ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume 69 44 Number 4, 2021

ANTIFUNGAL ACTIVITY OF A COMPOSITION OF SELENIUM AND IODINE NANOPARTICLES

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Link to this article: https://doi.org/10.11118/actaun.2021.044
Received: 12. 3. 2021, Accepted: 24. 6. 2021

To cite this article: VASYLCHENKO ANATOLII, DEREVIANKO STANISLAV. 2021. Antifungal Activity of a Composition of Selenium and Iodine Nanoparticles. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis,* 69(4): 491–500.

Abstract

The aim of this study was to investigate antifungal properties of the composition of Se and I nanoparticles (NPs) against strains of phytopathogenic fungi *Acremonium cucurbitacearum* 502, *Acremonium strictum* 048 and *Fusarium* sp. 072. It has been found that the composition of Se and I NPs has antifungal properties against these strains. The highest antifungal activity was against strain *A. cucurbitacearum* 502, manifesting as the decrease in the number of colonies (by 60.00–86.67%) and the decrease of the diameter of colonies (by 78.95–94.22%). Antifungal activity against strain *A. strictum* 048 manifested as the decrease in the diameter of colonies by 52.67–75.00%. The diameter of colonies the strain *Fusarium* sp. 072 decreased by 25.26–51.75%. Changes in the morphology of the colonies of the strain *A. strictum* 048 were also noticed. Thus, the composition of Se and I nanoparticles has antifungal activity against fungal strains *A. cucurbitacearum* 502, *A. strictum* 048 and *Fusarium* sp. 072, which are valuable plant pathogens. The composition of Se and I NPs can be recommended for the development of the measures for the control of phytopathogenic fungi.

Keywords: fungi, plant-pathogenic fungi, nanoparticles, antifungal activity, selenium, iodine, acremonium, fusarium

INTRODUCTION

The search for new antifungal substances and the development of modern antifungal preparations is one the most important tasks of mycology. A high interest in studying the nanoparticles (NPs) and their use in the development of antifungal preparations, especially for the control of the fungal pathogens of crop cultures is noticed recently. Nowadays NPs in various forms are used in human and veterinary medicine, agriculture, perfumery, textile and food industry. Many studies confirm antiviral, antibacterial and antifungal properties of NPs of metals, metal oxides and nonmetals.

Particular attention is paid to the influence of different NPs on fungi. It is known that NPs of metals, metal oxides, nonmetals, polymers and other substances has antifungal activity.

Thus, NPs are perspective for the use in human and veterinary medicine and agriculture as antifungal agents.

For instance, it is known that silver NPs has antifungal activity against broad spectrum of plant-pathogenic fungi: *Alternaria alternata*, *Alternaria brassicicola*, *Alternaria solani*, *Botrytis cinerea*, *Cladosporium cucumerinum*, *Corynespora cassiicola*, *Cylindrocarpon destructans*, *Didymella bryoniae*, *Fusarium oxysporum* f. sp. *cucumerinum*, *Fusarium oxysporum* f. sp. *lycopersici*, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium* sp., *Glomerella cingulata*, *Monosporascus cannoballus*, *Pythium aphanidermatum*, *Pythium spinosum* and *Stemphylium lycopersici* (Kim *et al.*, 2012).

Copper NPs show antifungal activity against phytopathogenic fungi *Phoma destructiva*, *Curvularia lunata*, *Alternaria alternata* and *Fusarium oxysporum*

(Kanhed *et al*., 2014). It was found that copper NPs has much higher antifungal activity against fungi *Phoma destructiva*, *Curvularia lunata*, *Alternaria alternata* and *Fusarium oxysporum* than such commercial preparations as bavistin, suppressing the growth of these fungi at very low concentrations (Kanhed *et al*., 2014).

Zinc oxide NPs has antifungal activity against phytopathogenic fungi *Botrytis cinerea* and *Penicillium expansum* (He *et al*., 2011).

