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PHYTOTOXIC PROPERTIES OF CULTURE FILTRATES OF MICROMYCETE *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY ISOLATES FROM THE PHYLLOSPHERE OF VARIOUS HOST PLANTS

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Abstract. *Phytotoxicity of isolates in S. sclerotiorum populations extracted from different geographic conditions and host plants has been established. The results of studies of the influence of culture filtrates of 42 isolates of the fungus Sclerotinia sclerotiorum (Lib.) de Bary of different geographical origin on the germination of seeds and growth of seedlings of winter wheat plants are presented. All the isolates tested showed a phytotoxic effect, which consisted of reduced seed germination, inhibition of root system growth and seedlings. S. sclerotiorum isolates extracted from soybean and rapeseed plants (Kyiv region, Vasylkiv district) showed the most negative impact on seed germination rates. Their phytotoxic effect caused a decrease in seed germination by 12.4-13.4 % less than in the control. S. sclerotiorum isolates also induced a reduction in the root length of the plant and inhibition of seedling growth compared to a control average of 1.0 to 57.9%. In particular, 71-100% of fungus isolates extracted from soybean and rapeseed plants (Kyiv region, Vasylkiv district) had a high degree of toxicity.*

Key words: *phytotoxicity, culture filtrate, isolates, population, Sclerotinia sclerotiorum, growth rates*

Introduction.

The necrotroph fungus *Sclerotinia sclerotiorum* (Lib.) de Bary is a cosmopolitan with a wide trophic specialization and is able to parasitize over 400 species of plants from different botanical families (Boland, G. J., Hall R., 1994), causing white mold. In Ukraine, it infects a variety of crops (Kyryk, M.M., Pikovskyi, M.Y., Azaiki, S., 2012), reducing yields and degrading its quality (Kirik, N.N., Koltunov, V.A. Brodai, V.V., 2001; Pikovskyi, M., Kyryk, M., Arnauta, N., 2018). *S. sclerotiorum*, as a causative agent of white mold of plants, has a wide range, occupies various ecological niches and is environmentally plastic. The study of its toxicogenic properties is relevant because of the great importance of this pathogen in various agroecosystems as a dangerous phytotroph. Significant plant damage, lack of high resistance to varieties and hybrids and high susceptibility to white mold caused by *S. sclerotiorum* require constant studies of various aspects of this pathogen (Bolton, M. D., Thomma, B. P. H. J., Nelson, B. D., 2006), including its phytotoxic effects (Sharma, P. C., Meena, P. D., Singh, Dhiraj, 2014).

Analysis of recent research and publications.

Phytotoxins are secondary metabolites, produced by individual pathogens, and have an important role in the interaction of pathogens with plants and the occurrence of the disease (Svabova, L., Lebeda, A., 2005). In the studies of Parveen, Shazia et al. (2019), germination of *Solanum lycopersicum* seeds was completely inhibited by the culture filtrate of the fungi *Trichothecium roseum* and *Alternaria alternata*. *Penicillium expansum* culture filtrate caused of the complete suppression of *Brassica rapa* seed germination. The authors also found that the filtrate of *Fusarium solani* culture at a low concentration increases the percentage of sprouted seeds (Parveen, Shazia & Wani, Ab. Hamid & Bhat, Mohd., 2019). Seed germination and subsequent growth of *Cuminum cyminum* seedlings slows down the *Fusarium equiseti* culture filtrate even at low concentrations (Suthar, Ramchandra & Bhatt, Daksha & Bhatt, Prashant, 2014). Some researchers noted inhibitory and stimulating effects of culture filtrates of *Penicillium* spp. on the germination of wheat seeds depending on the type of fungus (Ibatsam Khokhar et al., 2013). Corn seeds, treated with *Aspergillus niger* and *Penicillium chrysogenum* cultivated filtrates, reduced the germination rates to 65.33 % and 79.67 % respectively, whereas in the control, this indicator was 100 % (Garuba, T. et al., 2015). In studies by Sharma P. et al (2014), culture filtrates of 25 isolates of *S. sclerotiorum,* isolated from *Brassica juncea* cv. Rohini, reduced the germination of seeds at the varying degrees.

