mmhos/cm Ec, 0.80% organic matter and 8.22 pH.

Identification of the Isolated Fungus

Potato tubers from each location with visible sclerotia were washed thoroughly in tap water to remove any adhering soil particles, then rinsed in sterilized water and left it to dry. Isolation of the causal pathogen was carried from sclerotia, periderm tissue and parenchymatic potato tissues. The identification of the isolated fungus was made by phytopathological researchers of the Plant Pathology Research Institute, Agricultural research center, Giza, Egypt. This identification of the isolated fungus was done by using characteristics of mycelia of fungus as described by Barnett and Hunter (1972).

Biological control

Different biological agents as *Trichoderma virdi* and *Bacillius subtilis* were used under tests as a biological control of *Rhizoctonia solani*. *Trichoderma virdi* was obtained from Plant Pathology Department, Faculty of Agriculture, Zagazig University. While, *Bacillius subtilis* was obtained from Institute of plant pathology research, Agricultural research center, Giza, Egypt.

Chemical control

Rhizolex T50 is the most fungicide used for controlling soil borne diseases.

Experimental conditions

Earthenware pots of 35 cm inner diameter were used in these experiments in the

greenhouse at Faulty of Agriculture, Zagazig University. Each pot was filled with 10kg of soil.

Two experimental groups namely (A) and (B) were carried out and every treatment under these groups was replicated as following:

A- Untreated soil with *Rhizoctonia solani*.

B- Treated soil with *Rhizoctonia solani*.

Each group was cultivated by the following:

- Untreated seed tubers.
- Treated seed tubers with *Trichoderma virdi*.
- Treated seed tubers with *Bacillus subtilis*.
- Treated seed tubers with Rhizolex.

The soil which treated with *Rhizoctonia solani* fungus was inoculated at the rate of 3-5 % inoculum per kg soil. The antagonists of *Trichoderma virdi* and *Bacillus subtilis* were coated the seed tubers with spore's concentration of 1×10^6 spore/ml conc. and 1×10^8 cell/ml conc., respectively. While, Rhizolex fungicide was applied at the recommended doses of 3g/kg seed tubers.

Irrigation, fertilizing and weed control were the same in all treatments.

Measurements

Potato plants were taken out carefully at different ages of 30, 60 and 90 days after planting from the soil using stream water to ensure least losses of root system and then, each plant was separated into leaves, stems, roots and tubers.

Evaluation of the previous variables was carried out taking into consideration the following indicators:

Plant height

The plant height as a morphological character of potato plants was measured from the soil surface to the uppermost point of the plant.

Photosynthitic pigment

The photosynthitic pigment of chlorophyll A+B [chlorophyll A (yellow green) and chlorophyll B (blue green)] was extracted from the leaves of potato plant for each treatment using pure acetone according to Fadeel's method (Fadeel, 1962). The extract was filtered and the optical densities were measured spectrophotometrically using "spectronic-20" spectrophotometer at 662,664 and 440.5 nm for chlorophyll A and chlorophyll B, respectively. The pigment concentrations were calculated using wettsteins formula (Wettstein, 1957). The concentrations of pigments were calculated in mg/g fresh weight of leaves as follows:

Chl.A = (9.784 x E662)-(0.99 x E664), mg/liter.

Chl.B = (21.426 x E664)-(4.65x E662), mg/liter.

Where:

E: The reading of the optical density at given wave length.

The concentration of pigments was then expressed in mg/g fresh weight of leaves.

Proline concentration

The proline concentration was determined according to the method by Bates *et al.* (1973).

Total carbohydrates fractions

Carbohydrate fractions were determined in the dried tubers samples of all treatments photometrically according to Bernfeld (1955) and Miller (1959).

Starch content

Methods of AOAC (1990) were applied for the acid hydrolysis of starch to glucose and the later was determined by the method described by Dubois *et al.* (1956).

Total protein

The total protein was determined in potato tubers by multiplying total nitrogen concentration by the factor of 6.25 according to AOAC (1970).

Black scurf disease ratio

The disease ratio was calculated by the following equation:

$$\text{Disease ratio (\%)} = \frac{\text{Number of infected potato tubers}}{\text{Total number of potato tubers}} \times 100$$

Anatomical studies

Samples of potato tubers were taken in order to determine the effect of different treatments on thickness of phellem tissue of tubers as shown in Fig. 1.

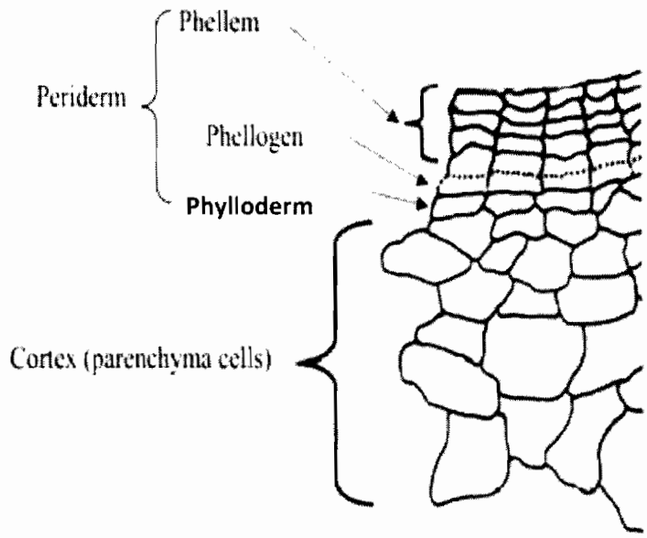

Fig. 1. Cross section of potato tuber

Statistical Analysis

All data were subjected to statistical analysis according to Snedecor and Cochran (1990). Means separation was done by L.S.D. at 0.05 level of probability. Data were analyzed as a complete randomized block design.

RESULTS AND DISCUSSION

The acquired results will be discussed under the following heads:

Plant Height

Fig. 2 and Table 1 showed that the effect of different biological agents and chemical fungicide had a significant effect on plant height of healthy and infected potato plants.

