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EFFECT OF PLANT EXTRACTS

against Alternaria tenuissima (Kunze) Wiltshir in vitro

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Goal. *To investigate the fungistatic effect of plant extracts on Alternaria tenuissima in vitro.* ***Methods****. The study was conducted in the Laboratory of Phytopathology of the Institute of Plant Protection of NAAS (IPP NAAS) and the Laboratory of Ecology and Pharmacognosy of Research Station of Medicinal Plants of the Institute of Agroecology and Environmental Management of NAAS (RSMP IAEM NAAS). Medicinal plant raw materials used in the research were grown and selected at the research sites of RSMP IAEM NAAS. Plant extracts were made on its basis. Determination of the activity of plant extracts on the growth of Alternaria tenuissima culture was performed in the laboratory of phytopathology of the IPP NAAS. Agar-disk diffusion method was used. The radial growth rate and the percentage of growth inhibition of colonies were determined.* ***Results****. On the 5th day after the start of the experiment, all studied extracts formed colonies of significantly smaller size compared to the control. On the 7th day, extracts of sage, thyme, annual wormwood, wormwood, echinacea root, and plume poppy significantly inhibited the development of Alternaria tenuissima colonies. On the 10th day, a significant reduction in the growth of colonies of the pathogen occurred with the use of extracts of sage, annual wormwood, echinacea roots and plume poppy. Inhibition of colony growth was highest for sage, annual wormwood and plume poppy and ranged from 84.3—99.5% on day 5 to 38.1—73.4% on day 10 after inoculation.* ***Conclusions****. According to our results, extracts of sage (Salvia officinalis L.), annual wormwood (Artemisia annua L.) and plume poppy (Macleaya cordata L.) showed a pronounced fungistatic effect against Alternaria tenuissima. These data suggest that extracts of these plants can be used in the future to develop plant protection products.*

plant extracts; *Alternaria tenuissima*; growth inhibition; radial growth rate; biological protection

Introduction. *Alternaria* spp. are one of the most common pathogens of plant diseases. They can cause leaf spots, and on cereals are also pathogens of «black point». According to research conducted in the last decade, *Alternaria tenuissima* (Kunze) Wiltshire predominates among the causative agents of sooty mould on winter wheat in Ukraine [1—3].

Given the need to apply environmental strategies in agriculture, there is growing interest in studying the possibilities of plant extracts. Compounds with fungicidal properties are widely present in nature, including those contained in essential oils, plant extracts, etc. [4, 5]. Plant-derived chemicals are structurally diverse, exhibit a wide range of biological activity, have lower environmental risk and toxicity to mammals, and may therefore play an important role in the development of botanical fungicides [6—10].

Studies of Devkota A. and Sahu A. [11] have shown that *Ageratum houstonianum* Mill leaf extract. inhibited the linear growth of colonies of *Alternaria brassicae* (Berk.) Sacc., *Botrytis cinerea* Pers., *Fusarium oxysporum* Schltdl., *Phytophthora capsici* Leonian and *Sclerotium rolfsii* Sacc. from 14 to 100% depending on the concentration.

Extract of green walnut husk showed a relatively high efficiency against the following pathogens: *Alternaria alternata* (Fries) Keissler, *Fusarium culmorum* (Smith) Saccardo, *Rhizoctonia solani* Kuhn, *Botrytis cinerea*, *Phytophthora infestans* (Mont.) De Bary. Inhibition of mycelial growth of these fungi *in vitro* reached 55—63% [12].

Another investigation showed that 4% aqueous extracts of the following plants inhibited more than 60% radial growth of *Alternaria solani* Sorauer: *Tephrosia purpurea* (L.) Pers (72%), *Capsicum annum* L. (70%), *Gliricidia sepium* (Jacq.) Steud. (70%), *Cleome viscosa* L. (69%), *Caesalpinia bonduc* (L.) Roxb (67%), *Cassia fistula* L. (63%), *Azadirachta indica* A. Juss (62%), *Cassia alata* (L.) Roxb (62%) [13].