Thus, the analysis of scientific publications shows the different influence of the culture filters of phytopathogenic fungi on the germination of seeds, which is related to the species and strain differences of microorganisms (Suthar, Ramchandra & Bhatt, Daksha & Bhatt, Prashant, 2014; Parveen, Shazia & Wani, Ab Hamid & Bhat, Mohd, 2019). At the same time, information on the phytotoxicity studies of *S. sclerotiorum* has not been reported in Ukraine over the last decades, and these issues have not been studied.

The purpose of the study was to determine the spectrum of phytotoxic activity of the culture filtrate of *S. sclerotiorum*

isolates from different host plants and to analyze their effect on winter wheat germination, root system growth and seedlings.

Materials and methods.

42 isolates of the fungus *S. sclerotiorum* were the objects of the study (Table 1). They have been removed from different geographical regions from the soybean, rapeseed, sunflower and dahlias. They were stored in a working collection of pure cultures of the Department of Phytopathology named after Academician V.F. Peresypkin of the National University of Life and Environmental Sciences of Ukraine (NULES of Ukraine).

The 5 mm diameter agar discs were cut out by a cork drill from the edge of a 7-day actively growing *S. sclerotiorum* colony. One mycelial disc was placed in a 250 ml conical flask with 100 ml of Richard's sterile liquid medium and incubated at 23 °C for 14 days (Sharma, P. C., Meena, P. D., Singh, & Dhiraj, 2014). Subsequently, the culture fluid was filtered and winter wheat seeds (variety Nationalna) soaked in it for 24 h (Bilay, V. I., 1982). A sterile nutrient medium as well as distilled and tap water were used for control. The seeds were laid out in Petri dishes on evenly moistened filter paper and incubated in a thermostat at 22-24 °C for 7 days.

The presence of phytotoxins in the culture filtrate was determined by the growth effects: seed germination and length of seedlings and roots. *S. sclerotiorum* isolates were moderately slightly toxic, if the inhibition of root growth or seedlings was 1-10 %, 11-29 % – medium toxic, when inhibition of growth of these organs was not less than 30 % – highly toxic. The experiment was repeated three times. Statistical processing of the experimental data was performed using Microsoft Office® for Microsoft Windows®.

Results and discussion.

As a result of the tests, it was found that all isolates of *S. sclerotiorum* fungus, isolated from different plants and

1. List of Sclerotinia sclerotiorum isolates used in the studies

Place of screening of the affected plants		Plants	Isolate number
regions, city	districts		
Kyiv	Vasylkivskyi	Soybeans (Glycine max L.)	Gm1, Gm2, Gm3, Gm4, Gm5, Gm6, Gm7
Zhytomyr	Popilnyanskyi	Sunflower (Helianthus annuus L.)	Han8, Han9, Han10, Han11, Han12, Han13, Han14
Kyiv	Bilotserkivskyi	Sunflower (Helianthus annuus L.)	Han15, Han16, Han17, Han18, Han19, Han20
Kyiv	Holosiivskyi	Dahlia (Dahlia Cav.)	D21, D22, D23, D24, D25, D26, D27
Kyiv	Vasylkivskyi	Winter rapeseed (Brassica napus L.)	Bn28, Bn29, Bn30, Bn31, Bn32
Ivano-Frankivsk	Gorodenkivskyi	Sowing peas, grain (Pisum sativum L.)	Ps33, Ps34, Ps35, Ps36 Ps37
Kyiv	Yagotinskyi	Sunflower (Helianthus annuus L.)	Han38, Han39, Han40, Han41, Han42

different geographical origin in Ukraine, led to a decrease in winter wheat seed germination, indicating that pathogenic phytotoxic metabolites were formed by the pathogen. Thus, laboratory germination of seeds in variants with the culture fluid filtrate (CFF) of Gm4, Han19 and Gm5 isolates decreased the most – up to 82-83 %, which is 18-19 % less than in the control (Table 2). Other fungus iso-