Using *T. virdi* as a biological control against the pathogen, the potato plant height was increased by 9.97% compared to infected plants with *R. solani*, this is in agreement with Mohammed *et al.* (2008). While, the shortest height of potato plants was 43.45 cm by *Rhizoctonia solani*. Above ground symptoms of black scurf disease such as plant stunting, chlorosis, purpling of leaves and the formation of aerial tubers may also be apparent (Banville and Carling, 2001). Chemical control at the recommended dose rate usually gave the good results. The height of infected plants with the pathogen was 47.78, 45.33 and 60.06 cm by using *Trichoderma*, *Bacillus* and Rhizolex, respectively.

Concerning the effect of different plant ages, data showed that plant height was increased by advancing plant age. Plant height was 46.04, 50.75 and 72.08 cm at 30, 60 and 90 days after planting.

Chlorophyll A+B

Chlorophyll is a green pigment found in cyanobacteria and the chloroplasts of algae and plants. Chlorophyll A+B as a photosynthetic pigment was determined as illustrated in Fig. 3 and Table 2. Data revealed that the rate of total chlorophyll content of A+B was 0.72, 0.53, 0.63 mg/g fresh wt. of leaves for healthy potato plants with *T. virdi*, *Bacillus* and Rhizolex, respectively. While, it was 0.49, 0.45 and 0.59 mg/g fresh wt. of leaves for infected plants by the pathogen with the previous treatments orders.

The highest rate of total chlorophyll was by Rhizolex treatment as a chemical fungicide, this may be due to more ability to resist the pathogen infection and thus, give the ability for plants to grow normally, take the needed nutrients and therefore, increased the chance for production of more chlorophyll, this is compatible with El-Kot (2008).

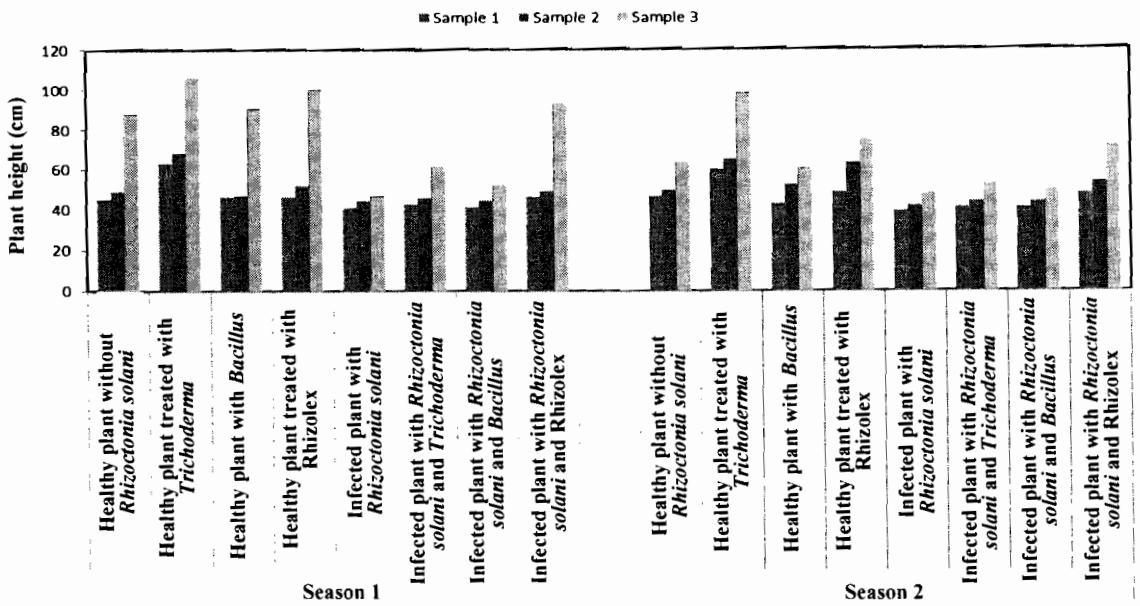

Fig. 2. Effect of biological agents (*Trichoderma virdi* and *Bacillus subtilis*) and chemical fungicide (Rhizolex) on plant height of healthy and infected potato plants at different plant ages of two growing seasons (2011/2012 and 2012/2013)

Table 1. Effect of biological agents (*Trichoderma virdi* and *Bacillus subtilis*) and chemical fungicide (Rhizolex) on plant height of healthy and infected potato plants at two growing seasons (2011/2012 and 2012/2013)

Seasons [A]	Plant ages (DAS) [B]	Healthy or infected potato plants [C]								Mean
		Healthy				Infected				
		1	2	3	4	5	6	7	8	
1st 2011/2012	30	45.00	63.00	46.00	46.67	41.00	42.67	41.33	46.00	46.46
	60	49.00	68.00	47.00	51.67	44.33	45.67	44.67	49.33	49.96
	90	87.67	105.67	90.33	99.67	47.00	61.33	52.33	92.67	79.58
	Mean	**60.56**	**78.89**	**61.11**	**66.00**	**44.11**	**49.89**	**46.11**	**62.67**	**58.67**
2nd 2012/2013	30	46.67	59.67	42.67	48.33	39.00	40.67	40.33	47.67	45.63
	60	49.67	65.00	52.00	63.00	41.67	44.00	43.67	53.33	51.54
	90	63.00	98.00	60.00	74.66	47.67	52.33	49.67	71.33	64.58
	Mean	**53.11**	**74.22**	**51.56**	**62.00**	**42.78**	**45.67**	**44.56**	**57.44**	**53.92**
		Impact of B, C or BC factors								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mean**
Plant ages (DAS)	30	45.84	61.34	44.34	47.50	40.00	41.67	40.83	46.84	46.04
	60	49.34	66.50	49.50	57.34	43.00	44.84	44.17	51.33	50.75
	90	75.34	101.84	75.17	87.17	47.34	56.83	51.00	82.00	72.08
	Mean	**56.84**	**76.56**	**56.33**	**64.00**	**43.45**	**47.78**	**45.33**	**60.06**	**56.29**
LSD at 0.05			A	B	AB	C	AC	BC	ABC	
			2.61	1.30	1.84	2.13	3.01	3.69	5.22	

Notes

DAS= 30, 60 and 90 days after sowing.