NPs of molybdenum and cobalt compounds also have high antifungal activity. Thus, molybdenum trioxide NPs have antifungal activity against *Candida albicans* and *Aspergillus niger* along with antibacterial and antioxidant activity (Fakhri and Nejad, 2016). Cobalt dithiocarbamate NPs have antifungal activity against *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger* (Nabipour *et al*., 2011).

Not only NPs of metals and their compounds, but also NPs of nonmetals has high scientific and industrial importance. Thus, surface-modified sulfur NPs have prominent antifungal activity against *Aspergillus niger* and *Fusarium oxysporum*, reducing their radial growth, spore formation and phospholipid content (Choudhury *et al*., 2011).

Selenium NPs have antifungal activity against such fungi as *Macrophomina phaseolina*, *Sclerotinia sclerotiorum*, *Diaporthe longicolla* (Vrandečić *et al.*, 2020), *Malassezia sympodialis*, *Malassezia furfur*, *Aspergillus terreus* (Shahverdi *et al.*, 2020), *Candida albicans*, *Aspergillus fumigatus*, *Aspergillus niger* (Shakibaie *et al.*, 2015; Eswarapriya and Jegatheesan, 2015), *Pyricularia grisea*, *Colletotrichum capsici*, *Alternaria solani* (Joshi *et al.*, 2019), *Trichophyton rubrum* (Yip *et al.*, 2014) and others.

Thus, NPs of such simple substances and chemical compounds as silver, copper, zinc oxide, molybdenum trioxide, cobalt dithiocarbamate, sulfur and selenium have antifungal activity against broad spectrum of fungi, which includes human pathogens as well as pathogens of animals and crop cultures.

Therefore, the aim of our study was to investigate antifungal activity of the composition of Se and I NPs against fungal strains *Acremonium cucurbitacearum* 502, *Acremonium strictum* 048 and *Fusarium* sp. 072, which are dangerous plant pathogens.

MATERIALS AND METHODS

Object and Subject of the Study

Object of the study – growth of the cultures of phytopathogenic fungi *in vitro* under the influence of the composition of Se and I NPs.

Subject of the study – antifungal activity of NPs against phytopathogenic fungi.

Materials of the Study

Sol of the composition of Se and I NPs, which was obtained by laser ablation (Kosinov and Kaplunenko, 2007), was kindly given to us by the head of “Nanomaterials and nanotechnologies” LTD, doctor of technical sciences, Kaplunenko Volodymyr Heorhiiovych.

Strains of phytopathogenic fungi *Acremonium cucurbitacearum* 502, *Acremonium strictum* 048 and *Fusarium* sp. 072 were kindly given to us by the head of the laboratory of plant-microorganism interactions of the Institute of Agricultural Microbiology and Agro-industrial Production of NAAS of Ukraine (IAMAP NAAS), doctor of biological sciences, professor, Nadkernychna Olena Volodymyrivna and chief researcher of the laboratory of plant-microorganism interactions of IAMAP NAAS, doctor of biological sciences, senior researcher, Kopylov Yevhenii Pavlovych.

Wort agar, the medium used in the study, was prepared from wort, agar and water. The wort was purchased from Chernihiv beer factory “Desna” (Ukraine) and the agar was purchased from Ferak Berlin GmbH (Germany).

Methods

Morphology of NPs was studied by transmission electron microscopy on the microscope JEOL JEM-1400 (Japan). Diluted sol of NPs was put on copper grids with carbon-formvar coating and dried in the air at room temperature.

Antifungal activity was studied by the addition of NPs to the culture medium, which was further used for fungi cultivation. 4.8% of sterile sol of the composition of Se and I NPs was added to the molten wort agar (40–50 °C). After the addition of NPs the medium was mixed properly and poured into Petri dishes (15 cm^3). Intact wort agar without NPs served as control variant.

After solidification of the medium, it was inoculated with cultures of fungi by means of inoculation needle. There were 5 Petri dishes for each variant of medium (5 repetitions). Each Petri dish was inoculated at 3 locations. Cultures were grown in thermostat at the temperature 25 °C. Diameters of colonies were measured at 2^{nd}, 3^{rd} and 4^{th} days of cultivation. Antifungal activity was measured by the decrease in the diameter of colonies or absence of fungal growth.

