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PLANT GROWTH REGULATORY ACTIVITY IN THE PHYTOPATHOGENIC FUNGUS *PLECTOSPHAERELLA MELONIS* STRAIN 502

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Aim. To investigate the ability of our phytopathogenic fungal strain 502, earlier preliminarily identified as the phytopathogen *Plectosphaerella melonis* (syn. *Acremonium cucurbitacearum*), to have phytotoxic and/or plant growth regulatory activity. **Methods.** The phytotoxicity of strain 502, was studied by bioassays using the test cultures of corn (*Zea mays* L.), garden cress (*Lepidium sativum* L.), cucumber (*Cucumis sativus* L.), and onion (*Allium cepa* L.). The cytotoxicity and genotoxicity of the fungus were estimated using the *Allium cepa*-test. The mitotic index of the, the duration of mitosis phases, and the frequency of aberrant ana-telophases of *Allium cepa* L. roots meristem was also investigated. For this purpose, strain 502, was grown in the following culture media: synthetic Raulin-Thom medium for 10 days at 26 ± 2 °C. Cell-free filtrate (culture fluid) was used for the study. Ethylene production was quantified in culture filtrate using gas-chromatography method. Ethylene measurement was performed every 7 days during 8 weeks. The determination was carried out using a gas chromatograph «Agilent Technologies 6850» (USA) fitted with a flame ionization detector, using commercial ethylene as a standard for identification and quantification Every experiment had three repeats. The reliability of experimental data was assessed by statistical methods using Statistica 12 (Stat-Soft Inc., USA). **Results**. Undiluted culture fluid (obtained by growing the fungus on liquid wort) of our strain 502 inhibited the growth of *Z. mays* seedlings by 14 %, *L. sativum* seedlings by 18 % (1 : 100 dilution) and stimulated the growth of *L. sativum* roots by 54 and 41 % (1 : 10 and 1 : 100 dilutions, respectively). The culture fluid, obtained by growing the fungus on Raulin-Thom's synthetic agar, demonstrated a slight inhibitory effect on the seedlings and roots of *L. sativum*, and at the dilution of 1 : 1000 stimulated growth by 30 %. Insignificant changes in the mitotic index of the meristem of *A. cepa* roots were revealed at the effect of the culture fluid of *P. melonis*, strain 502, diluted at the ratio of 1 : 100 and 1 : 1000. At the same time, the number of cells at the prophase stage decreased 1.7 times (1 : 100 dilution). There is a significant increase in the number of cells at the metaphase stage – 1.3 and 1.4 times (dilution 1 : 100 and 1 : 1000, respectively), the anaphase stage – 2.1 and 1.8 times (dilution 1 : 100 and 1 : 1000, respectively) and the telophase stage – 1.8 times (1 : 100 dilution), as compared with the positive control (culture medium). The frequencies of aberrant ana-telophases in the apical meristems of the initial roots were 5.0 and 2.2 % (at the culture fluid dilution of 1 : 100 and 1 : 1000, respectively). We researched the ability of *P. melonis* 502 to synthesize ethylene and the highest level of it was registered after 5 weeks of cultivation (111.78 nmol/h g). **Conclusions**: It was demonstrated by us that the culture fluid of strain 502 showed no phytotoxic effect on roots and seedlings of the investigated cultures, demonstrating the exclusion of phytotoxins from the possible range of effectors. No cytotoxic or genotoxic activity of the culture fluid was observed either. However, the culture fluid altered the dynamics of the cell cycle, in particular, shortened the prophase and stimulated the metaphase, anaphase, and telophase. The culture fluid of the fungus stimulated the growth of *L. sativum* roots depending on the nutrient medium, where the fungus was grown and cultivated. In particular, when growing the fungus on the liquid wort, the growth was higher by 54 and 41 % (dilution 1 : 10 and 1 : 100, respectively), when growing on synthetic Raulin-Thom's medium – by 30 %. This demonstrates the ability of strain 502 to possibly synthesize growth promoting substances. Also, we have shown the ability of this strain to synthetize ethylene in vitro (111.78 ± 13.27 nmol/h per g), which can act as virulence factor. We consider the obtained results to be the first stage of the study on the mechanism of the interaction between pathogenic strain 502 and plants.

Key words: *Plectosphaerella melonis*, Allium-test, culture fluid, *Cucumis sativus,* mitotic index, phytotoxicity, genotoxicity, cytotoxicity, growth-regulating activity, ethylene.

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INTRODUCTION

Pathogen metabolites play a prominent role in the initiation and development of the pathological process and serve as pathogenicity effectors (Rovenich H et al, 2014; Vleeshouwers VG, Oliver RP, 2014; Bulygina TV et al, 2015; Snelders NC et al, 2020; Roth MG et al, 2021). On the initial stages of the attack, the pathogens send chemical signals to host plants and enter the so called "molecular dialogue", aimed at penetrating a plant organism, inhibiting defense reactions, and using the content of plant cells as a nutrition source (Ökmen B, Doehlemann G, 2014). To colonize tissues, phytopathogens employ different strategies: necrotrophs kill their hosts with toxins and consume dead plant remains, while biotrophs colonize living tissues and manipulate the metabolism of plants (Doehlemann G et al, 2017). Toxins, enzymes, phytohormones, and other biologically active substances, interacting with host plant substances in a highly specific manner are pathogenicity factors.

Phytotoxins play an important role in the pathogenesis of plants, as they may determine the development of the disease (Zdorovenko EL et al, 2020; Portal González et al, 2021). The synthesis of phytotoxins is a characteristic feature of necrotrophic pathogens, which defines a wide spectrum of host plants (Medina R et al, 2019). The pathogens of endophytic nature, hemibiotrophs and biotrophs, are either incapable of synthesizing phytotoxic substances or synthesize them only in later stages of the pathogenesis (Mishra R et al, 2018).

