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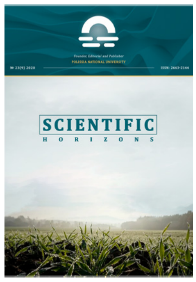

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Applying of Plants to Regulate Mycobiota of Winter Wheat Seeds

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Abstract. Plants, having a wide array of different substances that are natural to ecosystems, can be effective biofungicides. Most scientific studies have demonstrated the effectiveness of plant extracts against certain phytopathogenic species/genera in vitro. These results do not provide an answer to how certain substances might affect the systems of microorganisms. The aim of the work was to study the influence of plants on isolation of mycobiota representatives of winter wheat seeds, the peculiarities of its germination and seedling development. The mycocomplex of the seeds of the 2017 and 2019 harvests from the North-East of Ukraine was studied. The seeds were kept in aqueous plant solutions and spread on potato-glucose agar. The effects of *Citrus sinensis* L., *Citrus limon* L., *Zingiber officinale* Roscoe, *Larix decidua* Mill., and *Pinus sylvestris* L. were evaluated. All plant solutions changed the quantitative and qualitative composition of seed mycobiota. The negative effect of soaking seeds on the development of wheat seedlings was established, which will not allow them to be used to regulate the seed mycocomplex in the future. *C. limon* (67%) and *Z. officinale* (on average 52%) most effectively reduced the number of dominant *Alternaria* sp. Seed treatment increased the number of fungal colonies compared to the control, except for the 40% ginger solution, and when ginger was used, the spectrum of fungi was expanded, and when citrus and pine were used, it was narrowed. Plant solutions changed the dominance of *Alternaria* sp. in the seed mycobiota on the prevalence of *Penicillium* sp. and *Aureobasidium pullulans* (de Bary) G. Arnaud. The mycobiota of wheat seeds acquired the most significant changes under the influence of *L. decidua* and *P. sylvestris*

Keywords: biofungicides, citrus fruits, ginger, pine needles, seed treatment, fungal complex

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INTRODUCTION

Mycobiota of winter wheat seeds contains endophytic and phytopathogenic fungi. Endophytes are fungi that do not have a negative effect on plants (Chitnis *et al.*, 2020). Scientists are currently actively searching for biological plant protection agents among endophytes. Wheat seeds are a source of infection for phytopathogenic species. One of the elements of plant protection is the treatment of seeds with pesticides of various origins to regulate pathogens. The main purpose of seeds treatment is the destruction of fungi, which is effectively dealt with by modern chemical fungicides. The question of changes in the mycobiota of seeds when individual representatives disappear remains unexplored. Although chemicals are highly effective, they have a negative impact on human health (carcinogenic, teratogenic, acute toxicity) and the environment (prolonged decomposition leads to contamination of soil, water, etc.) (Soković *et al.*, 2013). Continued use of fungicides causes the emergence of resistant forms in populations of phytopathogens: resistance of *Fusarium* to prochloraz, tebuconazole and benomyl (Choi *et al.*, 2017); cross-resistance of *Alternaria alternata* to mancozeb and difenoconazole (Yang *et al.*, 2019). People require natural foods without dangerous substances to improve their health. To date, chemical manufacturers have regained their interest in natural compounds (Soković *et al.*, 2013). These companies used to attract natural compounds. Thus, the active ingredients of Falcon (manufactured by Bayer) include morpholines, which are derivatives of cinnamic acid.

In Ukraine, biological pesticides on wheat based only on microorganisms and their metabolites have been registered. Some plants contain a variety of biologically active chemicals that may be new components for biofungicides. Plant extracts, essential oils, gums, resins etc. are sources for finding natural ingredients. These products are more suitable for natural ecosystems. Plant substances have a specific target effect, without touching the beneficial antagonists, they are quickly destroyed, do not pollute the environment, easily accessible and cost-effective. Plant extracts and oils are most often used against fungal pathogens (Zaker, 2016). The biologically active substances of plants include alkaloids, glycosides, glucosinolates, lipids, phenols, terpenes, polythienes, polyacetylenes, etc. (Borges *et al.*, 2018).

An analysis of scientific studies on the effectiveness of plant extracts against seed fungi of various crops showed 100 percent inhibition of phytopathogens, with most researchers using aqueous plant extracts (Almeida *et al.*, 2021).