Statistical analysis was done in statsoft STATISTICA 10 software using ANOVA analysis. Weighted means (WM) were used to display average values. Duncan's new multiple range test (DMRT) was used to assess significance. 20–30 values (Valid N) were used for the analysis.

RESULTS

Morphology of NPs

According to the results of transmission electron microscopy it has been found that the composition of Se and I NPs contains separate triangular, irregular NPs and their aggregates. Triangular NPs have size of the dimension 30 nm. Irregular

NPs are 10–60 nm in size. Sizes of aggregates are 150–200 nm (Fig. 1a, b).

Antifungal Activity of NPs

According to the results of the study, the composition of Se and I NPs decrease the number and the diameter of the colonies of fungal strain *Acremonium cucurbitacearum* 502 and the diameter of colonies of strains *Acremonium strictum* 048 and *Fusarium* sp. 072.

Influence of the Composition of Se and I NPs on the Growth of the Strain *Acremonium cucurbitacearum* 502

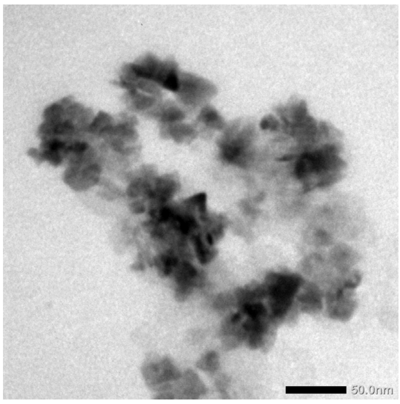
It has been found that on the 2nd day after inoculation the average number of *A. cucurbitacearum* 502 colonies on the control medium was 3.00, whereas on the medium with the composition of Se and I NPs it was 0.40 ± 0.25, which is less by 86.67%. On the 3rd day the average number of colonies on the medium with the composition of Se and I NPs was 1.00 ± 0.32 (by 66 67% less). On the 4th day the average number of colonies *A. cucurbitacearum* 502 on the control medium was 3.00, whereas on the medium with the composition of Se and I NPs it was 1.20 ± 0.37 (by 60.00% less) (Tab. I, Fig. 2, Fig. 3).

On the 2nd day the average diameter of *A. cucurbitacearum* 502 colonies on the medium with the composition of Se and I NPs was less than in control by 94.22%. On the 3rd day the difference was by 83.70%. On the 4th day – by 78.95% (Tab. II, Fig. 2, Fig. 3).

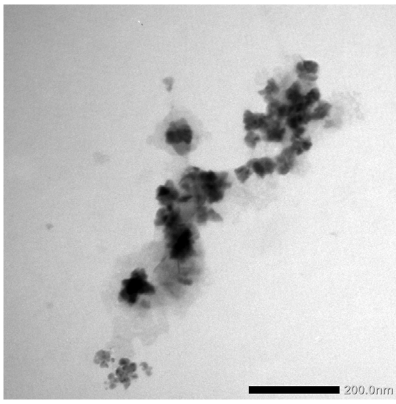
Influence of the Composition of Se and I NPs on the Growth of the Strain *Acremonium strictum* 048

The number of *Acremonium strictum* 048 colonies on the medium with the composition of Se and I NPs did not differ as compared to control.