The role of phytohormonal substances in the development of the pathological process of certain pathogens and defense reactions of the host is beyond any doubt (Huang S et al, 2020). Most phytohormones occurring in plants can also be synthesized by phytopathogens and may promote the penetration of the pathogen into tissues of host plants, create a niche for the pathogen (gall formation) and/or trigger other disease processes (Kazan K, Lyons R, 2014; Chanclud E et al, 2016; Kunkel & Johnson, 2021). Many fungi, colonizing plant roots (endophytes) synthesize auxins (Yin C et al, 2014; Kunkel BN, Harper CP, 2018; Kopilov E et al, 2020; Leontovyčová H et al, 2020), which change the levels of phytohormones in a plant, interfering with their biosynthesis or blocking the components of signaling pathways of phytohormones to trigger the disease (Han X, Kahmann R, 2019).

Earlier, isolate 502 was obtained in the Laboratory of Plant-Microbe Interactions at the Institute of Agricultural Microbiology and Agroindustrial Manufacture, NAAS from affected cucumber plants and preliminarily identified as *P. melonis* (syn *Acremonium cucurbitacearum*) (Kopilov E et al, 2021). The strain 502 was deposited in Depository of the Institute of Microbiology and Virology, NAS of Ukraine, with the number IMB F-100138. The partial ITS spacer 1/5.8S rRNA gene/partial ITS spacer 2 sequence of strain 502 (317 bp) was entered in the GenBank database under the number MK736305.1.

Since it is relevant to determine the role of secondary metabolites of phytopathogens in plant-microbe interactions in order to find out the mechanism of the pathological process, and there are insufficient data about the synthesis of growth regulating substances by *P. melonis*, our study was aimed at investigating phyto-, cytogenotoxicity and biosynthesis of the phytohormone and virulence factor ethylene of our phytopathogenic strain 502.

MATERIALS AND METHODS

Strain. A natural strain of a phytopathogenic fungus preliminarily identified as *P. melonis,* isolated from infected cucumber plants (*C. sativus)*), cv Koroliok, grown in covered soil, was used in our work (Kopilov E et al, 2021). The strain 502 was deposited in Depository of the Institute of Microbiology and Virology, NAS of Ukraine, with the number IMB F-100138. The partial ITS spacer 1/5.8S rRNA gene/partial ITS spacer 2 sequence of strain 502 (317 bp) was entered in the GenBank database under the number MK736305.1.

The phytotoxicity was studied using biotests according to Plokhynsky (1970) and Kopylov (2010). Corn seedlings (*Zea mays*, cv Kremin) and the seeds of garden cress (*Lepidium sativum*, cv Azhur), cucumber (*Cucumis sativus*, cv Kushchovy), and onion (*Allium cepa*, cv Khaltsedon) were used as test cultures. For this purpose, strain 502 was grown on culture media: synthetic Raulin-Thom's and water-diluted beer wort (NadkernichnySP, Kopilov YeP, 2010) for 10 days at 26 ± 2 °C. The culture fluid was filtered from mycelium by vacuum filtration method using a membrane filter and kept in the refrigerator at 5 ± 2 °C. Seedlings of *Zea mays* (root length 1 cm) were placed into the solutions for one hour, placed on the filtration paper moistened with sterile water; the changes in the root length were registered after 24 h. The seeds of test cultures (*L. sativum*, *C. sativus*, *A. cepa*) were placed on filter paper in Petri dishes – 50 seeds of garden cress, 25 seeds of each cucumber, and onion per dish. The filtration paper was moistened with sterile tap water (including the control variant), the culture medium of the relevant

dilution (negative control), and the culture fluid of the fungus in the relevant dilution. The dishes with seeds were placed into the thermostat at 26 ± 2 °C. The seeds were moistened with the same amount of the investigated solutions every day. The length of the roots and seedlings of test cultures was determined on Day 5.

The cytotoxicity and genotoxicity of the culture fluid were estimated using the *Allium cepa*-test (Tedesco S et al, 2012). The effect of the culture fluid of strain 502 on the mitotic index and the frequency of aberrant anatelophases was studied in the root meristem of onions *(A. cepa L.,* cv Khaltsedon) according to the method described in (Hostymskyi SA et al, 1974; Tedesco S et al, 2012). The onion seeds were grown in Petri dishes on sterile filter paper for five days, while being moistened with the culture fluid of strain 502, diluted at the ratio of 1 : 100 and 1 : 1000, every day. Seedlings with a root length of 0.7–0.9 cm were selected for the analysis. Squashed preparations were made by the standard method (Pausheva ZP, 1988) and studied under a light microscope (Delta Optical Evolution 300 Trino LED, Poland, magnification 400×). For this purpose, onion roots were placed into Clarke's fixative (3 parts of 96 % ethanol, 1 part of glacial acetic acid) and kept in glass vials at 3 °C. The maceration of tissues was done in 1 N solution of HCl for 8 min in water bath at 60 °C, then the tissues were stained with the acetocarmine solution (2 g carmine in 45 ml glacial acetic acid and 55 ml distilled water (Pausheva ZP, 1988) for 20 min. The roots were placed on specimen slides into a solution of acetic acid (45 %), the root tip was detached, and other tissues of root were removed. A cover slip and filter paper were placed on top of a slide onto which the root tip cells were placed. Lightly tapping on the coverslip, to align the cells of the root tip into one layer. The mitotic index was determined in the root meristem as the ratio between the dividing cells and the total number of cells in the microscopic field of vision. The mitotic index (MI, %) and the relative length of each phase of mitosis (prophase, metaphase, anaphase, telophase – P, M, A, T, respectively, %) was calculated according to the described methods (Tedesco S et al, 2012). The study on the genotoxicity of the culture fluid of *P. melonis,* 502, diluted at the ratio of 1 : 100 and 1 : 1000, was conducted by the ana-telophase method (Tedesco S et al, 2012), based on estimating the frequency of aberrant ana-telophases (F, %), defined as the percentage of cells at the stage of anaphase and telophase with aberrations from the total number of cells at these stages. In each repeat there were 10 roots. The experiment had three repeats.