Healthy or infected potato plants

1 = without *Rhizoctonia solani*, 2 = with *Trichoderma virdi*, 3 = with *Bacillus subtilis*,
4 = with Rhizolex, 5 = with *Rhizoctonia solani*, 6 = *Rhizoctonia solani* with *Trichoderma virdi*,
7 = *Rhizoctonia solani* with *Bacillus subtilis*, 8 = *Rhizoctonia solani* with Rhizolex.

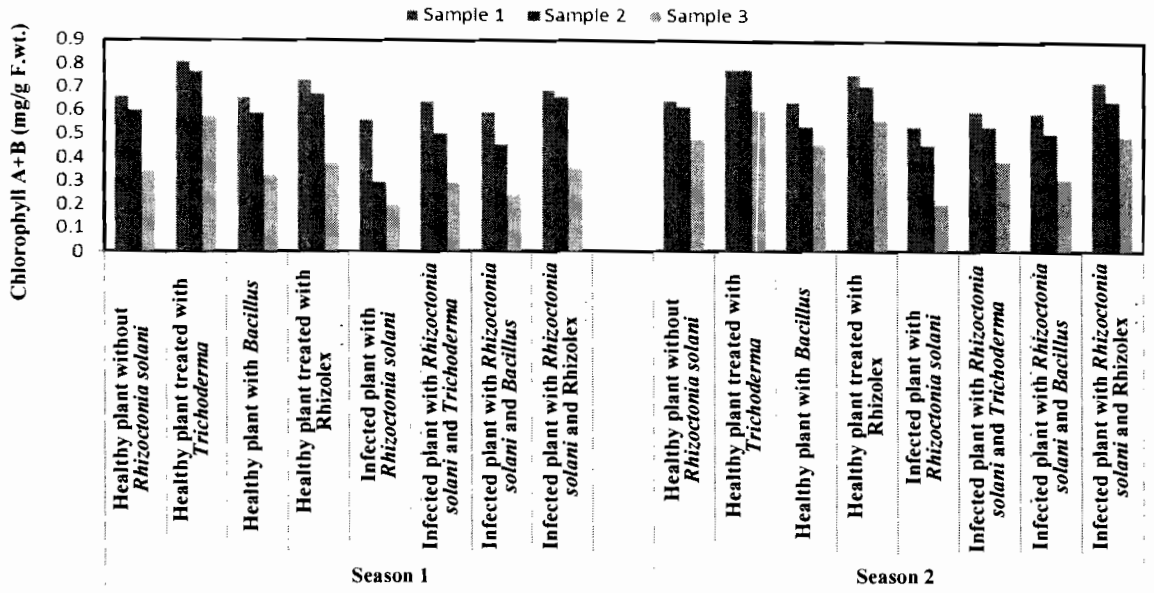

Fig. 3. Effect of biological agents (*Trichoderma virdi* and *Bacillus subtilis*) and chemical fungicide (Rhizolex) on chlorophyll A+B of healthy and infected potato plants at different plant ages of two growing seasons (2011/2012 and 2012/2013)

Table 2. Effect of biological agents (*Trichoderma virdi* and *Bacillus subtilis*) and chemical fungicide (Rhizolex) on chlorophyll A+B of healthy and infected potato plants at two growing seasons (2011/2012 and 2012/2013)

Seasons [A]	Plant ages (DAS) [B]	Healthy or infected potato plants [C]								Mean
		Healthy				Infected				
		1	2	3	4	5	6	7	8	
	30	0.66	0.81	0.65	0.73	0.56	0.64	0.59	0.69	0.67
1st	**60**	0.60	0.77	0.59	0.67	0.30	0.50	0.46	0.66	0.57
2011/2012	**90**	0.34	0.57	0.33	0.38	0.20	0.29	0.24	0.35	0.34
	Mean	**0.53**	**0.72**	**0.52**	**0.59**	**0.35**	**0.48**	**0.43**	**0.57**	**0.52**
	30	0.64	0.78	0.64	0.76	0.53	0.60	0.60	0.73	0.66
2nd	**60**	0.62	0.78	0.53	0.71	0.46	0.53	0.51	0.65	0.60
2012/2013	**90**	0.48	0.61	0.46	0.56	0.20	0.39	0.31	0.49	0.44
	Mean	**0.58**	**0.72**	**0.54**	**0.67**	**0.40**	**0.51**	**0.47**	**0.62**	**0.56**
		Impact of B, C or BC factors								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mean**
	30	0.65	0.80	0.65	0.74	0.55	0.62	0.59	0.71	0.66
Plant ages	**60**	0.61	0.77	0.56	0.69	0.38	0.52	0.48	0.65	0.58
(DAS)	**90**	0.41	0.59	0.39	0.47	0.20	0.34	0.28	0.42	0.39
	Mean	**0.56**	**0.72**	**0.53**	**0.63**	**0.37**	**0.49**	**0.45**	**0.59**	**0.54**
LSD			A	B	AB	C	AC	BC	ABC	
at 0.05			0.01	0.02	0.02	0.03	0.04	0.05	0.07	

Notes

DAS= 30, 60 and 90 days after sowing.

Healthy or infected potato plants

1 = without *Rhizoctonia solani*, 2 = with *Trichoderma virdi*, 3 = with *Bacillus subtilis*,
4 = with Rhizolex, 5 = with *Rhizoctonia solani*, 6 = *Rhizoctonia solani* with *Trichoderma virdi*,
7 = *Rhizoctonia solani* with *Bacillus subtilis*, 8 = *Rhizoctonia solani* with Rhizolex.

The pathogen affected the rate of chlorophyll in potato leaves. The least value was 0.37 mg/g fresh weight of leaves in infected plants with *Rhizoctonia solani*.

It was found from results that the rate of total chlorophyll (A+B) was decreased by advancing potato plants ages. The rate was 0.66, 0.58 and 0.39 mg/g fresh wt. of leaves by advancing the plant ages beginning with 30, 60 and 90 days after planting. It was noticed that the increasing in potato plants ages and closer to harvest time, the leaves of potato plants were been yellow and the chlorophyll break, which increases the appearance of other pigments such as carotenoids and appears the leaves colors other than green colors.