Mycobiota of wheat seeds consists of various representatives. As a rule, the most common or most harmful species are first isolated from seeds, and then the effectiveness of plant extracts against phytopathogens is studied in vitro. Thus, Z. Baka & M. Baka (2014) first identified the most common species in wheat seeds (*A. flavus*, *A. niger*, and *F. moniliforme*), and then determined the effectiveness of five medicinal plants before their growth on a nutrient medium. When studying the effectiveness of grain extracts and beekeeping products on fungi from the seeds of buckwheat, wheat, oats and corn, the most harmful species from the genus *Fusarium* were first isolated. And then the influence of the extracts on the growth of these fungi on PDA was studied (Keriene *et al.*, 2020).

Less often, researchers study the effectiveness of plant extracts *in vivo*. The study of four plants on the peculiarities of isolation of fungi from wheat seeds by the Standard Blotter Method (on filter paper) showed their high efficiency (Arshad Ali *et al.*, 2015). The efficiency of five plants was tested by seed treatment followed by incubation on filter paper and agar medium with control of 12 representatives of mycobiota of barley seeds (Ahmad *et al.*, 2016).

Thus, most often investigations do not take into account the changes that will occur in microbial complexes with a decrease in the number or extinction of a species. Important factors in determining the effectiveness of botanical fungicides are the chemical composition of plants, which depends on many factors, as well as the local population of phytopathogens. Therefore, *the goal of the research* was to determine not only the effectiveness of aqueous solutions of plants against certain representatives of the mycobiota of winter wheat seeds, but also the nature of changes in it, taking into account the impact on seed germination and plant development.

MATERIALS AND METHODS

Winter wheat (Bohdana variety) was grown in the North-East of Ukraine (Sumy region, educational and scientific production complex of Sumy National Agrarian University). The seeds of the 2017 and 2019 harvests were analysed.

Lemons, oranges and ginger were bought in the supermarket. Pine and larch needles were torn from the trees of the SNAU arboretum. Lemon and orange fruits were squeezed to obtain juice. Sterilized water was added to the juice to obtain a 10% aqueous solution. The ginger rhizome was crushed in a blender, and then the juice was squeezed through gauze. By adding sterile water, a 40% aqueous solution was obtained. Larch and pine needles (4.5 g) were ground with the addition of water. Then was added 100 ml of sterile water. The seeds were kept in aqueous solutions for 2-3 hours. It was soaked in sterilized water in the control variant. After drying on filter paper, the seeds were spread on potato-glucose agar. Petri dishes were incubated in a thermostat at 22-24°C for seven days. Fungi were identified by the biological method based on colony morphology, mycelium and asexual sporulation based on (Warham *et al.,* 1997; Watanabe, 2002; Leslie & Summerell, 2006; Schubert *et al.,* 2007; Zalar *et al.,* 2008; Walther *et al.,* 2013). The second step was to study the effect of solutions on seed germination: the number of germinated seeds (on the 7th day) and average length of seedlings were calculated. The length of seedlings to study the effectiveness of solutions of ginger and conifers was measured on the 14th day.

Statistical analysis of the results was performed by the method of one-way analysis of variance in Excel, calculating the LSD_{05}.

RESULTS AND DISCUSSION

The dominance of Alternaria in the mycobiota of winter wheat seeds in the conditions of the North-East of Ukraine was established, as evidenced by other studies (Rozhkova *et al.,* 2021). In addition to these fungi, two genera (*Penicillium* sp. and *Trichoderma* sp.) and five species (*Cladosporium herbarum* (Pers.: Fr.) Link, *Neurospora sitophila* Shear, *Mucor mucedo* L., *Nigrospora oryzae* (Berkeley et Broome) Petch., and *Aureobasidium pullulans* (de Bary) G. Arnaud) were identified in the control. Fungi that germinated from wheat seeds without sporulation, but only in the form of mycelium, were not identified.

Citrus was chosen to regulate the mycobiota of seeds based on their properties: they have an antiseptic effect and contain a significant amount of ascorbic acid (in orange 66, lemon – 52-60 mg). When soaking the seeds for two hours, the number of fungal colonies on variants with the use of plants increased compared to the control (Table 1).

***Table 1**. The effect of aqueous extracts of plants of the genus Citrus on the isolation of fungi mycobiota seeds and the development of seedlings of winter wheat (2017)*

Variants	Isolation of fungal colonies, pcs	Number of colonies, pcs	Seed germination, %	The average length of seedlings, mm
Control (water)	*Alternaria* sp. 78 *Penicillium* sp. 21 *C. herbarum* 9 *N. sitophila* 3 *M. mucedo* 2	113	100	51.4
Orange (10% aqueous solution of fruit juice)	*Alternaria* sp. 65 *Penicillium* sp. 61 *M. mucedo* 5	131	100	40.5
Lemon (10% aqueous solution of fruit juice)	*Penicillium* sp. 105 *Alternaria* sp. 26 *M. mucedo* 6	137	100	20.5
LSD_{05}	*Alternaria* sp. 4.8 *Penicillium* sp. 6.8	10.6	-	4.4

Regarding the effect of lemon and orange on the mycobiota of wheat seeds, the similarity of their action was noted: they reduced the range of its species and genera, the amount of *Alternaria* fungi and increased the number of *Penicillium* sp. These fungi inhibited the development of wheat germ. Moreover, this negative effect was greater in the variant with the use of lemon, where the amount of *Penicillium* fungi was 105 colonies – this is the maximum indicative isolation among all authors' studies.