However, the diameter of colonies differed significantly. According to the results of the study it was shown that on the 2nd day the average diameter of *A. strictum* 048 colonies on the medium with the composition of Se and I NPs was less than in control by 75.00%. On the 3rd day the difference was 60.84%, on the 4th – 52.67% (Tab. III, Fig. 5).



a



b

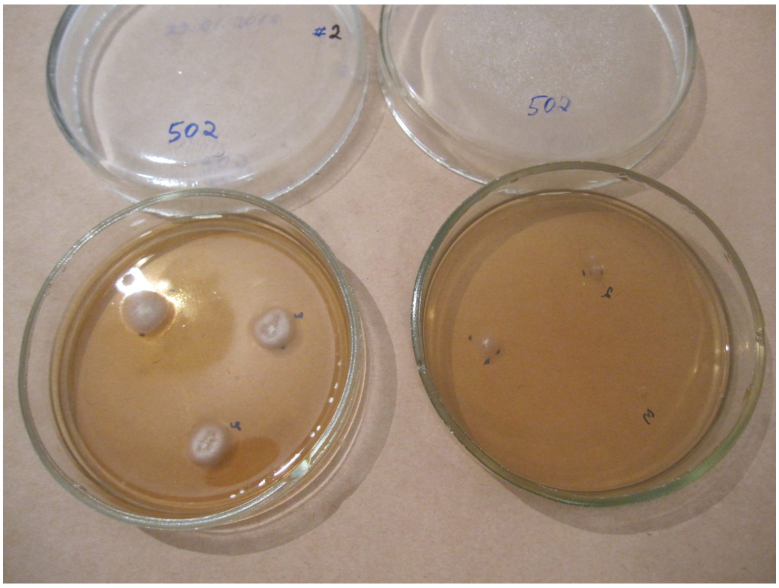
1: *Electron micrographs of the composition of Se and I NPs. a, b – aggregates of Se and I NPs.*

I: *Influence of the composition of Se and I NPs on the number of colonies of the strain A. cucurbitacearum 502*

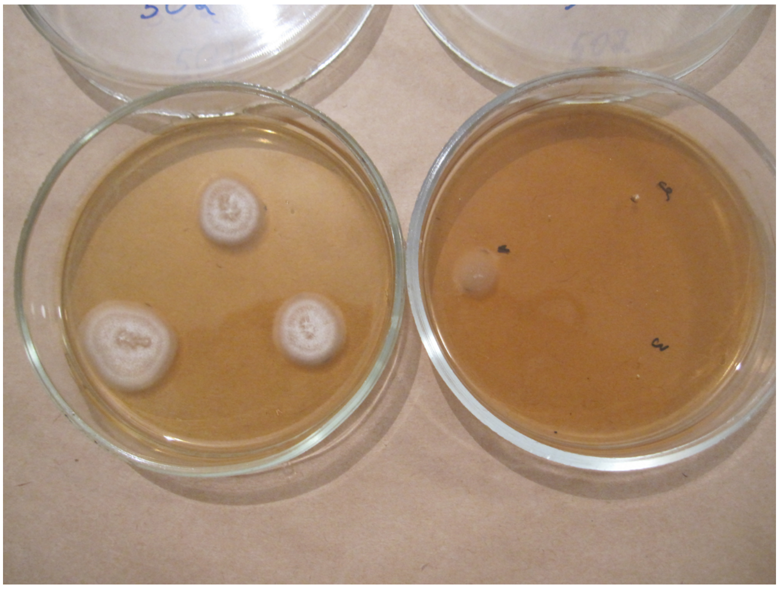
Duration of cultivation	Average number of colonies (Control)	Average number of colonies (Se and I NPs)	Difference with control, %	Significance, p (DMRT)
2 days	3.00 ± 0.00	0.40 ± 0.25	-86.67	< 0.0003
3 days	3.00 ± 0.00	1.00 ± 0.32	-66.67	< 0.0005
4 days	3.00 ± 0.00	1.20 ± 0.37	-60.00	< 0.002

II: *Influence of the composition of Se and I NPs on the diameter of A. cucurbitacearum 502 colonies*

Duration of cultivation	Average diameter of colonies (Control), cm	Average diameter of colonies (Se and I NPs), cm	Difference with control, %	Significance, p (DMRT)
2 days	3.75 ± 0.10	0.22 ± 0.10	-94.22	< 0.000005
3 days	6.57 ± 0.16	1.10 ± 0.31	-83.70	< 0.00002
4 days	9.50 ± 0.19	2.00 ± 0.56	-78.95	< 0.000005