Ethylene production in vitro (in the culture filtrate) was determined using gas-chromatography according to Liu *et al*, 2011. Strain 502 cultured in 30 ml glass vials, containing 10 ml of Czapek-Dox medium: 30.0 g glucose, 2.0 g $NaNO_3$, 1.0 g K_2HPO_4, 0.5 g $MgSO_4$, 0.5 g KCl, 0.01 $FeSO_4$. This medium was inoculated by a spore suspension (T = 1×10^6 CFU), in the amount of 5 % of volume and cultivated in a thermostat in dark conditions at 26 °C. Ethylene measurement was performed every 7 days during 8 weeks. The vial was tightly closed with rubber stopper and incubated for 24 h at room temperature. The accumulation of ethylene in each tube was determined from three different samples of 1 ml taken with a syringe from the tube. The determination was carried out in a gas chromatograph «Agilent Technologies 6850» (USA) fitted with a flame ionization detector.

Statistical analyses. Every experiment had three repeats. The reliability of experimental data was assessed by statistical methods using Statistica 12 (Stat-Soft Inc., USA).

For biotests the mean arithmetic and the mean square deviation (SD) were calculated at the significance level of $p < 0.05$ and $p < 0.001$ and the *t*-test and the Mann-Whitney U-test were used for comparison.

The distribution of data for ethylene checked for normality using the Shapiro-Wilk's W-test and the homogeneity of variance checked using Brown-Forsythe's test. Particularly, Duncan Multiple Range Test (DMRT) used to check if each parameter differs significantly under different values of ethylene. The level of significance has been set to $p < 0.05$.

RESULTS

The initial study on plant growth regulating activity of the culture fluid of strain 502 was conducted on the seedlings of *Z. mays* (Fig. 1, *a*). There was an insignificant inhibition of corn roots when undiluted culture fluid (CF) of the fungus was used (14 %). The biometric indices of *C. sativus* roots when using diluted (10 and 100×) CF, obtained by growing *P. melonis,* strain 502, on wort, are presented in Fig. 1, *b*. The data demonstrate that at the effect of the CF of *P. melonis*, strain 502 the root length of cucumbers does not differ from the negative control (wort only). There was, however, an inhibitory effect of the nutrient medium (the 10 and 100× diluted wort) as compared to the control variant, by 39 and 20 %, respectively.

The seedlings of *L. sativum* were found to be more sensitive to the effect of strain 502 (Fig. 2). There was