Proline Concentration

Results of proline concentration of potato plants under various treatments were presented in Fig. 4 and Table 3. It is evident from data that proline concentration was high in plants grown in soil which inoculated with the fungal of *R. solani* rather than other treatments. The concentration of proline in infected plants was 5.50, 4.87, 5.07 and 3.69% with *R. solani*, *Trichoderma*, *Bacillus* and Rhizolex, respectively.

The proline accumulation was the highest in infected plants with *R. solani*; this might be due to the induction of resistance against infection. On the other hand, lowest proline content was showed under using Rhizolex as a chemical fungicide against the fungal, this reduction of proline might be due to minimization of the effect of *R. solani* by the added fungicide.

Noticeable, from untreated potato plants results that the highest proline concentration was 3.95% under using *Bacillus*. *Bacillus subtilis* synthesizes large amounts of the compatible solute proline as a cellular defense against high osmolarity to ensure a physiologically appropriate level of hydration of the cytoplasm and turgor (Hoffmann *et al.*, 2012).

The influence of plant ages was a significant effect on proline concentration. It was 3.48, 4.24 and 4.89% at 30, 60 and 90 days after planting, respectively.

Total carbohydrates, starch and protein concentrations in tubers

Effect of different treatments on total carbohydrates, starch and protein concentrations in tubers was illustrated in Fig. 5 and Table 4.

Bacillus gave 86.70% of carbohydrate concentration in tubers for healthy plants, while in infected plants; *Bacillus* gave the highest concentration of 81.28%. *Bacillus subtilis* was an effective biological agent in improving seed quality such as seed germination and nutritional quality such as protein content and carbohydrate content (Prathibha and Siddalingeshwara, 2013).

The starch concentration in infected plants was 65.61, 71.19, 66.74 and 78.59% with *Rhizoctonia solani*, *T. virdi*, *Bacillus* and Rhizolex, respectively.

With regard to protein concentration, Rhizoctonia decreased the concentration by 18.79% compared to control treatment. The highest concentration was obtained by Rhizolex, it was 13.82%.

Black scurf disease ratio

Fig. 6 and Table 5 showed the effect of different treatments on black scurf disease ratio of potato plants.

R. solani increased the disease ratio by 63.01 and 69.01% in the first and second seasons, respectively. The effect of *R. solani* is in agreement with Lutomirska (2010).

It was noticed from results that using fungicide was the best treatment to resist the pathogen. Disease ratio in infected plants by using Rhizolex was 10.17 and 14.00% in the first and second seasons, on the same order. Add to that, the percentage of disease ratio was decreased under using *T. virdi* as a biological control by 25.60% in the first season and 35.11% in second season compared to the infected plants with *Rhizoctonia solani*.

Anatomical sections of potato plant tubers

The anatomical studies of potato plant tubers were done to determine the effect of different treatments on thickness of phellem as shown in Figs. 7 and 8 and Table 6.

Phellem (the cork) consists of cells that are dead at maturity and their primary walls become covered from the inside by the secondary wall

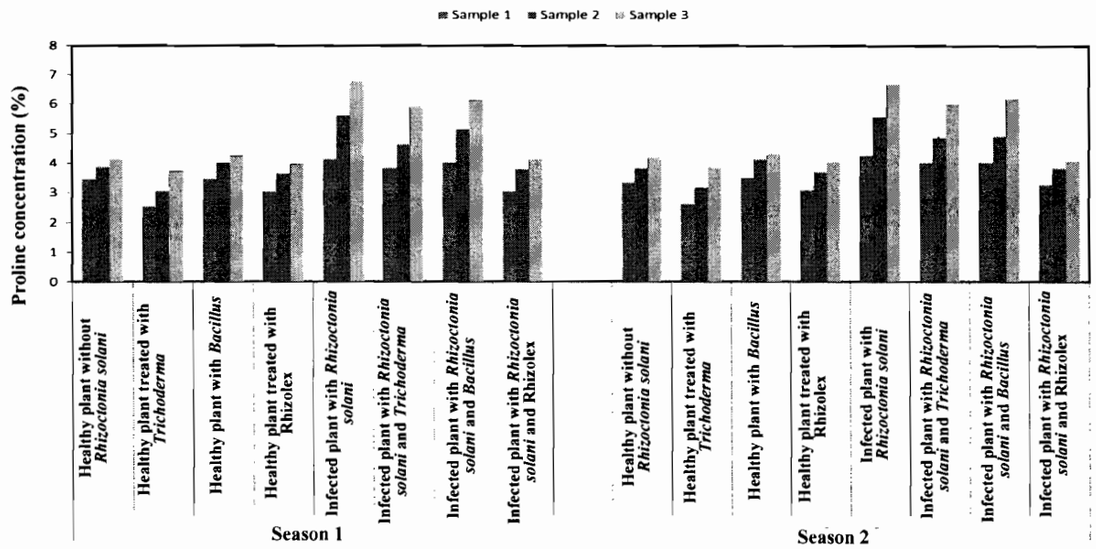

Fig. 4. Effect of biological agents (*Trichoderma virdi* and *Bacillus subtilis*) and chemical fungicide (Rhizolex) on proline concentration of healthy and infected potato plants at different plant ages of two growing seasons (2011/2012 and 2012/2013)

Table 3. Effect of biological agents (*Trichoderma virdi* and *Bacillus subtilis*) and chemical fungicide (Rhizolex) on proline concentration of healthy and infected potato plants at two growing seasons (2011/2012 and 2012/2013)