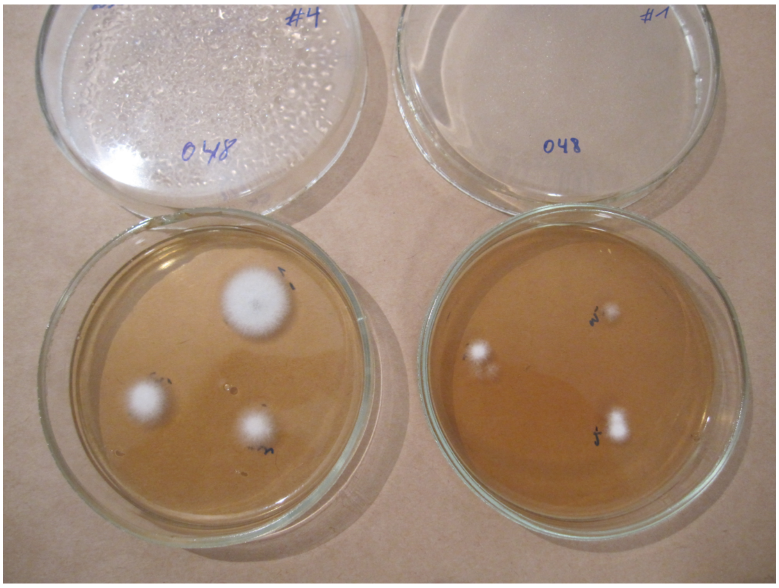
2: *Colonies of A. cucurbitacearum 502 in control (left) and on the medium with the composition of Se and I NPs (right). 3rd day of cultivation.*



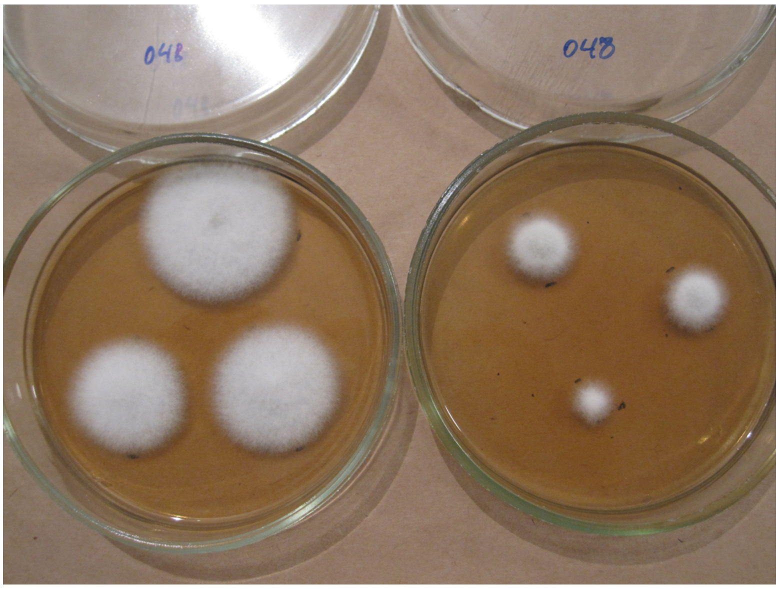
3: *Colonies of A. cucurbitacearum 502 in control (left) and on the medium with the composition of Se and I NPs (right). 4th day of cultivation.*

III: *Influence of the composition of Se and I NPs on the diameter of A. strictum 048 colonies*

Duration of cultivation	Average diameter of colonies (Control), cm	Average diameter of colonies (Se and I NPs), cm	Difference with control, %	Significance, p (DMRT)
2 days	4.60 ± 0.45	1.15 ± 0.23	-75.00	< 0.0002
3 days	9.92 ± 0.44	3.88 ± 0.27	-60.84	< 0.000006
4 days	15.95 ± 0.47	7.55 ± 0.32	-52.67	< 0.000004