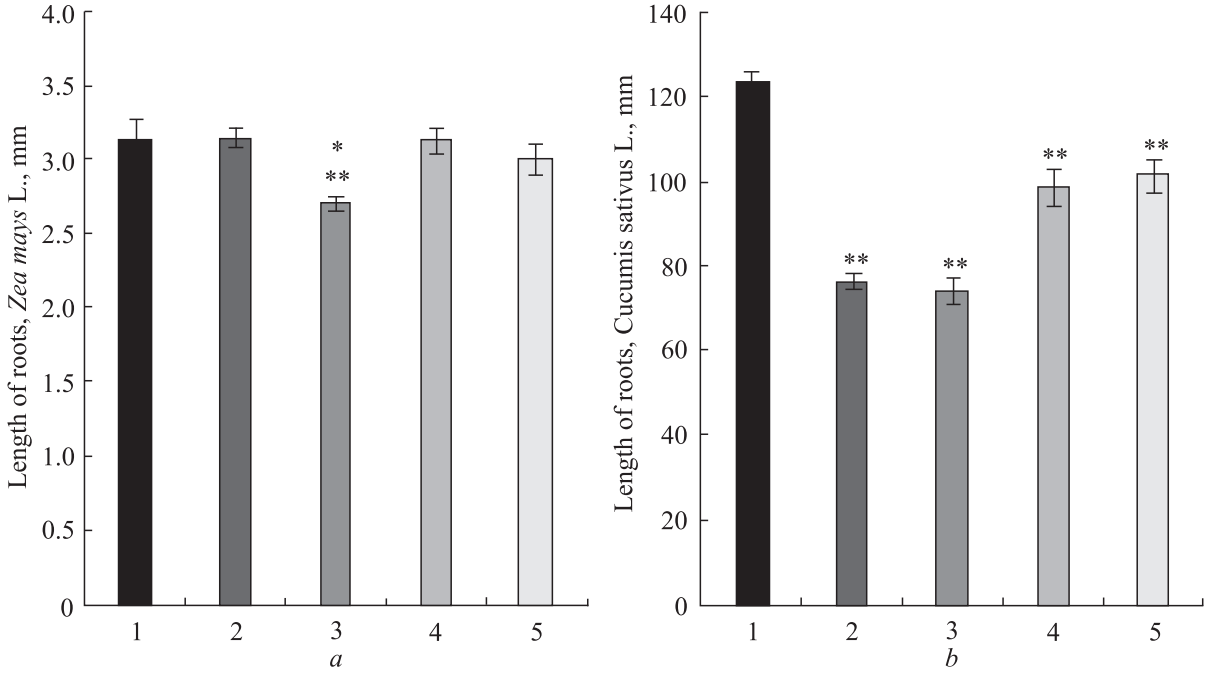

Fig. 1. The effect of the culture fluid of strain 502, on the growth of *Z. mays* seedlings, (n = 10 plants), (*a*) (nutrient medium: wort): 1 – water (control); 2 – wort; 3 – native culture fluid of the fungus; 4 – wort, 1:10 dilution; 5 – culture fluid of the fungus, 1 : 10 dilution. The effect of the culture fluid of *P. melonis,* strain 502, on the growth of *C. sativus* roots (n = 20 plants) (*b*) (nutrient medium: wort): 1 – water (control); 2 – wort, 1 : 10 dilution; 3 – culture fluid, 1 : 10 dilution; 4 – wort, 1 : 100 dilution; 5 – culture fluid of the fungus, 1 : 100 dilution. (*a*) *significance at $p < 0.05$ according to *t*-test as compared to the positive control (nutrient medium: wort of the corresponding dilution) **significance at $p < 0.05$ according to *t*-test as compared to the negative control (water). (*b*) **significance at $p < 0.001$ according to the Mann-Whitney U-test as compared to the negative control (water)

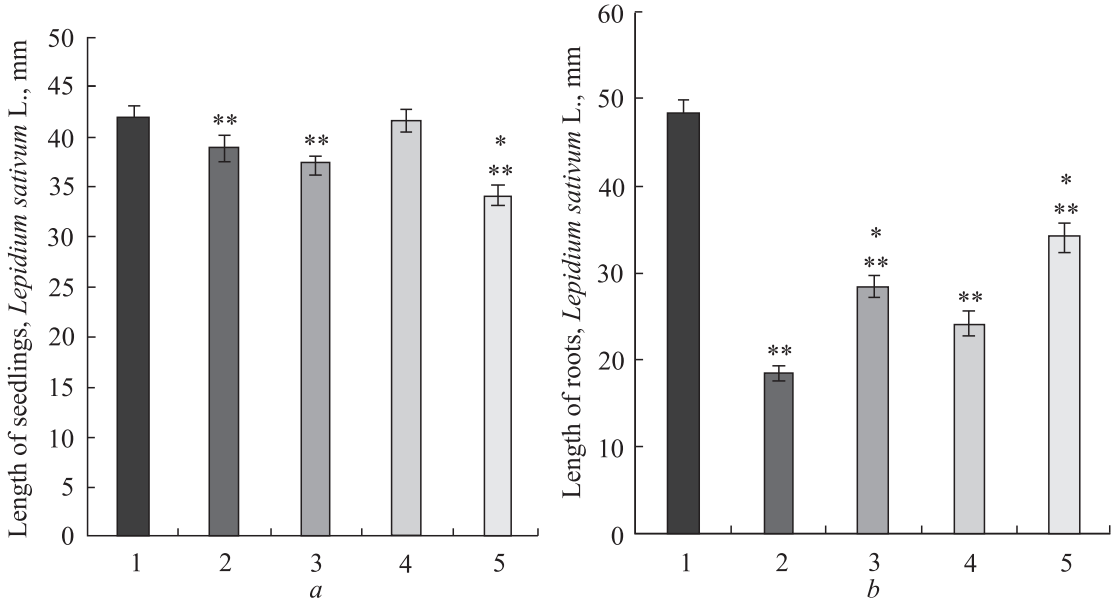

Fig. 2. The effect of the culture fluid of strain 502, on the growth of seedlings (n = 25 plants) (*a*) and roots (n = 25 plants) (*b*) of *L. sativum* (nutrient medium: wort): 1 – water (control); 2 – wort, 1 : 10 dilution; 3 – culture fluid, 1 : 10 dilution; 4 – wort, 1 : 100 dilution; 5 – culture fluid, 1 : 100 dilution. (*a*) *significance at $p < 0.05$ according to the Mann-Whitney U-test as compared to the positive control (nutrient medium: wort of the corresponding dilution) **significance at $p < 0.001$ according to the Mann-Whitney U-test as compared to the negative control (water). (*b*) *significance at $p < 0.001$ according to *t*-test as compared to the positive control (nutrient medium: wort of the corresponding dilution) **significance at $p < 0.001$ according to *t*-test as compared to the negative control (water)