Alternaria fungi are dangerous pathogens for citrus crops, so most modern research is aimed at studying their species composition, pathogenicity and more. Information on studies on the effectiveness of citrus extracts against fungi was not found, but the results of testing essential oils are known. An in vitro study of the antifungal activity of lemon (*C. limon*) essential oil against three pathogenic fungi that infect grapevine (*Eutypa* sp., *Botryosphaeria dothidea* and *Fomitiporia mediterranea*) has shown its significant effectiveness against all tested fungi. Eutypa sp. was the most sensitive to the essential oil of *C. limon*. The composition of EO was also studied. Ten volatile components were identified by gas chromatography, the most common of which were hydrocarbon monoterpenes (Ammad *et al.*, 2018). The effectiveness of citrus essential oils on *Aspergillus* and *Penicillium* fungi has been studied. The essential oil of *C. sinensis* caused complete inhibition of the growth of *Aspergillus niger* (L.) at 3.0 $\mu g \cdot ml^{-1}$ on agar plates. The oil showed fungistatic activity at 1.5 $\mu g \cdot ml^{-1}$ with approximately 79% growth inhibition after 7 days of incubation and delayed conidiation compared to control. Its use also caused bifurcation of apical hyphae and abundant budding of vegetative hyphae, which led to complete loss of cytoplasm by hyphae (Sharma & Tripathi, 2008). Earlier fungitoxic studies of *C. sinensis* essential oil have shown the antifungal efficacy of citrus oils against *Penicillium digitatum* and *Penicillium italicum* and have shown that P. digitatum has been demonstrated to be the most sensitive (Caccioni *et al.*, 1998).

Thus, citrus changes the mycobiota of winter wheat seeds, reducing the number of *Alternaria* sp., but increasing the number of *Penicillium* sp., which inhibits the development of wheat seedlings (especially lemon).

The influence of garden ginger on the mycobiota of wheat seeds and the peculiarities of the development of its seedlings was carried out in 2017 and 2019 (Table 2).

***Table 2**. The effect of garden ginger (Z. officinale) on the mycobiota of winter seeds and the development of wheat seedlings*

Variant	Isolation of fungal colonies, pcs	Number of colonies, pcs	The average length of seedlings on the 7th/14th day, mm
2017			
Control (water)	*Alternaria* sp. 78 *Penicillium* sp. 21 *C. herbarum* 9 *N. sitophila* 3 *M. mucedo* 2	113	51.4/-
Ginger (100% juice)	*Penicillium* sp. 64 *Alternaria* sp. 44 *N. sitophila* 4 *C. herbarum* 3 *M. mucedo* 2 *Fusarium* sp. 1 Other fungal colonies 1	119	45.3/-
LSD_{05}	*Alternaria* sp. 6 *Penicillium* sp. 5.3 *C. herbarum* 3.7	6	8.1
2019			
Control (water)	*Alternaria* sp. 43 *Penicillium* sp. 16 *N. oryzae* 12 *A. pullulans* 10 *Trichoderma* sp. 5 Other fungal colonies 29	115	40.4/97.5
Ginger (40% aqueous solution)	*Penicillium* sp. 18 *Alternaria* sp. 17 *A. pullulans* 16 *N. oryzae* 6 *Arthrinium arundinis* (Corda) Dyko & B. Sutton 1 *N. sitophila* 1 *Fusarium* sp. 1 Other fungal colonies 38	98	28.2/78.9
LSD_{05}	*Alternaria* sp. 5.3 *N. oryzae* 7.7 *A. pullulans* 3.2	8	5.6/8.1

In 2017, the effect of undiluted rhizome juice was investigated, which showed a slight increase in colonies compared to the control.

The use of ginger reduced the amount of *Alternaria* sp. by 43.6%, but increased the number of *Penicillium* sp. (Fig. 1).