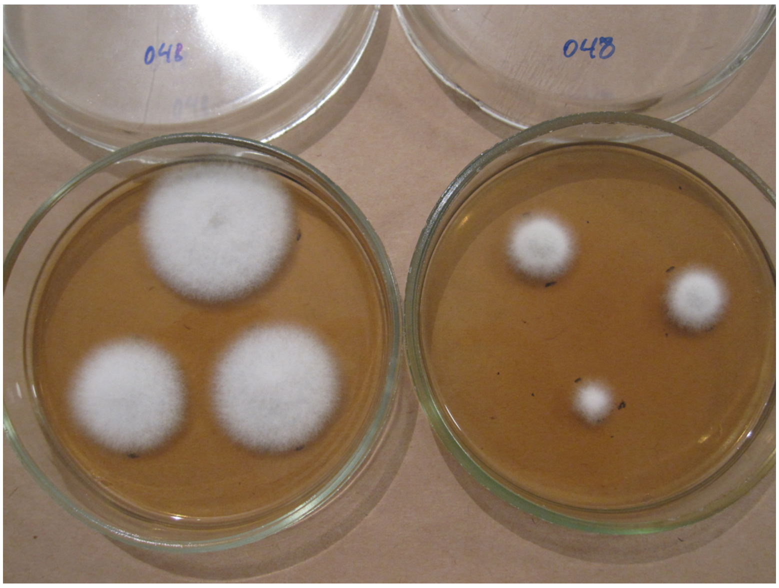
4: *Colonies of A. strictum 048 in control (left) and on the medium with the composition of Se and I NPs (right). 3 days after inoculation.*



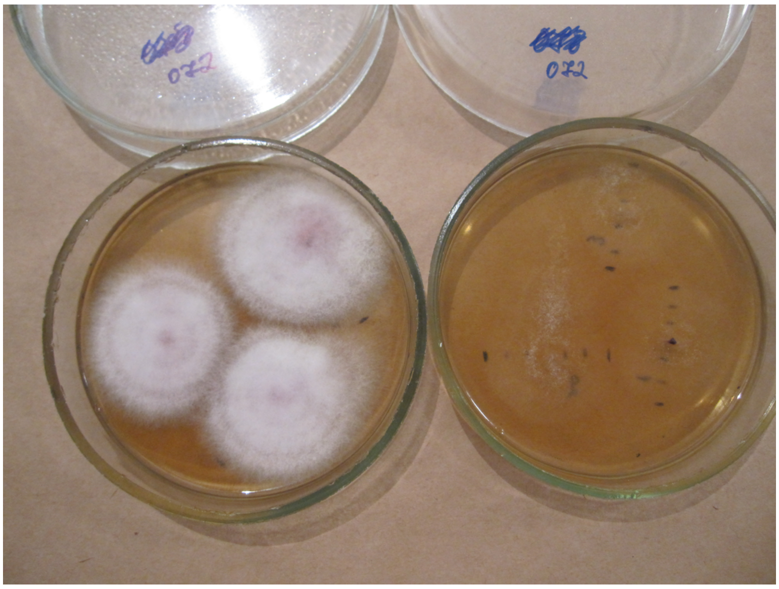
5: *Colonies of A. strictum 048 in control (left) and on the medium with the composition of Se and I NPs (right). 4 days after inoculation.*

IV: *Influence of the composition of Se and I NPs on the diameter of Fusarium sp. 072 colonies*

Duration of cultivation	Average diameter of colonies (Control), cm	Average diameter of colonies (Se and I NPs), cm	Difference with control, %	Significance, p (DMRT)
2 days	9.89 ± 0.23	4.78 ± 0.19	-51.75	< 0.000004
3 days	16.73 ± 0.27	11.27 ± 0.41	-32.63	< 0.000005
4 days	22.19 ± 0.65	16.58 ± 0.46	-25.26	< 0.000005



6: *Colonies of Fusarium sp. 072 in control (left) and on the medium with the composition of Se and I NPs (right). 3 days after inoculation.*



7: *Colonies of Fusarium sp. 072 in control (left) and on the medium with the composition of Se and I NPs (right). 4 days after inoculation.*

Influence of the Composition of Se and I NPs on the Growth of the Strain *Fusarium* sp. 072

The number of the *Fusarium* sp. 072 colonies on the medium with the composition of Se and I NPs did not differ as compared to control.