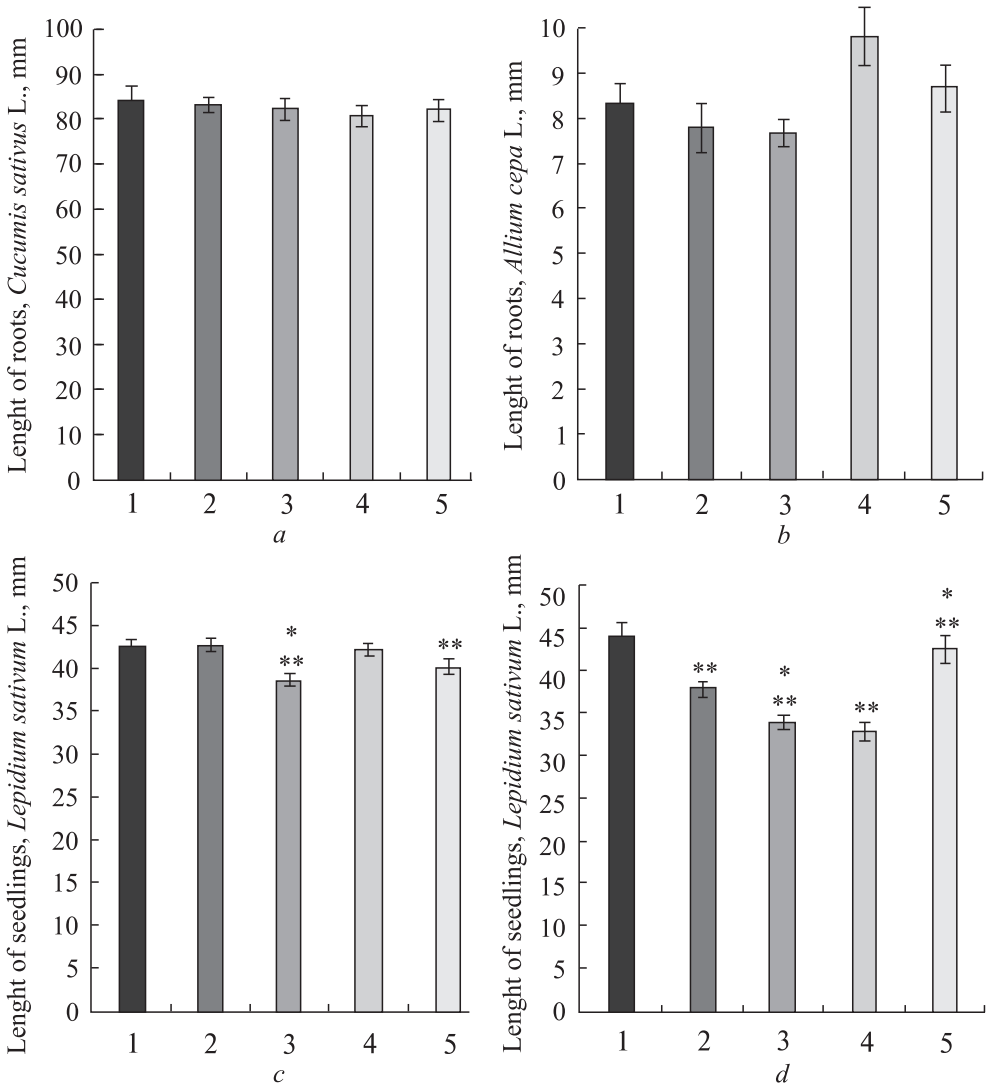

Fig. 3. The effect of the culture fluid of strain 502, on the growth of the roots of *C. sativus* (n = 20 plants) (*a*), *A. cepa* (n = 10 plants) (*b*) and seedlings (n = 25 plants) (*c*) and roots (n = 25 plants) (d) of *L. sativum* (Raulin -Thom's medium): 1 – water (control); 2 – Raulin-Thom's medium, 1 : 100 dilution; 3 – culture fluid of the fungus, 1 : 100 dilution; 4 – Raulin-Thom's agar, 1 : 1000 dilution; 5 – culture fluid of the fungus, 1 : 1000 dilution. (*c*) *significance at $p < 0.05$ according to the Mann-Whitney U-test as compared to the positive control (nutrient medium: wort of the corresponding dilution) **significance at $p < 0.001$ according to the Mann-Whitney U-test as compared to the negative control (water). (*d*) *significance at $p < 0.001$ according to the Mann-Whitney U-test as compared to the positive control (nutrient medium: wort of the corresponding dilution) **significance at $p < 0.001$ according to the Mann-Whitney U-test as compared to the negative control (water)

insignificant inhibition of the aboveground part of the plants by the culture fluid of the fungus, diluted 100 times (18 %). There was an increase in the root length by 54 and 41 % at the effect of the culture fluid of strain 502, diluted 10 and 100 times, respectively. The inhibitory effect of the wort on the roots was manifested in this test culture too. Since the impact of secondary metabolites of the fungus on test plants could have been masked by the inhibitory effect of the wort, we studied the impact of the culture fluid of the fungus, grown on the Raulin-Thom's medium.

The effect of the culture fluid of strain 502, grown on Raulin-Thom's medium, on the test plants is presented in Fig. 3. The data demonstrate the absence of any phytotoxic effect of the CF of strain 502, diluted 100 and 1000 times, on the roots of *C. sativus* (Fig. 3, *a*) and *A. cepa* (Fig. 3, *b*), since no significant inhibition of the root length of the abovementioned test-cultures was registered. Similar to the previous variant, the seedlings of *L. sativum* were more sensitive to the effect of the CF of strain 502 (Fig. 3, *c*, *d*). The culture fluid of the fungus, diluted 100 times, demonstrated insignifi-

cant inhibitory effect on the seedlings (10 %) and roots (10 %) of *L. sativum*, and at 1 : 1000 dilution stimulated the root growth by 30 %.

The study of cytogenetic indices of the onion root meristem at the effect of CF of *P. melonis,* strain 502, is presented in Table. The information obtained demonstrates insignificant effect on the mitotic index of strain 502.

The dynamics of the cell cycles changed, in particular, the pro-phase index at the effect of CF of the fungus, diluted at 1 : 100, decreased 1.7 times, and at the dilution of 1 : 1000 – did not change as compared with the positive control. CF of *P. melonis,* strain 502, diluted at 1 : 100 and 1 : 1000, promoted the increase in the number of cells in the metaphase (1.3 and 1.4 times, respectively), anaphase (2.1 and 1.8 times, respectively) and telophase – 1.8 times (1 : 100 dilution), which demonstrates the delay in the division of cells at these phases.

The ana-telophase analysis was applied to check the ability of CF of *P. melonis,* strain 502, to induce aberrations in the cells of the root meristem of *A. cepa* (Table 1). The frequencies of aberrant ana-telophases in apical meristems of the initial roots at the effect of CF of *P. melonis,* strain 502, diluted at 1 : 100 and 1 : 1000, are 5.0 and 2.2 %, respectively, which demonstrates the absence of the genotoxic effect, since the frequencies of aberrant cells are within the indices of the nutrient medium (4.8).