The use of aqueous extracts of *Azadirachta indica* A. Juss. and *Datura stramonium* L. against purple blotch of onion (*Alternaria porri* (Ellis Cif.)) under greenhouse conditions reduced the disease severity by 70% and 61%, respectively, and against Stemphylium blight (*Stemphylium vesicarium* (Wallr.) Simmons) — by 89 and 85% [14].

Extracts from the seeds of *Chenopodium album* L. inhibited the germination of spores of *Alternaria alternata* (Fr.) Keissl and *Bipolaris sorokiniana* Shoem. by 14—16% and 32—34%, respectively [15].

Also inhibiting effect on the

growth of mycelium of *Alternaria* spp. was observed for essential oils from tea tree (decrease in colony diameter was 33—62% depending on the concentration), sage (29—46%) and eucalyptus (19—36%) [16].

Pal G. K., Kumar B., Shahi S.K. [17], studied *in vitro* the effects of extracts of *Achyranthes aspera* L., *Parthenium hysterophorus* L., *Cannabis sativa* L., *Calotropis gigantean* (L.) W.T. Aiton, *Chenopodium album* L., *Phalaris minor* Retz., *Cynodon dactylon* (L.) Pers., *Argemone mexicana* L., *Ageratum conyzoides* L., *Lantana camara* L. on the development of colonies of *Alternaria* spp. and found that extracts of *Ageratum conyzoides* and *Parthenium hysterophorus* had the highest level of inhibition of mycelial growth of the pathogen.

Chaudhari et al. [18] evaluated the efficiency of the essential oil of *Origanum majorana* L. as a mean for preventing the accumulation of aflatoxin in corn grain during storage and found that the minimum inhibitory concentration for *Aspergillus flavus* Link is 2.5 µl/ml, and at a concentration of 1.5 µl/ml stopped the production of aflatoxin. In addition, it showed fungitoxicity against other pathogens (*Cladosporium cladosporoides* (Fresen.) G.A. de Vries, *Penicillium italicum* Wehmer, *Penicillium chrysogenum* Thom, *Fusarium poae* (Peck) Wollenw., *Alternaria alternata* (Fr.) Keissl.) [18]. Also quite effective (inhibition of mycelial growth by 65—86%) against *Alternaria* spp. were essential oils of *Origanum vulgare* subsp. *hirtum* (Link) Ietsw and *Origanum vulgare* subsp. *vulgare* L. [19].

Essential oil of *Thymus vulgaris* L. completely inhibited the growth of mycelium of *Alternaria* spp., *Botrytis* spp., *Colletotrichum* spp. for 7 days at a concentration of 400 µl/l [20].

Essential oil of *Litsea cubeba* (Lour.) Pers. can act as a fungicide against *Fusarium moniliforme* J. Sheld., *Fusarium solani* (Mart.) Sacc., *Alternaria alternata* and *Aspergillus niger* Tiegh. damaging their cell walls and cell membranes [21] .

Extracts of garlic, ginger and neem have shown high efficacy against seed infection of *Alternaria* spp., *Bipolaris sorokiniana*, *Curvularia lunata*, *Fusarium* spp. on wheat [22]. Ginger and garlic oil was found to be as effective as the synthetic fungicide against *A. solani* and *P. infestans* [23].

Methanol extract of *Eclipta alba* (L.) Hassk. showed good fungicidal activity against sorghum pathogens such as *Fusarium thapsinum* Klittich, J.F. Leslie, P.E. Nelson & Marasas, *Alternaria alternata*, *Epicoccum sorghinum* (Sacc.) Aveskamp, Gruyter & Verkley, *Curvularia lunata* (Wakker) Boedijn. Significant disease protection of 95% was observed in greenhouse and 66% disease protection was noticed in field experiments [24].