2. Effect of culture filtrate of S. sclerotiorum isolates on seed germination and growth of winter wheat seedlings

Isolates	Laboratory germination of seeds,%	Length, cm		Decrease in length			
		roots	seedlings	roots		seedlings	
				cm	%	cm	%
1	2	3	4	5	6	7	8
Gm1	88	6,58	3,24	3,00	45,6	1,86	57,4
Gm2	84	7,25	3,65	2,35	32,4	1,45	39,7
Gm3	93	8,01	4,75	1,59	19,9	0,35	7,37
Gm4	82	6,78	3,38	2,82	41,6	1,72	50,9
Gm5	83	6,99	4,44	2,61	37,3	0,66	14,9
Gm6	85	7,65	4,55	1,95	25,5	0,55	12,0
Gm7	91	6,6	4,38	3,00	45,5	0,72	1,6
Han8	95	9,20	4,47	0,40	4,40	0,63	14,0
Han9	89	6,08	3,84	3,52	57,9	1,26	32,8
Han10	98	8,43	4,41	1,17	13,9	0,69	15,7
Han11	97	8,39	4,42	1,21	14,4	0,68	15,4
Han12	84	7,77	4,70	1,83	23,6	0,40	8,5
Han13	86	7,35	4,53	2,25	30,6	0,57	12,6
Han14	89	8,17	4,18	1,43	17,5	0,92	22,0
Han15	95	8,71	4,70	0,89	4,10	0,35	7,5
Han16	95	9,22	4,75	0,38	9,90	0,66	13,9
Han17	98	8,75	4,44	0,87	9,90	0,66	14,9
Han 18	89	7,99	4,45	1,61	20,2	0,65	14,6
Han 19	82	6,36	4,75	3,24	50,9	0,35	7,4
Han 20	85	8,85	5,12	0,75	8,50	0,20	3,9
D21	96	9,12	4,62	0,48	5,30	0,48	10,4
D22	94	8,21	4,61	1,39	16,9	0,49	10,6
D23	95	8,61	4,89	0,99	11,5	0,21	4,3
D24	95	8,70	5,0	0,90	10,3	0,10	2,0
D25	90	7,90	4,32	1,70	21,5	0,78	18,0
D26	89	7,88	4,29	1,72	21,8	0,81	18,9
D27	90	8,28	5,00	1,32	15,9	0,1	2,0
Bn28	89	6,97	4,43	2,63	37,7	0,67	15,1
Bn29	84	6,25	4,21	3,35	53,6	0,89	21,1
Bn30	89	4,78	3,64	4,82	38,0	1,46	40,1
Bn31	86	6,80	3,76	2,80	41,2	1,34	35,6
Bn32	90	7,20	4,19	2,40	33,3	0,91	21,7
Ps33	98	9,67	4,72	0,69	7,70	0,38	8,0

2. Effect of culture filtrate of S. sclerotiorum isolates on seed germination and growth of winter wheat seedlings *(continuation)*

1	2	3	4	5	6	7	8
Ps34	91	7,02	4,31	2,58	36,8	0,79	18,3
Ps35	90	6,98	4,69	2,62	37,5	0,41	1,9
Ps36	94	7,85	5,00	1,85	23,6	0,1	2,0
Ps37	95	8,28	4,53	1,32	15,9	0,57	12,6
Han38	98	9,71	4,43	0,10	1,00	0,67	15,1
Han39	97	8,54	4,91	1,00	11,7	0,19	3,9
Han40	95	9,13	5,00	0,47	5,50	0,1	2,0
Han41	96	8,02	5,33	1,58	19,7	0,1	1,9
Han42	92	9,21	4,70	0,39	4,20	0,4	8,5
Control (nutrient medium)	100	9,6	5,1				
Control (H2O tap)	100	8,83	4,87				
Control (H2O distilled)	100	10,54	5,43				
ID05	0,30	0,07	0,05				