Strain 502 synthesized ethylene at the start of cultivation (Fig. 4). In the first week it amounted 4.89 ± 0.63 nmol/h per g. The highest amount of ethylene was 5 weeks after cultivation and it was 111.78 ± 13.27 nmol/h per g. Subsequent cultivation of strain 502 showed the reduction in the amount of synthetized ethylene that may be explained by switch from stationary growth phase, when physiological processes in the fungus are the highest, to aging and dying phase. After 9 weeks of cultivation, the amount of ethylene decreased to 5.13 ± 0.74 nmol/h per g due to the decreased metabolism and switch of strain 502 to an apparent survival phase.

DISCUSSION

The data obtained demonstrate that the seedlings of *L. sativum* were more sensitive to the filtrate of the culture fluid of strain 502, preliminarily identified as *Plectosphaerella melonis* (Kopilov E et al, 2021), as compared to *Z. mays*, *A. cepa* and *C. sativus*. In addition, there was an inhibitory effect of the nutrient medium (wort) on test-cultures, which is in good agreement with the results of other researchers (Ogórek R et al, 2020).

The results of the study on the effect of the culture fluid of phytopathogens are ambiguous. For instance, Abbas T et al, (2017) demonstrated that phytotoxins had both inhibitory and stimulating effects depending on the concentration. However, most studies highlight the inhibitory effect of the phytotoxic substances of pathogenic fungi. Javaid A, Adrees H (2009) investigated the growth regulating activity of the culture fluid of 10 phytopathogenic fungi (*Alternaria alternata, Fusarium oxysporum*, *F. solani* and others), which, similar to *P. melonis,* strain 502, are root rot fungi, damaging diverse agricultural crops, and demonstrated the inhibition of germination and of growth of seedlings and roots of plants. Other studies

The cytogenetic indices of the root meristem of *A. cepa* after the treatment with the culture fluid of *P. melonis,* strain 502, measured after 5 days (n = 1000 cells)

Variant	Mitotic index (MI), Mean ± SD	MI, % regarding the positive control	Prophase, %	Metaphase, %	Anaphase, %	Telophase, %	Aberrant ana-telophases, %
Water (control)	5.65 ± 0.94	–	39.9	16.8	9.1	34.3	3.2
Raulin-Thom's medium, 1 : 100 dilution	6.69 ± 0.49	–	57.1	22.4	6.3	14.1	4.8
Culture fluid, 1 : 100 dilution	6.45 ± 0.40	96	32.4	29.6	12.5	25.5	5.0
Raulin-Thom's medium, 1 : 1000 dilution	8.41 ± 0.85	–	26.3	21.7	7.1	45.0	4.8
Culture fluid, 1 : 1000 dilution	5.68 ± 0.67	68	25.4	30.3	7.0	37.3	2.2

also demonstrated the phytotoxic effect of the culture fluid of *A. alternata*, *F. solani* (Parveen S et al, 2019), *F. oxysporum f. sp. lycopersici* (Khurshid S et al, 2014) and *F. equiseti* (Suthar R et al, 2014) on the seedlings of test plants. The culture fluid of phytopathogenic fungi *F. oxysporum, F. sulphureum*, *Gibberella avenacea, G. intricans, Haematonectria haematococca*, which are necrotrophs, inhibited the growth of *L. sativum* seedlings (Ogórek R, 2016).

As for our study, we registered insignificant inhibition of *Z. mays* roots (14 %) and *L. sativum* seedlings (18 %) when using culture fluid of *P. melonis,* strain 502, which demonstrates that the growth-regulating substances that are formed, are not toxic because the inhibition is low. The stimulation of *L. sativum* roots, obtained by us at the effect of the culture fluid of *P. melonis,* strain 502, is likely to demonstrate the ability of the fungus to produce substances of phytohormonal, rather than phytotoxic, nature, since subsequent cultivation of the culture fluid conditioned the increase in the root length of *L. sativum* by 54 % (Hadacek F et al, 2011; Simlat M et al, 2019; García-Gómez P et al, 2020). The synthesis of phytohormonal substances by phytopathogens is one of relevant mechanisms of their impact on the host plant which allows phytopathogens to unbalance the defense systems of the plant and penetrate the tissues (Kazan K, Lyons R, 2014; Chanclud E et al, 2016; Di X et al, 2016; Han X, Kahmann R, 2019; Kunkel BN, Johnson JM, 2021).

We did not register any inhibition or stimulation of the growth of *A. cepa* roots, but we observed some disruption in the dynamics of the cell cycle – asynchrony of phases of the mitotic division, including delayed mitosis in the metaphase, anaphase, and telophase. It was demonstrated that the accumulation of many metaphase cells resulted from the delayed karyokinesis on this stage and was related to the disrupted function of the spindle of the division and the process of phragmoplast formation – so called colchicine effect (Barman MRS, Ray S, 2020). Similar results on delayed mitosis in the metaphase were obtained while testing antibiotics (Kantsavaya I, Alekseenko O, 2020), and while studying phytohormonal substances, in anaphase and telophase – indole derivatives (Belousova Z, Selezneva E, 2004).