Seasons [A]	Plant ages (DAS) [B]	Healthy or infected potato plants [C]								Mean
		Healthy				Infected				
		1	2	3	4	5	6	7	8	
1st 2011/2012	1	3.44	2.54	3.48	3.03	4.15	3.83	4.02	3.04	3.44
	2	3.89	3.06	4.02	3.65	5.61	4.64	5.14	3.79	4.23
	3	4.13	3.74	4.24	3.97	6.76	5.91	6.13	4.11	4.87
	Mean	**3.82**	**3.11**	**3.91**	**3.55**	**5.51**	**4.79**	**5.10**	**3.65**	**4.18**
2nd 2012/2013	1	3.35	2.64	3.49	3.08	4.24	4.00	4.04	3.28	3.52
	2	3.81	3.17	4.13	3.70	5.57	4.86	4.91	3.84	4.25
	3	4.18	3.85	4.32	4.03	6.67	5.99	6.20	4.08	4.92
	Mean	**3.78**	**3.22**	**3.98**	**3.60**	**5.49**	**4.95**	**5.05**	**3.73**	**4.23**
		Impact of B, C or BC factors								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mean**
Plant ages (DAS)	1	3.40	2.59	3.49	3.06	4.20	3.92	4.03	3.16	3.48
	2	3.85	3.12	4.08	3.68	5.59	4.75	5.03	3.82	4.24
	3	4.16	3.80	4.28	4.00	6.72	5.95	6.17	4.10	4.89
	Mean	**3.8**	**3.17**	**3.95**	**3.58**	**5.50**	**4.87**	**5.07**	**3.69**	**4.20**
LSD at 0.05			A	B	AB	C	AC	BC	ABC	
			0.18	0.06	NS	0.10	0.14	0.18	NS	

Notes

DAS= 30, 60 and 90 days after sowing.

Healthy or infected potato plants

1 = without *Rhizoctonia solani*, 2 = with *Trichoderma virdi*, 3 = with *Bacillus subtilis,*
4 = with Rhizolex, 5 = with *Rhizoctonia solani*, 6 = *Rhizoctonia solani* with *Trichoderma virdi*,
7 = *Rhizoctonia solani* with *Bacillus subtilis,* 8 = *Rhizoctonia solani* with Rhizolex.
NS = Not significant at 0.05 probability.

Table 4. Effect of biological agents (*Trichoderma virdi* and *Bacillus subtilis*) and chemical fungicide (Rhizolex) on carbohydrates, starch and protein of healthy and infected potato plants after 90 days from planting at two growing seasons (2011/2012 and 2012/ 2013)

Seasons [A]	Healthy or Infected potato plants [B]		Carbohydrate in tubers (%)	Starch in tubers (%)	Protein in tubers (%)
1st 2011/2012	Healthy potato plants	1	80.53	72.37	13.08
		2	88.25	60.06	13.98
		3	86.81	60.38	19.66
	Infected potato plants	4	73.77	69.28	12.55
		5	70.74	65.85	10.55
		6	75.23	71.04	8.94
		7	81.52	66.78	12.30
		8	79.74	78.56	13.65
	Mean		**79.57**	**68.04**	**13.09**
2nd 2012/2013	Healthy potato plants	1	81.05	72.49	13.00
		2	86.05	60.00	14.05
		3	86.58	60.52	19.59
	Infected potato plants	4	72.18	69.91	12.43
		5	71.43	65.36	10.62
		6	75.22	71.33	8.83
		7	81.04	66.70	12.14
		8	79.48	78.61	13.98
	Mean		**79.13**	**68.12**	**13.08**
Healthy potato plants		1	80.79	72.43	13.04
		2	87.15	60.03	14.02
		3	86.70	60.45	19.63
Infected potato plants		4	72.98	69.60	12.49
		5	71.09	65.61	10.59
		6	75.23	71.19	8.89
		7	81.28	66.74	12.22
		8	79.61	78.59	13.82
LSD at 0.05		**A**	0.75	0.23	NS
		B	3.27	2.19	1.76
		AB	NS	NS	NS

Notes

DAS= 30, 60 and 90 days after sowing.

Healthy or infected potato plants

1 = without *Rhizoctonia solani*, 2 = with *Trichoderma virdi*, 3 = with *Bacillus subtilis*,
4 = with Rhizolex, 5 = with *Rhizoctonia solani*, 6 = *Rhizoctonia solani* with *Trichoderma virdi*,
7 = *Rhizoctonia solani* with *Bacillus subtilis*, 8 = *Rhizoctonia solani* with Rhizolex.
NS = Not significant at 0.05 probability.

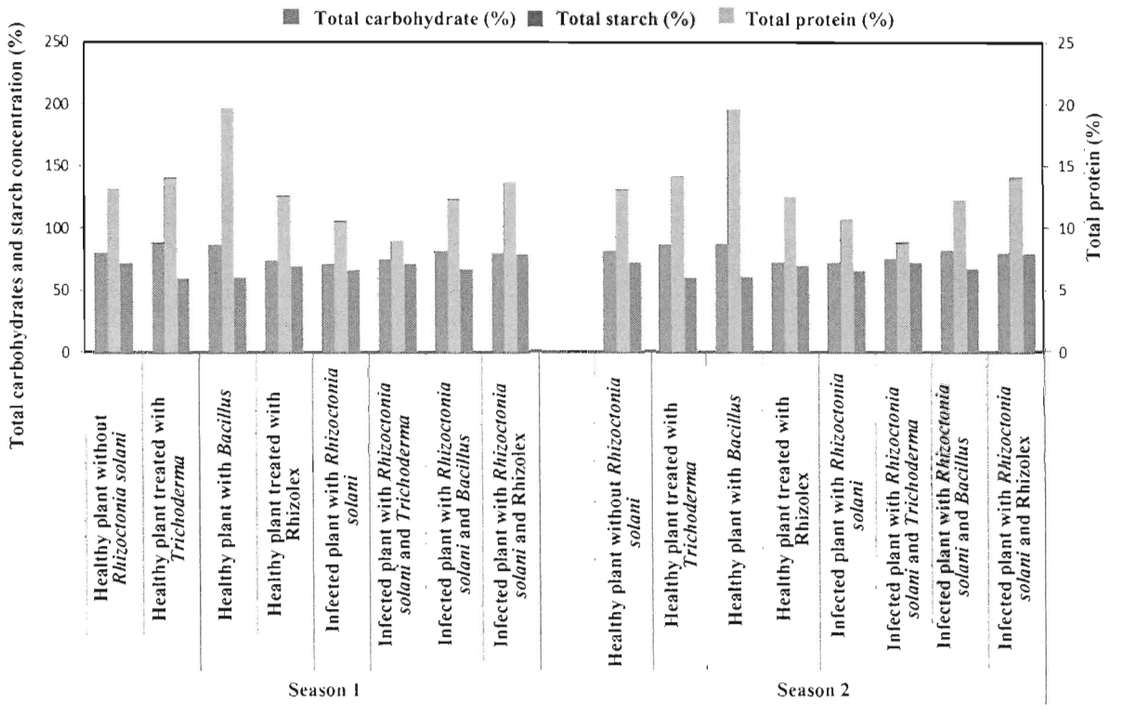

Fig. 5. Effect of biological agents (*Trichoderma virdi* and *Bacillus subtilis*) and chemical fungicide (Rhizolex) on total carbohydrates, starch and protein concentrations in tubers for healthy and infected potato plants at two growing seasons (2011/2012 and 2012/2013)