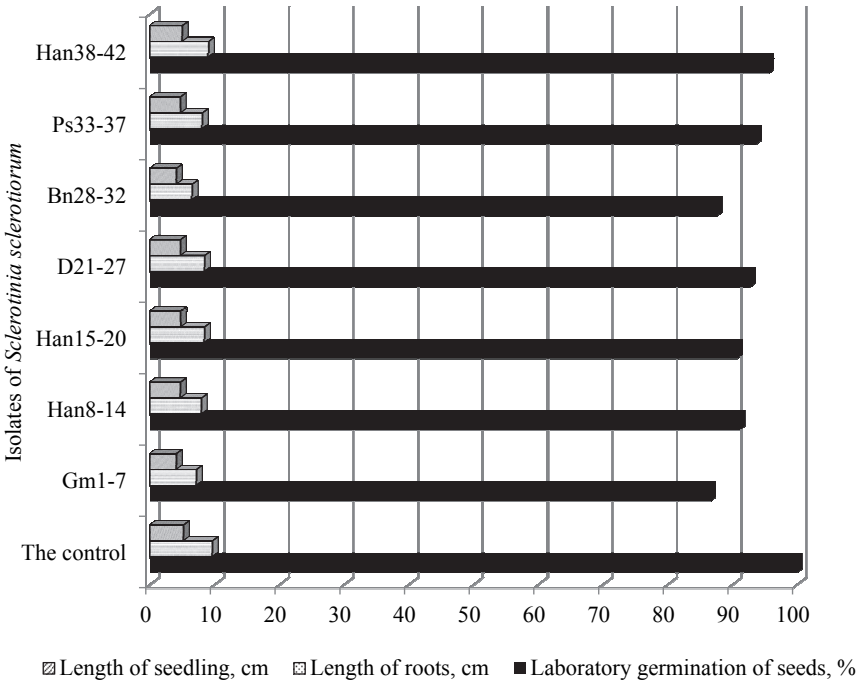

Fig. 1. Results of phytotoxic action of isolates on winter wheat seeds

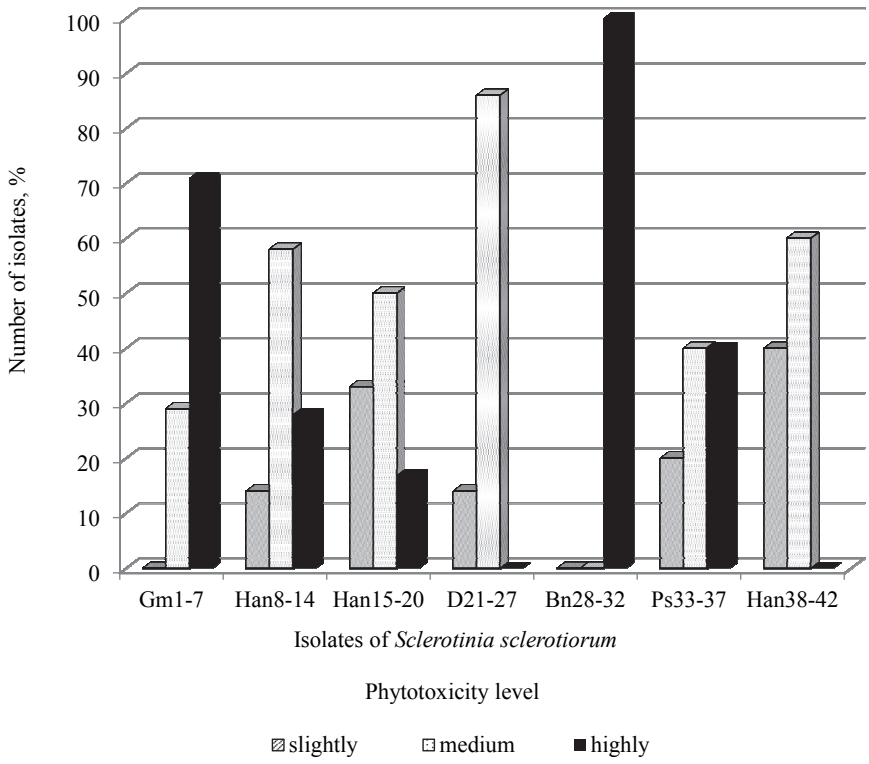

Fig. 2. Differentiation of phytotoxicity of isolates in Sclerotinia sclerotiorum populations, extracted from different geographical conditions and host plants

lates caused a 2-17 % decrease in laboratory germination of seeds.

In addition to inhibition of seed germination, it has found inhibition of the length of the root system and seedlings of winter wheat plants. All the isolates of the *S. sclerotiorum* fungus under study had this effect. In particular, the reduction in root length of the plant compared to the control (sterile liquid nutrient medium) ranged from 0.1 to 4.8 cm or 1.0-57.9 %. The metabolites of the fungus adversely affected on the seedlings of the plants, causing inhibition of their growth by 0.1-1.86 cm (1.6-57.4 %).