However, the diameter of the colonies on the medium with the addition of the composition of Se and I NPs was significantly less than in control. Thus, on the 2nd day the average diameter of the colonies on the medium with the composition of Se and I NPs was less than in control by 51.75%. On the 3rd day the difference was 32.63%, on the 4th – 25.26% (Tab. IV, Fig. 6, Fig. 7).

Influence of the Composition of Se and I NPs on the Morphology of *A. strictum* 048 Colonies

Significant difference in morphological properties between *A. strictum* 048 colonies on the control medium and on the medium with the composition of Se and I NPs were observed (Fig. 8). The formation of the droplets of dark-green and reddish-green exudate by the mycelium was observed on the medium with the composition of Se and I NPs. Also the colonies differed in color and shape. Under the influence of the composition of Se and I NPs colonies of *A. strictum* 048 were prominently umbonate. Dense greenish protuberances were formed at the centres of colonies. Concentric circles of clear dark-

a b

8: *Morphological properties of the A. strictum 048 colonies on the control medium (a) and on the medium with the addition of the composition of Se and I NPs (b). 7 days after inoculation.*

green exudate were form around the protuberances (Fig. 8b). Such changes in morphological properties of the colonies can be a result of changes in fungus metabolism, caused by the presence of NPs in culture medium. The influence of the composition of Se and I NPs on the morphology of *A. strictum* 048 colonies and the metabolism of the fungus deserves further investigation.

Thus, the composition of Se and I NPs affects the strains of plant-pathogenic fungi *Acremonium cucurbitacearum* 502, *Acremonium strictum* 048 and *Fusarium* sp. 072, suppressing their growth and influencing morphological properties.

DISCUSSION

Thus, the results of our study show that the composition of Se and I NPs inhibits growth of fungal strains *Acremonium cucurbitacearum* 502, *Acremonium strictum* 048 and *Fusarium* sp. 072.

These results agree with results of other authors. It is known that Se NPs have antifungal activity (Vrandečić *et al.*, 2020; Shahverdi *et al.*, 2020; Shakibaie *et al.*, 2015; Eswarapriya and Jegatheesan, 2015; Joshi *et al.*, 2019; Yip *et al.*, 2014). It is also known that soluble compounds of iodine have antifungal activity against such fungi as *Venturia inaequalis*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Bipolaris sorokiniana*, *Fusarium moniliforme* (Efimov *et al.*, 2020), *Candida albicans*, *C. parapsilosis*, *C. glabrata*, *C. tropicalis*, *C. lusitaniae*, *C. guilliermondii*, *C. krusei* (Kondo *et al.*, 2012), *Oligoporus placenta*, *Gloeophyllum trabeum*, *Coniophora puteana*, *Trametes versicolor* (Ihssen *et al.*, 2014), other fungi and particularly against *Acremonium* species (Farrag *et al.*, 2012). Thus, the antifungal activity of the composition of Se and I NPs is caused by its components and agrees with existing data.

The mechanisms of antifungal activity of Se NPs are not studied sufficiently. However, they can be understood from the mechanisms of antibacterial activity of Se NPs. Thus, it has been found that quercetin and acetylcholine coated Se NPs significantly increase reactive oxygen species (ROS) production in bacterial cells. And it is supposed that one of the mechanisms of antibacterial activity of Se NPs is associated with ROS production (Huang *et al.*, 2016). Similarly to antibacterial activity, antifungal activity of Se NPs can be caused by ROS production, since high amounts of ROS are dangerous for most living cells. It has been also noticed that Se NPs damage cell membranes of bacterial cells, which can be also a mechanism of antifungal activity (Huang *et al.*, 2016). Moreover, Se NPs can deplete internal ATP and affect membrane potential (Huang *et al.*, 2019).