Extracts of *Acacia nilotica* (L.) Delile, *Achillea fragrantissima* (Forssk.) Sch.Bip. and *Calotropis procera* (Aiton) W. T. Aiton inhibited the linear mycelial growth in 1.5—3 times and reduced conidium germination of *A. solani in vitro* in 2—6 times. In a plot experiment, these extracts reduced disease severity by 55—80%. Tomato fruit yield was increased by 44—92% [25].

Thus, plant extracts and oils are able to inhibit the development of pathogenic fungi, in particular *Alternaria* spp.

Objective: to investigate the fungistatic effect of plant extracts on *Alternaria tenuissima in vitro.*

Methods of investigation. The investigation was conducted in the Laboratory of Phytopathology of the Institute of Plant Protection of NAAS (IPP NAAS) and the Laboratory of Ecology and Pharmacognosy of Research Station of Medicinal Plants of the Institute of Agroecology and Environmental Management of NAAS (RSMP IAEM NAAS).

Medicinal plant raw materials (MPRM) used in the research were grown and selected at the research plots of RSMP IAEM NAAS. The following MPRM was used: sage (*Salvia officinalis* L.) leaves; plume poppy (*Macleaya cordata* (Willd) R. Br.) leaves; echinacea (*Echinacea purpurea* (L.) Moench) roots; echinacea herba; peppermint (*Mentha piperita* L.) leaves; thyme (*Thymus vulgaris* L.) herba; chamomile (*Matricaria recutita* L.) flowers; jimsonweed (*Datura stramonium* L.) seeds; oregano (*Origanum vulgare* L.) herba; hindu datura (*Datura metel* L.) seeds; yarrow (*Achillea millefolium* L.) herba; indian hemp (*Apocynum cannabinum* L.) roots; annual wormwood (*Artemisia annua* L.) herba; wormwood (*Artemisia absinthium* L.) herba; greater celandine (*Chelidonium majus* L.) herba.

Plant extracts were made on the basis of the above mentioned raw materials. The extractant was a water-alcohol mixture in a ratio of 1:1 (ethyl alcohol 96° and distilled water, respectively). The extract was prepared in a ratio of 1:10 — raw material: extractant. The raw material had an average grinding with a particle size (0.5—0.7) cm. Extraction was performed with boiling water-alcohol mixture under constant heating for 30 minutes with the connection of a cooling system to prevent evaporation of the extractant and loss of active substances. Separation of the precipitate from the finished extract was performed using standard paper filters «red ribbon». The extracts formed were evaporated 4 times.

Evaluation of the activity of plant extracts on the growth of *Alternaria tenuissima* culture was performed in the laboratory of phytopathology of the IPP NAAS. Agar disk-diffusion method was used for assessing the sensitivity of fungus. Potato-dextrose agar with the addition of the antibiotic gentamicin at the rate of 10 mg per 1 liter of medium was used. Monospore isolates were used for the experiment. Agar plates were inoculated by placing of fungal mycelium particles [26].

Filter paper discs (10 mm in diameter) were sterilized in a Petri dish wrapped in paper in a dry oven for two hours at 160°C. Sterile water was used to prepare solutions with different concentrations. Paper disks were poured in the solution and then placed on agar plates directly on the inoculum particles. Dishes with inoculum particles covered with filter paper moistened with sterile water were used as controls. Three disks were placed in each Petri dish. Number of plates for each concentration — three. The experiment was repeated twice.

Culture of *Alternaria tenuissima* was kept in a thermostat at 20°C. Assessments were performed on the 5th, 7th and 10th day. Linear dimensions were measured and the area of colonies was determined. The diameter of the colonies was measured in two perpendicular directions without opening the Petri dishes. Inhibition of fungal colony growth was calculated by the formula [26]:

$$E = \frac{S_к - S_0}{S_к} \times 100,$$

E — the inhibition of colony growth, %; $S_к$ — area of the colony

in control; S_0 — area of the colony in the experiment.