S. sclerotiorum isolates from soybean and rapeseed plants in the Kyiv region of Vasylkivskyi district showed the most negative effect on the seed germination index (Fig. 1). So, the seed germination was 86.6-87.6 % on average, due to the effect of phytotoxicity, it was less than in the control on 12.4-13.4 %. Cultural filtrate of fungus isolates from sunflower plants (Zhytomyr region, Popilnyanskyi district) reduced seed germination by 8.9%. Pathogen population from the same culture, but from Kyiv region, Bilotserkivskyi district, also had a negative effect on the similarity, which was 90.7 % (less than the control by 9.3 %). *S. sclerotiorum* isolates, removed from dahlia plants in Kyiv, reduced of the seed germination by 7.3 % and from peas (Ivano-Frankivsk region, Gorodenkivskyi district) – by 6.4 %. Isolates of the fungus population extracted from

sunflower plants (Kyiv region, Yagotinsky district), were the least phytotoxic in influencing the germination of wheat seeds and reduced the laboratory germination of seeds by only 4.4 %.

Analysis of the phytotoxicity ratio of isolates in *S. sclerotiorum* populations, removed from different geographical conditions and host plants, showed that all pathogen isolates (100%) from rapeseed plants (Kyiv region, Vasylkiv district) and 71% of isolates from plants were highly toxic soybeans (Fig. 2). Fungus isolates from sunflower plants (Kyiv region, Yagotinsky district) and dahlia (Kyiv) did not characterize of high toxicity against winter wheat seeds. The medium-toxic isolates (86 and 40 % respectively) were dominated in these populations of white mold pathogen.

Conclusions.

It was found that all 42 isolates of *S. sclerotiorum* fungus isolated from soybean, sunflower, rapeseed, dahlia and pea plants of different geographical origin in Ukraine had phytotoxic effects on seed germination, root system growth and winter wheat seedlings.

The conducted studies revealed the intraspecific differences of individual *S. sclerotiorum* isolates by the degree and spectrum of phytotoxicity. Strong phytotoxic activity was shown by *S. sclerotiorum* isolates, extracted from soybean and rapeseed plants (Kyiv region, Vasylkiv district), which reduced seed germination by an average of 12.4-13.4% compared to control. Isolates of the fungus populations extracted from sunflower plants (Kyiv region, Yagotinsky district) were the least phytotoxic in the germination of wheat seeds and reduced the laboratory germination of seeds by only 4.4%.

The culture filtrate of the fungus *S. sclerotiorum* caused a decrease in the length of the root system of the plants compared to the control (sterile liquid nutrient medium) by 1.0-57.9%, and seedlings of seeds, respectively, by 1.6-57,4 %.

It was found that all the isolates of the pathogen extracted from rapeseed plants under conditions of the Kyiv region of Vasylkiv district were highly toxic. Among the isolates of the fungus from the affected sunflower plants (Kyiv region, Yagotinsky district) and dahlia (Kyiv) were not very toxic for winter wheat seeds. In these populations, the white rot pathogen was dominated by medium-toxic isolates, 86 and 40 %, respectively.

It have been shown, that secondary metabolites of *S. sclerotiorum* culture fluid filtrate had a prolonged action: the laboratory germination decreased on 2.5-18.3 % compared to the control, the length of the root system and seedlings, in average respectively – on 53.6 and 57.4 %.

The high toxigenic activity some of *S. sclerotiorum* isolates, as a component of a complex infection of many plants, should be taken into account as a criterion for the pathogen's harmfulness and taken into account during the development of artificial infectious backgrounds and for the organization of protection measures.

Further studies should address the nature of fungal inhibitory substances and their effect on plant morphometric parameters.

References

1. Boland, G. J., Hall R. (1994). Index of Plant Hosts of Sclerotinia sclerotiorum. Canadian Journal of Plant Pathology. 16. 93-108.