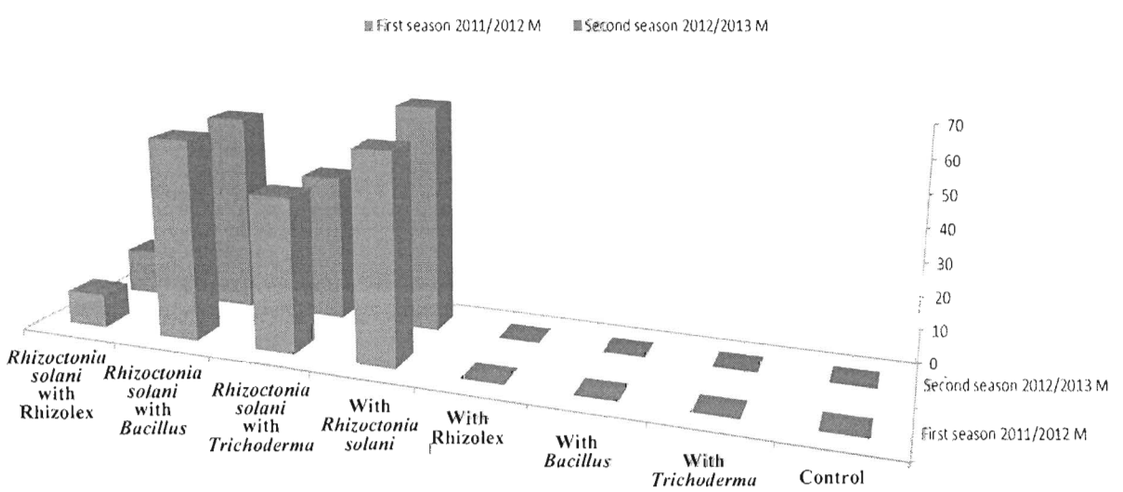

Fig. 6. Effect of biological agents (*Trichoderma virdi* and *Bacillus subtilis*) and chemical fungicide (Rhizolex) on black scurf disease ratio for healthy and infected potato plants at two growing seasons (2011/2012 and 2012/2013)

Table 5. Effect of biological agents (*Trichoderma virdi* and *Bacillus subtilis*) and chemical fungicide (Rhizolex) on black scurf disease ratio of healthy and infected potato plants at two growing seasons (2011/2012 and 2012/2013)

Seasons	Healthy or infected potato plants		Disease ratio (%)
1st 2011/2012	Healthy potato plants	1	0.00
		2	0.00
		3	0.00
		4	0.00
	Infected potato plants	5	63.01
		6	46.88
		7	61.19
		8	10.17
2nd 2012/2013	Healthy potato plants	1	0.00
		2	0.00
		3	0.00
		4	0.00
	Infected potato plants	5	69.01
		6	44.78
		7	61.11
		8	14.00

Notes

Healthy or infected potato plants

1 = without *Rhizoctonia solani*, 2 = with *Trichoderma virdi*, 3 = with *Bacillus subtilis*, 4 = with Rhizolex, 5 = with *Rhizoctonia solani*, 6 = *Rhizoctonia solani* with *Trichoderma virdi*, 7 = *Rhizoctonia solani* with *Bacillus subtilis*, 8 = *Rhizoctonia solani* with Rhizolex.

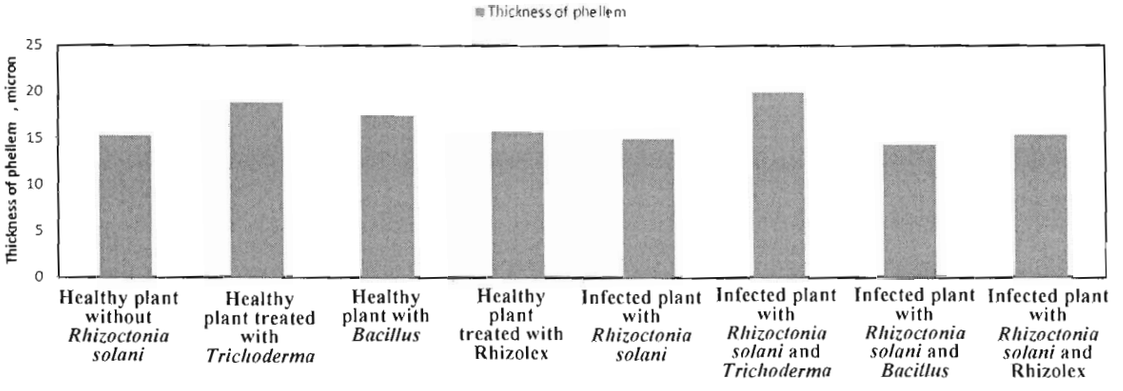

Fig. 7. Effect of different treatments on thickness of phellem of potato tubers

Table 6. Effect of biological agents (*Trichoderma virdi* and *Bacillus subtilis*) and chemical fungicide (Rhizolex) on anatomical studies of potato tubers after 90 days from planting of healthy and infected potato plants

Anatomical studies of potato tubers	Healthy or infected potato plants							
	Healthy				Infected			
	1	2	3	4	5	6	7	8
Thickness of phellem	15.25	18.80	17.38	15.61	14.90	19.86	14.19	15.25

Notes

Healthy or infected potato plants

1 = without *Rhizoctonia solani*, 2 = with *Trichoderma virdi*, 3 = with *Bacillus subtilis*, 4 = with Rhizolex, 5 = with *Rhizoctonia solani*, 6 = *Rhizoctonia solani* with *Trichoderma virdi*, 7 = *Rhizoctonia solani* with *Bacillus subtilis*, 8 = *Rhizoctonia solani* with Rhizolex.