The radial growth rate of colonies was determined by the formula:

$$V = \frac{R - R_0}{T} \times 100,$$

V — radial growth rate of the colony, mm/day; R — radius of the colony at the end, mm; R_0 — radius of the colony at the initial time, mm; T — time interval between measurements, days.

Results. The first assessment of the size of the colonies of the pathogen was performed on the 5th day after the start of the experiment. At this time, all studied extracts formed colonies significantly smaller than the control (fig. 1, 2). Growth inhibition was higher in the variants with extracts of sage, annual wormwood and plume poppy — 84.3—99.5% (table 1). In other variants, the area of the colonies was less than the control by 18.8—51.8%.

On the 7th day of the experiment, the increase in the size of the colonies occurred in both the control plates and the experimental ones. In some cases, there was a loss of inhibitory effect on the pathogen. In plates with extracts of peppermint, chamomile, oregano, yarrow, echinacea, jimsonweed, hindu datura, indian hemp, greater celandine an active growth of mycelium was observed. The radial growth rate of colonies for the period from the 5th to the 7th day of the experiment on these variants exceeded the control by 0.3—1.3 mm/day. As a result, during the second assessment the size of the colonies did not differ from the control. Only sage, thyme, annual wormwood, wormwood, echinacea (root) and plume poppy extracts significantly inhibited the development of colonies of *Alternaria tenuissima* (fig. 3, table 1).

On the 10th day, a significant reduction in the growth of colonies of the pathogen occurred when extracts of sage, annual wormwood, echinacea roots and plume poppy were used (fig. 4). Only in these variants the radial growth rate of the mycelium was significantly lower than in control (fig. 5). Inhibition of colony growth was highest for sage, anual wormwood and plume poppy (38.1—73.4%) (table 1).

These results are in agreement with data of many researches, who reported that of plant extracts have inhibitory effect on the growth of mycelium and the germination of spores of pathogenic fungi. In particular, it was found that sage extract inhibited the growth of *B. cinerea* (40—54%) [27]. Its essential oils were also effective against *A. alternata, A. solani, Fusarium solani, F. oxysporum* f.sp. *lycopersici, P. infestans, Rhizoctonia solani, B. cinerea, Colletotrichum coccodes* (Wallr.) S. Hughes, *Verticillium albo-atrum* Reinke & Berthold, *Colletotrichum acutatum* J.H. Simmonds, *Clavibacter michiganensis* Smith, *Xanthomonas campestris* (Pammel) Dowson, *Pseudomonas savastanoi* Janse, *P. syringae*