In studies with *Candida glabrata* it has been found that Se NPs attach and agglomerate on the surface of cells, cause loss of membrane's smoothness, appearance of bulges and impairment of membrane's integrity (Lotfali *et al.*, 2021). Higher concentrations cause dramatic changes in cell morphology and membranes' breakdown.

It is known that iodine has strong fungicidal, bactericidal and virucidal activity. It is supposed that iodine penetrates cells of microorganisms and interacts with proteins (especially with cysteine and methionine residues), fatty acids and nucleotides, which leads to cell death (McDonnell and Russell, 1999; Springthorpe and Sattar, 1990). Iodine has high affinity to fatty acids, can bind to carbon-carbon double bond (C=C) of unsaturated fats and cause membrane immobilization (Apostolov *et al.*, 1980). In our opinion, similar mechanisms can cause antifungal activity of iodine.

Mechanisms of antifungal activity of the composition of Se and I NPs need further studies in order to obtain the exact data on the mechanisms of the composition's activity.

Thus, our results are consistent with existing data. The composition of Se and I NPs inhibits growth of phytopathogenic fungi significantly, which makes it a promising antifungal material. Nanoparticles has especially high importance today due to several circumstances. For instance, one of the problems with conventional fungicides today is fungal resistance to them. A vast number fungi, including plant pathogens, are reported to develop resistance to modern conventional fungicides (McDonald *et al.*, 2019; Yang *et al.*, 2019; Cook *et al.*, 2021; Sevastos *et al.*, 2018).

Also conventional fungicides are shown to be toxic for humans (Lv *et al.*, 2017; Knebel *et al.*, 2019), animals (Gupta *et al.*, 2018; Syromyatnikov *et al.*, 2017; Wade *et al.*, 2019; Kumar *et al.*, 2020; Wu *et al.*, 2018) and plants, including agricultural crops (Shahid *et al.*, 2018; Fedotov *et al.*, 2019).

All these circumstances make NPs, and particularly the composition of Se and I NPs, a promising tool for plant pathogenic fungi control.

CONCLUSION

It has been found that the composition of Se and I NPs suppresses the growth of the strain *Acremonium cucurbitacearum* 502 significantly, decreasing both the number (by 60.00–86.67%) and the diameter of the colonies (by 78.95–94.22%).

The composition of Se and I NPs suppresses the growth of the strain *Acremonium strictum* 048, decreasing the diameter of colonies by 52.67–75.00%.

The composition of Se and I NPs suppresses the growth of the strain *Fusarium* sp. 072, decreasing the diameter of the colonies by 25.26–51.75%.

The composition of Se and I NPs influences the morphology of the colonies of the strain *Acremonium strictum* 048, causing the appearance of exudate and changes in color.

The composition of Se and I NPs can be recommended for the development measures for the control of plant-pathogenic fungi.

Acknowledgements

We express our sincere gratitude to the head of "Nanomaterials and nanotechnologies" LTD, doctor of technical sciences, Kaplunenko Volodymyr Heorhiiovych for providing us the composition of Se and I NPs.

We express our sincere gratitude to the transmission electron microscope operator, senior researcher of the Institute of microbiology and virology of NAS of Ukraine, philosophy doctor, Kharchuk Maksym Serhiiovych for electron microscopy study of NPs.

We express our sincere gratitude to the head of the laboratory of plant-microorganism interactions of IAMAP NAAS, doctor of biological sciences, professor, Nadkernychna Olena Volodymyrivna and chief researcher of the laboratory of plant-microorganism interactions of IAMAP NAAS, doctor of biological sciences, senior researcher, Kopylov Yevhenii Pavlovych for providing us strains of plant-pathogenic fungi.

We express our sincere gratitude to the senior researcher of the laboratory of plant-microorganism interactions of IAMAP NAAS, candidate of biological sciences, Tsekhmister Hanna Viktorivna for the help in the conduction of the study.

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