Although the frequency of aberrant ana-telophases in the apical meristem of *A. cepa* (2.2–5.0 %), registered by us, exceeds the negative control – water (3.2 %), it is within the control indices of frequencies in other studies – 2.26 % (Karaismailoglu MC, 2014) and 6.8 % (Liman R et al, 2021). And the frequencies of chromosomal aberrations and nuclear anomalies were much higher in plants, infected by genotoxic phytopathogen *Botrytis allii* (10.43–14.74 %) (Bonciu E et al, 2018). Phytopathogens affect the morphology and physiology of host-plants, producing phytohormonal compounds. Intense synthesis of phytohormones by phytopathogen is associated with tissue necrosis and their hydrolysis to simple compounds (Volynets AP, Poliakova NV, 2012). In some plants, they will induce resistance, and in others – will be virulence factors, suppressing the immune potential and inducing sensitivity to phytopathogens (Yamada T, 1993). The nature of the interaction between the host-plant and the pathogen at the hormonal level will depend on the type of fungus trophicity (Shakirova FM, 2001). For example, biotrophic and hemibiotrophic parasites synthesize phytohormones that allow to bypass the plant defence systems and exist in the plant without provoking an immune response (Bushnell WR, Rowell JB, 1981; Volynets AP, Polyakova, 2012; Maksymov IV, 2005). One such phytohormone is ethylene (ET) (Broekaert WF et al, 2006). It is an active component of the plant regulatory system, the quantitative change of which affects the content of other phytohormones (Lawton KA et al, 1994; Taverner EA et al, 1999). Also, there are studies that show that exogenous ET can both induce resistance in plants and reduce it depending on the pathogen and the host plant. For example, ET treatment of citrus fruits reduced their resistance to *Diplodia natalen-*

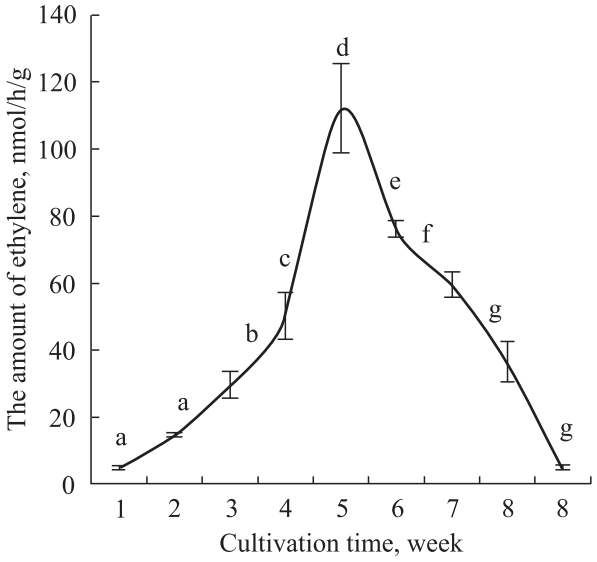

Fig. 4. Ethylene biosynthesis by strain 502. The values represent the average ± SD of three replicates. Mean growth rates in the same column followed by different letters and significantly different according to Duncan Multiple Range Test (DMRT) test at $p \leq 0.05$. The letter shows the differences between the weeks of cultivation

sis (Brown GE, Lee HS, 1993) and disruption of the ET synthesis pathway in *Arabidopsis* increases its sensitivity to the necrotrophic fungus *P. cucumerina* (Sánchez-Vallet A et al, 2012). It is also reported that the hypersynthesis of ET is characteristic of those phytopathogenic bacteria that do not produce phytotoxins. (Stall RE, Hall CB, 1984). Plant cells are sensitive to ethylene only at a certain stage of development (Lutova LA et al, 2010). Also, it is known that ET activates and accelerates the aging process of plant tissues (Mattoo AK, Aharoni N, 1988; Mattoo and Handa, 2004), at the same time, they show increased activity of pectinase and cellulase, which leads to the destruction of intercellular connections (Lutova LA et al, 2010). ET promotes the fall of leaves, flowers, set, etc. (Abeles FB et al, 1992), which is associated with protein gene expression involved in formation of separating layer (Bleecker AB, 1999). ET is formed in the tissues of the root of the batata (*Ipomoea batatas*) during injury and infection (Hyodo H et al, 2003). ET also disrupts the induction of acid invertase in damaged tissues, inhibits the formation of a layer of lignin on the surface of damaged tissue, stimulates the activity of cytochrome c oxidase, succinate dehydrogenase and leads to increased peroxidase activity without causing systemic immunity in plants (Velykanova LL, 1985). Other study demonstrated that ET emitted by maize seed facilitates *Aspergillus flavus* colonization but not mycotoxin production, indicating that maize seed-derived ET modulates plant susceptibility and fungal growth (Wang S et al, 2017). Also host-derived ET provide fungal colonization, reproduction, and mycotoxin production and that host ET biosynthesis may be manipulated by *F. verticillioides* via fungal G-protein signalling to subsequently promote its virulence (Park YS et al, 2021). Significant influence on the biosynthesis of ET is played by environmental factors such as pH, temperature and substrate, which has been shown for *A. falciforme* (Arshad M, Frankenberger JWT, 1989). The ability of the pathogen of anthracnose of banana *C. musae* to *in vitro* ET biosynthesis was shown (Daundasekera M et al, 2003). Also, it was found that the necrotrophic phytopathogen *B. cinerea* synthesizes ET *in vitro*, but does not induce an increase in ET in the tomato pathogen system (Cristescu SM et al, 2002). ET biosynthesis is inherent not only in phytopathogenic fungi, but also in bacteria (El-Kazzaz MK et al, 1983). Different strains of the necrotrophic bacterium *Pseudomonas syringae* pv. *pisi* produce ET (1.5–115.8 nmol h g), and the level of ethylene biosynthesis for them correlates with the aggressiveness of the strains (Dankevich LA, 2013).