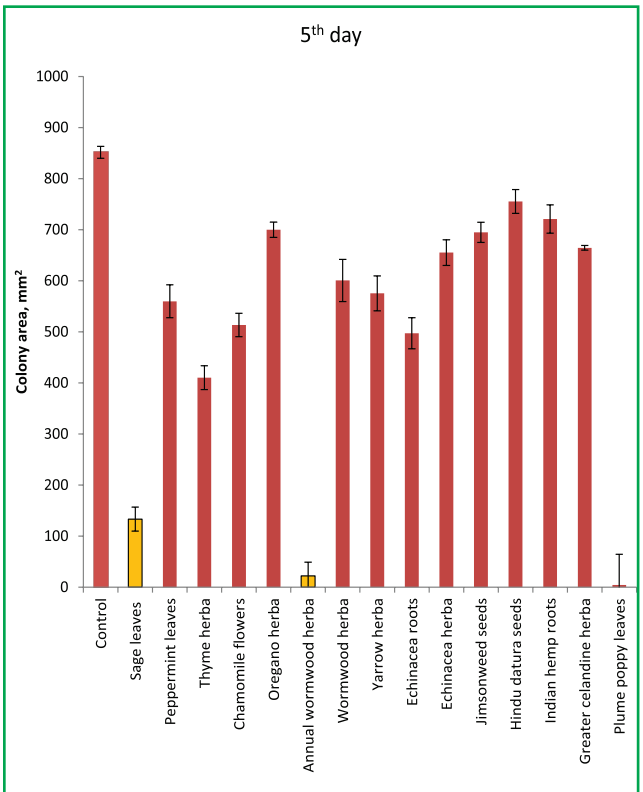
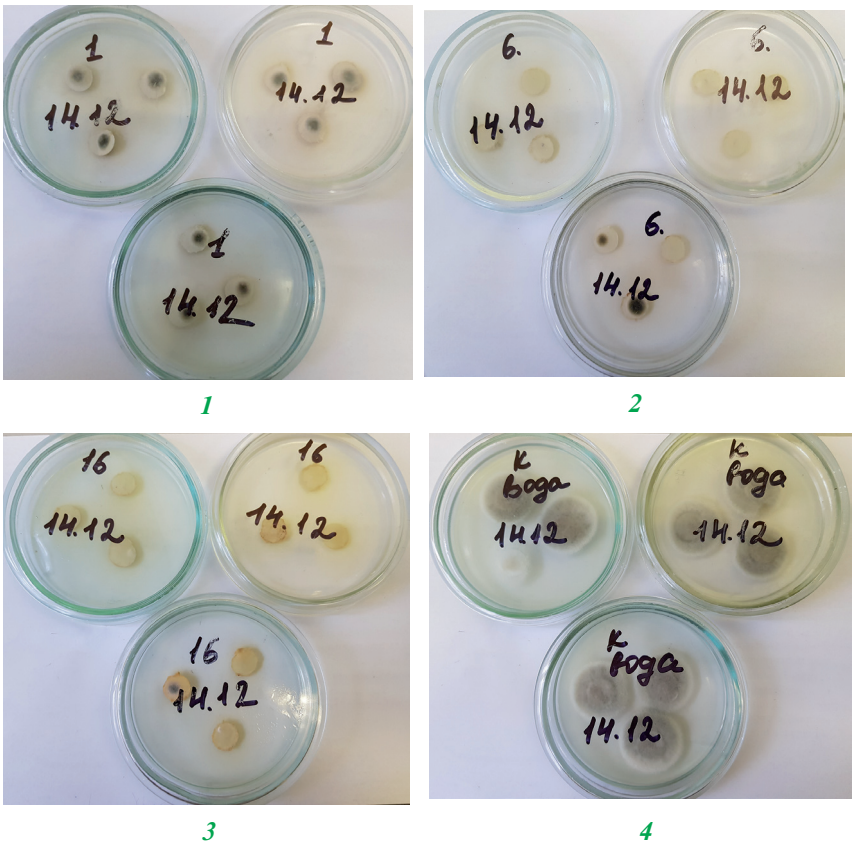


Fig. 1. Area of colonies of Alternaria tenuissima on the 5th day of experiment (bars on diagram are standard errors, LSD_{05} = 80.3)



1 *2*

3 *4*

Fig. 2. Colonies of Alternaria tenuissima on the 5th day of: 1 — Sage; 2 — Annual wormwood; 3 — Plume poppy; 4 — Control (water)

Table 1. Inhibition of colony growth (%)

Extracts	5th day	7th day	10th day
Sage leaves	84.3	54.8	38.1
Peppermint leaves	34.2	7.1	2.1
Thyme herba	51.8	15.6	7.6
Chamomile flowers	39.7	8.1	1.8
Oregano herba	18.8	0	0
Annual wormwood herba	97.4	73.3	56.0
Wormwood herba	29.5	13.2	5.8
Yarrow herba	32.4	8.0	5.3
Echinacea roots	41.6	18.6	17.7
Echinacea herba	23.1	8.4	3.1
Jimsonweed seeds	18.4	5.7	0
Hindu datura seeds	11.3	4.1	0
Indian hemp roots	15.3	1.4	0
Greater celandine herba	21.9	8.9	7.8
Plume poppy leaves	99.5	87.4	73.4

pv. *phaseolicola* van Hall [28—38]. Caffeic and rosmarinic acid in the extracts could be related to the ability to control the development of phytopathogens and are reported to be the most common phenolic compounds in sage extracts [28, 39].