2. Kyryk, M.M., Pikovskyi, M.Y., Azaiki, S. (2012). Diagnostic signs of diseases of vegetable crops and potato. Kyiv: Phenix. 175.
3. Kirik, N. N., Koltunov, V. A., Borodai, V. V. (2001). Patologicheskiye izmeneniya v korneplodakh morkovi pod vliyaniyem vozbuditeley gniley v period khraneniya. [Pathological changes in rootsof carrot causal by fungi during the storage]. Mikologiya i fitopatologiya. 35. 6. 48-51. (In Russian).
4. Pikovskyi, M., Kyryk, M., Arnauta, N. (2018). Shkidlyvist' biloyi hnyli horokhu. [The harmfulness of white mold peas]. Zahist i karantin roslin. 64. 143-149. (In Ukrainian).
5. Bolton, M. D., Thomma, B. P. H. J., Nelson, B. D. (2006). Sclerotinia sclerotiorum (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen. Mol. Plant Pathol. 7. 1. 1-16. doi: 10.1111/j.1364-3703.2005.00316.x PMID: 20507424
6. Sharma, P. C., Meena, P. D., Singh, Dhiraj. (2014). Effect of Sclerotinia sclerotiorum culture filtrate on seed germination and seedling vigour of Indian mustard (Brassica juncea cv. Rohini). Journal of Oilseed Brassica. 5. 2. 158-161.
7. Svabova L., Lebeda A. (2005). In vitro selection for improved plant resistance to toxin-producing pathogens. J. Phytopathol. 153. 52-64. https://doi.org/10.1111/j.1439-0434.2004.00928.x
8. Parveen, Shazia & Wani, Ab. Hamid & Bhat, Mohd. (2019). Effect of culture filtrates of pathogenic and antagonistic fungi on seed germination of some economically important vegetables. Brazilian Journal of Botany. 6. 133-139. https://doi.org/10.21472/bjbs.061212
9. Suthar, Ramchandra & Bhatt, Daksha & Bhatt, Prashant. (2014). Effect of culture filtrate of Fusarium equiseti on seed germination and seedling growth of cumin (Cuminum cyminum). Indian Phytopath. 67.2. 193-194.
10. Ibatsam Khokhar, Muhammad Saleem Haider, Irum Mukhtar, Amna Ali, Sobia Mushtaq, Muhammad Ashfaq. (2013). Effect of Penicillium species culture filtrate on seedling growth of wheat. International Research Journal of Agricultural Science and Soil Science. 3.1. 24-29.
11. Garuba, T., Abdulrahaman, A.-A, Olahan, G. S., Abdulkareem, K. A., Amadi, J. E. (2015). Effects of Fungal Filtrates on Seed Germination and Leaf Anatomy of Maize Seedlings (Zea mays L., Poaceae). Journal of Applied Sciences and Environmental Management. 18. 4. 662-667. http://dx.doi.org/10.4314/jasem.v18i4.15
12. Bilay, V. I., editor. (1982). Metody eksperimetal'noy mikologii. [Methods of Experimental Mycology]. Kiev: Nauk. Dumka. 550 p. (In Russian).

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Анотація. *Представлено результати досліджень впливу культуральних фільтратів 42 ізолятів гриба Sclerotinia sclerotiorum (Lib.) de Bary різного географічного походження на схожість насіння та ріст проростків рослин пшениці озимої. Усі досліджувані ізоляти проявляли фітотоксичну дію, яка полягала у знижені схожості насіння, інгібуванні росту кореневої системи та проростків. Найбільш негативний вплив на показники схожості*

насіння проявляли ізоляти S. sclerotiorum, вилучені із рослин сої та ріпаку в умовах Київської області Васильківського району. Їхня фітотоксична дія зумовлювала зниження схожості насіння на 12,4-13,4 % менше порівняно з контролем. Досліджувані ізоляти S. sclerotiorum спонукали також зменшення довжини кореневої системи рослин і пригнічення росту проростків порівняно із контролем у середньому від 1,0 до 57,9 %.

Установлено диференціацію ступенів фітотоксичності ізолятів у популяціях S. sclerotiorum, вилучених із різних географічних умов та рослин-господарів. Зокрема, високий ступінь фітотоксичності мали 71-100 % ізолятів гриба, вилучених із рослин сої та ріпаку в умовах Київської області Васильківського району. Ізоляти патогену із уражених рослин соняшнику (Яготинський район Київської області) та жоржини (м. Київ) характеризувалися слабкою та середньою фітотоксичністю.

Ключові слова: *фітотоксичність, фільтрат культуральної рідини, ізоляти, популяція, Sclerotinia sclerotiorum, ростові показники*