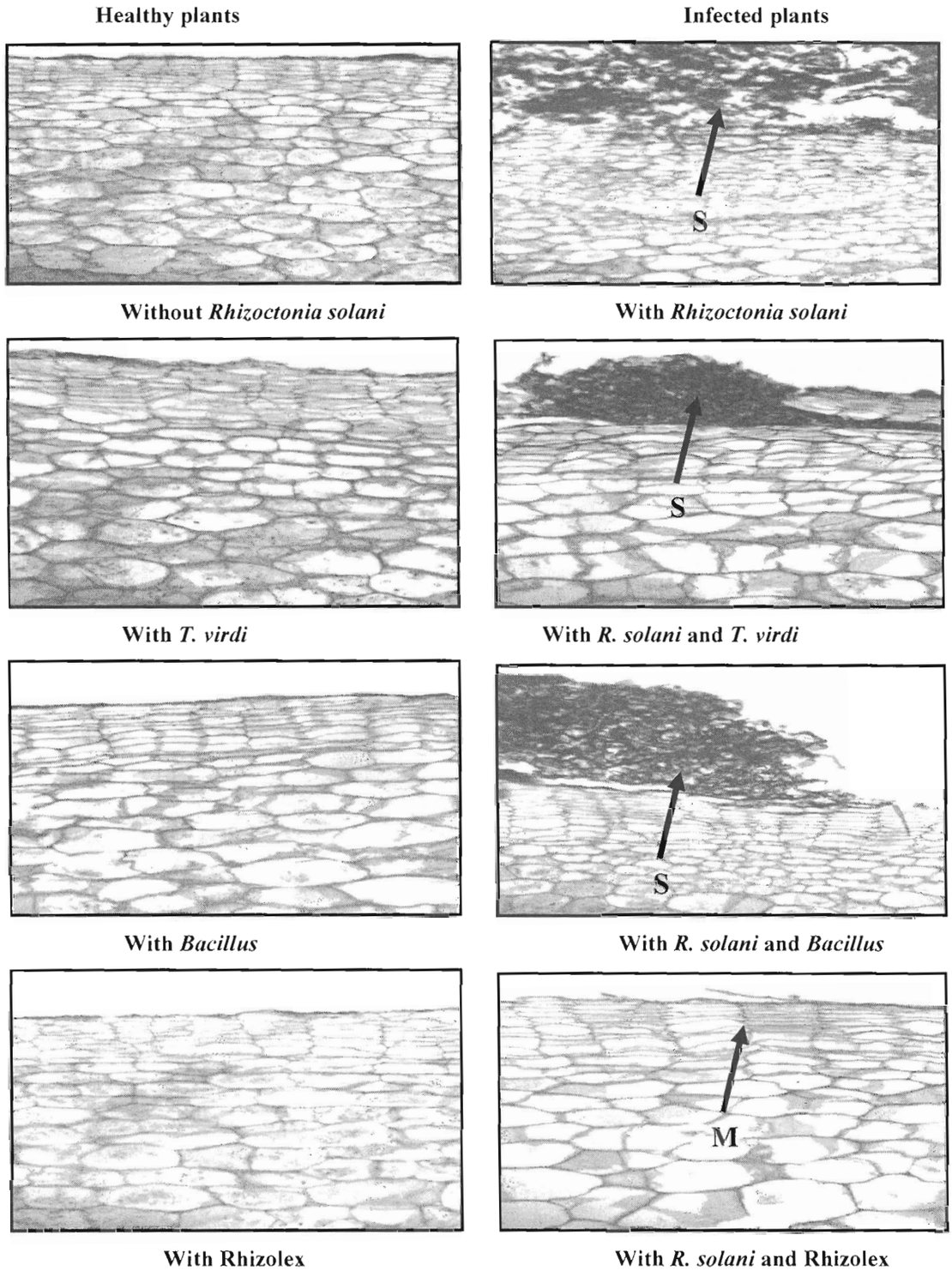

Fig. 8. Anatomical sections of potato plant tubers

Notes: S: Sclerotia of *Rhizoctonia solani* M: Mycelium of *Rhizoctonia solani*

which consists of parallel suberin lamellae alternating with wax layers.

R. solani decreased the thickness of phellem by 2.30 % compared to control treatments.

On the hand, the highest thickness of phellem was by using *T. virdi*. It was 19.86 micron with infected plants by the pathogen, while the thickness was 18.80 micron with healthy potato plants.

Conclusion

Based on the obtained results in this study, the following recommendations can be drawn:

1. Using *Trichoderma virdi* as an effective biological control against the pathogen which motivates the growth of potato plants.
2. Rhizolex as a chemical control to *Rhizoctonia solani* fungus at the dose recommended that reduces the disease ratio of potato tubers.

REFERENCES

AOAC (1970). Official methods of analysis of the association of official agricultural chemists. 11th Ed. Pub. By the AOAC, P.O. Box 540, Washington.

AOAC (1990). Official methods of analysis of the association of official analytical chemists, vol. II, 15th Ed. Sec. 985.29. The Association: Arlington, VA.

Banville, G.J. and D.E. Carling (2001). *Rhizoctonia* canker and black scurf. In: Stevenson, W.R., Loria, R., Franc, G.D., Weingartner, D.P. (Eds.), Compendium of Potato Diseases, second Ed. APS Press, St. Paul, MN, 36 –37.

Barnett, H.L. and B.B. Hunter (1972). Illustrated genera of imperfect fungi. Third Ed. Burgress Publ. company minneopolis, Minneosta, 2411.

Bates, L.S., R.P. Waldren and I.D. Teare (1973). Rapid determination of free proline for water stress studies. Plant and Soil, 39: 205 – 207.