Essential oil of annual wormwood inhibited the development of *Fusarium oxysporum, Fusarium solani, Cylindrocarpon destrutans* (Zinssm.) Scholten, *Alternaria solani, Sclerotinia sclerotiorum* (Lib.) de Bary [40—45]. Studies with the major components present in *A. annua* essential oil have shown that artemisia-ketone and α-terpineol are compounds with the highest antimicrobial activity [41, 46].

The essential oil of *Macleaya cordata* has shown an inhibitory effect on a number of pathogenic microorganisms, including *Aspergillus niger, Aspergillus flavus* [46, 47]. Studies have shown that this effect is due to the presence of quaternary benzophenanthridine alkaloids (sanguinarine and chelerythrin) and protopine alkaloids (protopine and allocryptopine). They showed fungistatic activity with EC_{50} values in the range of 5—11 μg/ml and a minimum inhibitory concentration of 8—32 μg/ml against *Botryosphaeria berengeriana* De Not., *Botrytis cinerea*, *Fusarium graminearum* Schwabe, *Fusarium oxysporum*, *Magnaporthe oryzae* B.C. Couch, *Rhizoctonia solani* [47, 48].

CONCLUSION

According to our results, extracts of sage (*Salvia officinalis* L.), annual wormwood (*Artemisia annua* L.) and plume poppy (*Macleaya cordata* L.) showed a pronounced fungistatic effect against Alternaria tenuissima. These data suggest that extracts of these plants can be used in the future to develop plant protection products.

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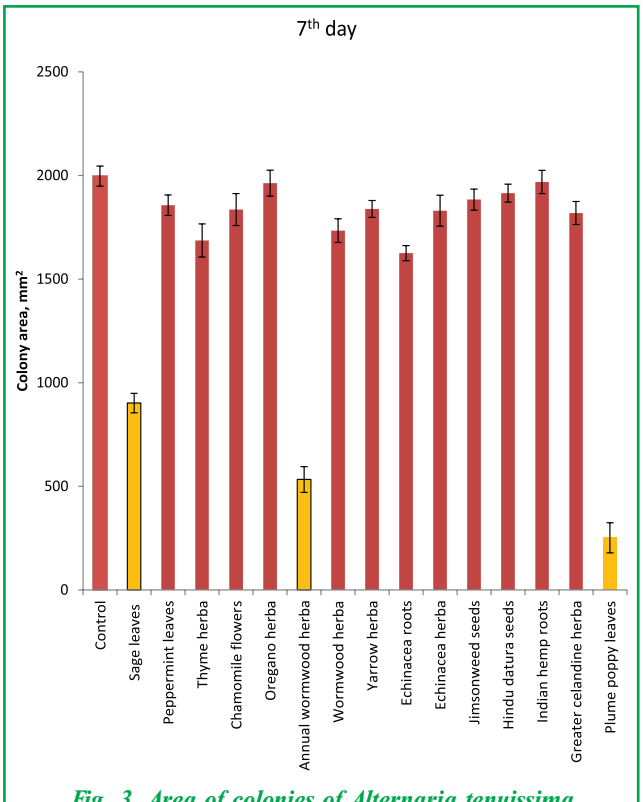


Fig. 3. Area of colonies of Alternaria tenuissima on the 7th day of experiment (bars on diagram are standard errors, LSD_{05} = 184.2)