CONCLUSIONS

Previous studies of *P. melonis* have focused on the description of disease symptoms, varietal susceptibility, histotrophic localization or phylogenetic relationships, and did not study of secondary metabolites of the pathogen, which determine the development of the pathological process. Here we report on the investigation of the growth regulating activity of the culture fluid of a phytopathogenic strain (502) isolated from melon and earlier preliminarily identified by us as *P. melonis*. It was demonstrated by us that the culture fluid of strain 502 showed no phytotoxic effect on the roots and seedlings of the investigated cultures. No cytotoxic or genotoxic activity of the culture fluid was registered either. The culture fluid of the fungus stimulated the growth of *L. sativum* roots depending on the nutrient medium, where the fungus was grown and cultivated. For instance, when growing the fungus on the liquid wort, the growth was higher by 54 and 41 % (dilution 1 : 10 and 1 : 100, respectively), when growing on synthetic Raulin-Thom's medium – by 30 % (dilution 1 : 1000). This demonstrates the ability of strain 502 to possibly synthesize growth promoting substances. Also, we have shown the ability of strain 502 to synthetize ethylene (111.78 ± 13.27 nmol/h per g), which can be virulence factor. We consider the obtained results to be the first stage of the study on the mechanism of the interaction between the abovementioned pathogen and plants.

Adherence to ethical principles. All the experiments, described in this paper, did not involve animals.

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Здатність фітопатогенного гриба *Plectosphaerella melonis* виявляти ріст-регулюючу активність у рослин

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Мета. Дослідити фітотоксичну та/або ріст-регулюючу активність фітопатогена огірків *Plectosphaerella melonis* штам 502 у рослин. **Методи.** Фітотоксичність *P. melonis* штам 502 вивчали методом біотестів з використанням

тест-культур кукурудзи (*Zea mays* L.), крес-салату (*Lepidium sativum* L.), огірка посівного (*Cucumis sativus* L.) та цибулі ріпчастої (*Allium cepa* L.). Цитотоксичність та генотоксичність гриба оцінювали за допомогою Allium-test. Досліджували мітотичний індекс, тривалість фаз мітозу та частоту аберантних ана-телофаз меристеми коренів *Allium cepa* L. Для цього *P. melonis* штам 502 поверхнево вирощували на поживних середовищах: синтетичному середовищі Ролена-Тома та розведеному водою пивному суслі (7° за Баллінгом) упродовж 10 діб за температури 26 ± 2 °С. Для досліджень брали безклітинний фільтрат (культуральну рідину). Кількісне визначення етилену оцінили за допомогою методу газової хроматографії. Вимірювання етилену проводили кожні 7 днів упродовж 8 тижнів. Визначення проводили на газовому хроматографі «Agilent Technologies 6850» (США), оснащеному полум'яно-іонізаційним детектором, з використанням комерційного етилену як стандарту для ідентифікації та кількісної оцінки. Кожен експеримент мав три повторення. Достовірність експериментальних даних оцінювали статистичними методами за допомогою Statistica 12 (Stat-Soft Inc., США). **Результати**. Культуральна рідина *P. melonis* штам 502 (одержана за вирощування гриба на рідкому суслі) інгібувала ріст проростків *Z. mays* на 14 %, пагонів *L. sativum* на 18 % (розведення 1 : 100) та стимулювала ріст коренів *L. sativum* на 54 і 41 % (розведення 1 : 10 та 1 : 100 відповідно). Культуральна рідина, одержана за вирощування гриба на синтетичному середовищі Ролена-Тома виявляла незначну інгібуючу дію на проростки та корені *L. sativum*, а у розведенні 1 : 1000 стимулювала ризогенез на 30 %. Виявлено недостовірні зміни мітотичного індексу меристеми коренів *A. cepa*. За дії культуральної рідини *P. melonis* штам 502, розбавленої у співвідношенні 1 : 100 і 1 : 1000. В той же час кількість клітин на стадії профази зменшилась у 1,7 раза (розведення 1 : 100). Значно збільшилась кількість клітин на стадії метафази у 1,3 та 1,4 рази (розведення 1 : 100 та 1 : 1000 відповідно), анафази – 2,1 та 1,8 рази (розведення 1 : 100 та 1 : 1000 відповідно) та телофази у 1,8 раза (розведення 1 : 100) порівняно з позитивним контролем (поживним середовищем). Частоти аберантних ана-телофаз в апікальних меристемах первинних коренів становили 5,0 та 2,2 % (за розведення культуральної рідини у співвідношенні 1 : 100 та 1 : 1000 відповідно). Досліджено здатність *P. melonis* 502 синтезувати етилен і найвищий його рівень зареєстровано через 5 тижнів культивування (111,78 нмоль/год г). **Висновки**. Показано, що культуральна рідина *P. melonis* штам 502 не виявила фітотоксичної дії на корені та проростки досліджених тест-культур, що свідчить про виключення фітотоксинів з можливого кола ефекторів. Цитотоксичної або генотоксичної активності культуральної рідини також не спостерігалося. Проте культуральна рідина змінювала динаміку клітинного циклу, зокрема, скорочувала профазу та стимулювала метафазу, анафазу і телофазу. Культуральна рідина гриба стимулювала ріст коренів *L. sativum* залежно від поживного середовища, де вирощували та культивували гриб. Зокрема, при вирощуванні гриба на рідкому суслі приріст був вищий на 54 і 41 % (розведення 1 : 10 і 1 : 100 відповідно), при вирощуванні на синтетичному середовищі Ролена-Тома – на 30 %. Це свідчить про можливу здатність *P. melonis* штам 502 синтезувати речовини, що стимулюють ріст. Показано здатність *P. melonis* 502 синтезувати етилен (111,78 ± 13,27 нмоль/год на г), який може бути фактором вірулентності. Отримані результати є першим етапом дослідження механізму взаємодії зазначеного збудника з рослинами.

Ключові слова: *Plectosphaerella melonis*, Allium-test, культуральна рідина, *Cucumis sativus,* мітотичний індекс, фітотоксичність, генотоксичність, цитотоксичність, ріст-регулююча активність, етилен.

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