Bernfeld, P. (1955). Methods in enzymology, 1: 149 – 154.Acad, Press, Inc; New York.

Dubois, M., K. Gilles, J. Hamilton, P. Rebers and F. Smith (1956). Colorimetric method for determination of sugars and related substances. Analytical Chem., 28 (3):350 – 356.

El-Kot, G.A.N. (2008). Biological control of black scurf and dry rot of potato. Egypt. J. Phytopathol; 36 (1– 2): 45-56.

Fadeel, A.A. (1962). Location and properties of chloroplasts and pigment determination in roots. Physiol. Plant, 15: 130 – 147.

FAO (2010). Food and Agriculture organization of the United Nations. FAO statistics division. FAO state. http://faostat. fao.org/ site/ 567/ DesktopDefault.aspx?PageID=567#ancor.

FAO (2011). Food and Agriculture organization of the United Nations. FAO statistics division. FAO state. http://faostat. fao. org/ site/ 339/default.aspx.

Hoffmann, T., C. Von Blohn, A. Stanek, S. Moses, H. Barzantny and E. Bremer (2012). Synthesis, release and recapture of compatible solute proline by osmotically stressed *Bacillus subtilis* cells. Appl. Environ Microbiol., 78 (16): 5753 – 5762.

Lutomirska, B. (2010). Influence of seed tuber infestation with black scurf on potato yield and symptoms of *Rhizoctonia solani* Kuhn on plant and progeny tubers. [Polish], Progress in Plant Protection, 50 (1): 230-235.

Miller, G.L. (1959). Use of di nitro salicylic acid reagent for determination of reducing sugar. Ann, Chem., 31: 426 – 428.

Mohammed, A.S., S.M. El-Hassan, M.M.A. Elballa and E.A.E. El-Sheikha (2008). The role of *Trichoderma*, VA mycorrhiza and dry yeast in the control of *Rhizoctonia* disease of potato (*Solanum tuberosum* L.). U. of K. J. Agric. Sci., 16 (2): 285 – 301.

Prathibha, K.S. and K.G. Siddalingeshwara (2013). Effect of plant growth promoting *Bacillus subtilis* and *Pseudomonas fluorescence* as Rhizobacteria on seed quality of sorghum.

Snedecor, G.W. and W.G. Cochran (1990). Statistical methods. "8th Ed. Iowa state Univ., Press, Ames Iowa, U.S.A.

Wettstein, D. (1957). Chlorophyll-Lethal under submink roskopische formivechoel der plastiden. Exp. cell. Res., 12: 427 – 433.

Woodhall, J.W., B. Lutomirska, J.C. Peters and P.S. Wharton (2012). *Rhizoctonia solani* anastomosis group 3 is predominant in potato tubers in Poland. Plant Disease. http:// dx.doi.org/10.1094/PDIS-05-12-0482-PDN.

دراســـات تشـــريحيـة مـرضيـة علـى مـرض القشـرة السـوداء فـى نبـاتـات البطـاطـس

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تعتبر البطاطس من محاصيل الخضر ذات الأهمية الغذائية الكبيرة حيث توجد بها العناصر الغذائية بصورة متوازنة فعلاوة على أنها مصدرا هاما للمواد الكربوهيدراتية فإنها أيضا تحتوي على كميات لا بأس بها من البروتين وبعض العناصر المعدنية مثل البوتاسيوم والفوسفور والحديد إلا أنها فقيرة في الكالسيوم كما أنها غنية في فيتامين جـ وتوجد بها كميات ضئيلة من فيتامين أ، ب كما أنها تحتل المرتبة الرابعة بعد محاصيل الحبوب فى التصدير والمرتبة الأولى بالنسبة لمحاصيل الخضر المصدرة، يعتبر مرض القشرة السوداء من أهم الأمراض التى تصيب محصول البطاطس والذى يسببه فطر الريزوكتونيا سولانى والتى تتمثل بعض أعراضه فى تكوين أجسام حجرية فوق قشرة الدرنة غير منتظمة الشكل على الدرنة كما تظهر تقرحات بنية اللون عند قاعدة الساق الهوائية، أجريت التجارب خلال موسمى ٢٠١٠/٢٠١١ و ٢٠١١/٢٠١٢ م بصوبة التجارب بكلية الزراعة – جامعة الزقازيق بمحافظة الشرقية وذلك لدراسة تأثير مرض القشرة السوداء والذى يسببه فطر الريزوكتونيا سولانى على جودة وإنتاجية محصول البطاطس حيث تم استخدام صنف سبونتا لإجراء التجارب عليه وكذلك لتقييم افضل الطرق لمكافحة هذا المرض، تم تقسيم التجربة الى مجموعتين (A) و(B): تربة غير مصابة بفطر الريزوكتونيا سولانى و تربة مصابة بالفطر على الترتيب وتم زراعة كل مجموعة بكلا مما يأتى: درنات بطاطس غير معاملة بالفطر الممرض ودرنات بطاطس معاملة بفطر الترايكوديرما فيردى ودرنات بطاطس معاملة بالبكتيريا باسيلس سابتيلس ودرنات معاملة بمبيد الريزولكس، تم تقييم أثر الفطر وطرق المكافحة البيولوجية والمبيد الفطرى على نباتات البطاطس من حيث: طول النبات (خواص مورفولوجية) – تقدير الصبغات كلوروفيل A+B (خواص فسيولوجيه)– تقدير البرولين والكربوهيدرات والنشا والبروتين وكذلك على التركيب التشريحى لنبات البطاطس، وقد أوضحت النتائج المتحصل عليها أنه للحصول على أعلى انتاجية لمحصول البطاطس وأفضل جودة للدرنات فيوصى بأحد المعاملات الآتية: استخدام فطر الترايكوديرما كأحد طرق المكافحة البيولوجية للفطر حيث وجد أنه يحفز نبات البطاطس على النمو، استخدام مبيد الريزولكس كأحد المبيدات الفطرية بالجرعة الموصى بها والتى تقلل من نسبة الاصابة بمرض القشرة السوداء.

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