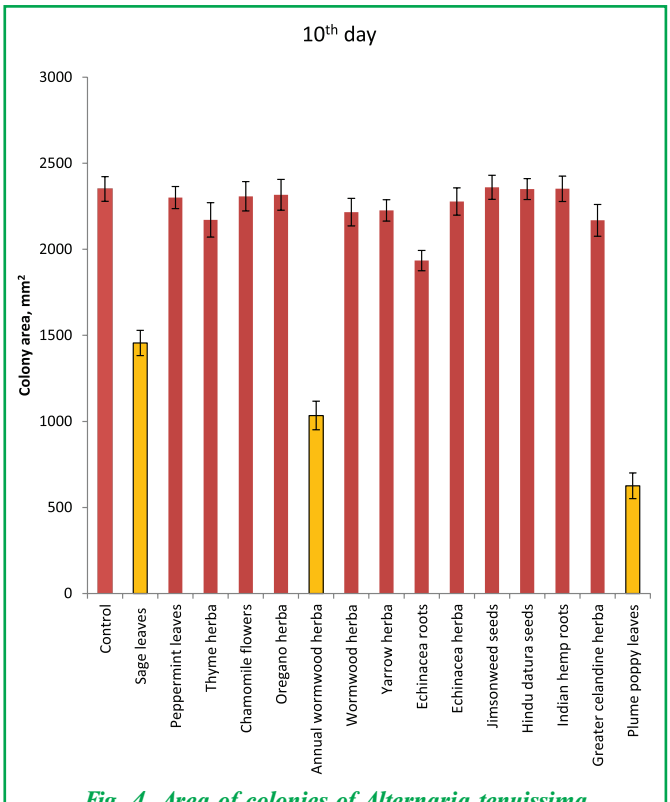
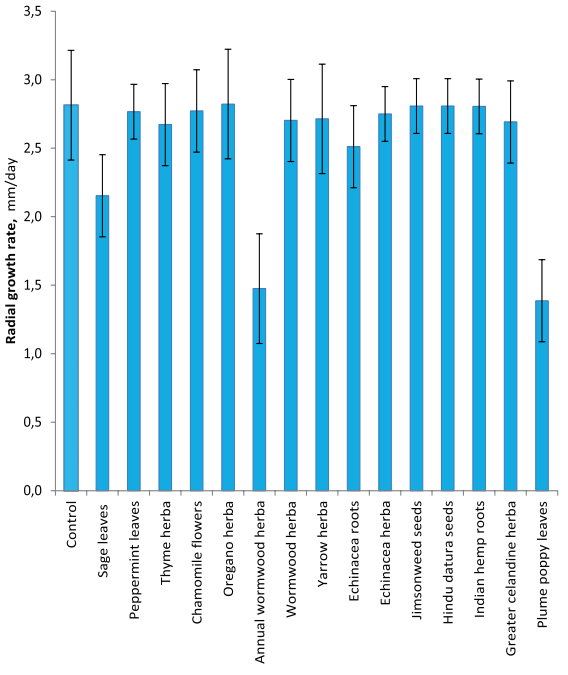


Fig. 4. Area of colonies of Alternaria tenuissima on the 10th day of experiment (bars on diagram are standard errors, LSD_{05} = 262.7)



Puc. 5. Radial growth rate of colonies of Alternaria tenuissima (bars on diagram are standard errors, LSD_{05} = 0.3)

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Вплив рослинних екстрактів на *Alternaria tenuissima* (Kunze) Wiltshire в умовах *in vitro*

Мета: *дослідити фунгістатичну дію рослинних екстрактів щодо Alternaria tenuissima в умовах in vitro.* **Методика.** *Дослідження проведено в лабораторії фітопатології Інституту захисту рослин НААН (ІЗР НААН) та лабораторії екології і фармакогнозії Дослідної станції лікарських рослин Інституту агроекології і природокористування НААН (ДСЛР ІАП НААН). Лікарська рослинна сировина, що використовувалась у дослідженнях, була вирощена та відібрана на дослідних ділянках ДСЛР ІАП НААН. На її основі виготовлено рослинні екстракти. Визначення активності рослинних екстрактів щодо росту культури Alternaria tenuissima проводили в лабораторії фітопатології ІЗР НААН. Використано метод оцінки чутливості грибів з використанням агаризованого живильного середовища. Визначали радіальну швидкість росту та відсоток гальмування росту колоній.* **Результати**. *На 5-й день після закладання досліду всі досліджувані екстракти формували колонії істотно меншого розміру порівняно з контролем. На 7-й день істотно пригнічували розвиток колоній Alternaria tenuissima екстракти шавлії, чебрецю, полину однорічного, полину гіркого, кореня ехінацеї, маклеї. На 10-й день достовірне зниження росту колоній збудника відбувалося за застосування екстрактів шавлії, полину однорічного, коріння ехінацеї та маклеї. Гальмування росту колоній було найвищим для шавлії, полину однорічного та маклеї і становило від 84,3—99,5% на 5-й день до 38,1—73,4% на 10-й день після інокуляції.* **Висновки**. *За даними проведених досліджень виражену фунгістатичну дію проти збудника Alternaria tenuissima проявили екстракти шавлії лікарської (Salvia officinalis L.), полину однорічного (Artemisia annua L.) та маклеї серцеподібної (Macleaya cordata L.). Ці дані свідчать про те, що екстракти даних рослин можуть бути використані в подальшому для розроблення засобів захисту рослин від альтернаріозу.*

рослинні екстракти, *Alternaria tenuissima*, інгібування росту, радіальна швидкість росту, біологічний захист

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Влияние растительных экстрактов на *Alternaria tenuissima* (Kunze) Wiltshire в условиях *in vitro*

Цель. *Исследовать фунгистатическое действие растительных экстрактов на Alternaria tenuissima в условиях in vitro.* **Методика.** *Исследования проведены в лаборатории фитопатологии Института защиты растений НААН (ИЗР НААН) и лаборатории экологии и фармакогнозии Опытной станции лекарственных растений Института агроэкологии и природопользования НААН (ОСЛР ИАП НААН). Лекарственное растительное сырье, которое использовали в исследованиях, было выращено и отобрано на исследовательских участках ОСЛР ИАП НААН. На его основе были изготовлены растительные экстракты. Определение активности растительных экстрактов на рост культуры Alternaria tenuissima проводили в лаборатории фитопатологии ИЗР НААН. Использован метод оценки чувствительности грибов с использованием агаризированной питательной среды. Определяли радиальную скорость роста и процент торможения роста колоний.* **Результаты.** *На 5-й день после закладки опыта все изучаемые экстракты формировали колонии существенно меньшего размера по сравнению с контролем. На 7-й день подавляли развитие колоний Alternaria tenuissima экстракты шалфея, чабреца, полыни однолетней, полыни горькой, корня эхинацеи, маклеи. На 10-й день достоверное снижение роста колоний возбудителя происходило в вариантах применения экстрактов шалфея, полыни однолетней, корня эхинацеи и маклеи. Торможение роста колоний было самым высоким для шалфея, полыни однолетней, маклеи и составляло от 84,3—99,5% на 5-й день до 38,1—73,4% на 10-й день после инокуляции.* **Выводы**. *По результатам проведенных исследований выраженное фунгистатическое действие против возбудителя Alternaria tenuissima проявили экстракты шалфея лекарственного (Salvia officinalis L.), полыни однолетней (Artemisia annua L.) и макли сердцевидной (Macleaya cordata (Willd) R. Br.). Эти данные свидетельствуют о том, что экстракты данных растений могут использоваться в дальнейшем для разработки средств защиты растений от альтернариоза.*

растительные экстракты; *Alternaria tenuissima*; ингибирование роста; радиальная скорость роста; биологическая защита

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