Figure 1. *Mycobiota of winter wheat seeds treated with 100% ginger juice (Bogdana variety, 2017)*

Suppression of seedling development was also recorded. In 2019, the seeds were kept in 40% aqueous solution, after which the number of fungal colonies decreased. Uncertain species occupied a dominant position in the mycobiota, and the number of *Alternaria* fungi decreased by 60% compared to the control. The species composition has changed: instead of *Trichoderma* sp. sprouted other fungi (*A. arundinis* (Fig. 2), *N. sitophila*, and *Fusarium sp*.). An inhibitory effect on seedling length on both the 7th and 14th day was also observed.

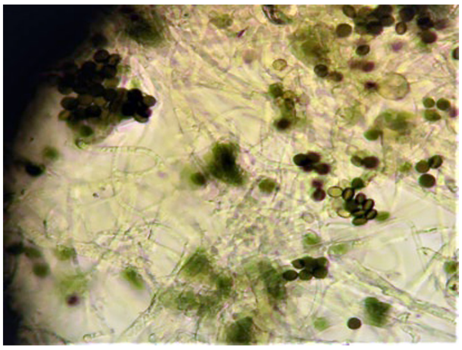

Figure 2. *Mycelium and conidial sporulation of A. arundinis*

A significant effect of aqueous solutions of ginger (15.10 and 5%) on the growth of *Alternaria alternata* (Fr.) Keissl. from tomatoes in Sudan has been shown. The highest concentration contributed to the best inhibition of the fungus. Ginger restrained mycelial growth by 57%, 35% and 20% compared to controls (Osman & Algam, 2016). The effect of aqueous extracts of five different plants on *A. alternata* and *Fusarium oxysporum* was studied in vitro. Ginger solutions have been effective in inhibiting the growth of tested fungi (Fawzi *et al.*, 2009). Ethanol extract of ginger of various concentrations (0.5, 1.5-5.5%) showed high efficiency of inhibition of mycelial growth and sporulation of *A. alternata* – the causative agent of spinach spot, in vitro. The highest concentration (5.5%) was 90.4% inhibition of mycelial growth. The germination of spores was completely suppressed by both 4.5% and 5.5% concentration (Rizwana, 2015). The effect of aqueous, ethanol and methanol extracts and ginger essential oil on the radial growth of *Alternaria solani* and *Phytophthora infestans* was studied. Ginger oil was as effective as a synthetic fungicide. That is, this plant has strong antifungal properties with a high potential for use as a biofungicide (Mugao *et al.*, 2021). A study of the effectiveness of extracts from five plants against *Alternaria alternata* in vitro showed significant antifungal activity of these plants, one of which was ginger. The zone of inhibition of *Z. officinale* ranged from 30 to 48 mm. The highest antifungal value of ginger extract was observed at a concentration of 2.5 $\mu l \cdot ml^{-1}$ – 87.04% (Ghalem *et al.*, 2020).

Thus, ginger alters the mycobiota of wheat seeds, most reducing the amount of *Alternaria* fungi, but this increases the number of *Penicillium* sp. 100% juice and 40% aqueous solution inhibits the development of wheat seedlings.

It was established that *C. limon* and *Z. officinale* are active *Alternaria* reducers. Scientists around the world are actively searching for chemical compounds to regulate the number of these fungi. In Uruguay, a study of 10 plant species found that extracts of *Salvia sclarea* L., *S. officinalis* L., and *Rosmarinus officinalis* L. were effective against *Alternaria* sp. at the level of chemical fungicides (Dellavalle *et al.*, 2011). In Mexico, the effectiveness of 12 different plants was found to inhibit the growth of *A. alternata* in the range of 2.02-69.07%. *Adenophyllum aurantium* L. was the most effective against this fungus (Lira-De León *et al.*, 2014). In India, aqueous and methanol extracts of *Aloe vera* L., *Polygonum perfoliatum* L., *Cymbopogon citratus* (DC.) Stapf, *Lantana camara* L., and *Mimosa pudica* L. have been shown to have the ability to significantly inhibit the growth of *A. alternata* and *A. tenuissima* (Srivastava *et al.*, 2012).

Quite unexpected results were obtained by studying the effect of aqueous solutions of conifers on the mycobiota of wheat seeds. Soaking the seeds in aqueous extracts of pine and larch needles showed quite similar results (Table 3).

***Table 3**. The effect of aqueous solutions of needles on the mycobiota of winter wheat seeds (Bohdana variety of the 2019 harvest)*

Variant	Isolation of fungal colonies, pcs	Number of colonies, pcs
Control (water)	*Alternaria* sp. 30 *A. pullulans* 18 *N. oryzae* 9 *Trichoderma* sp. 7 *Penicillium* sp. 6 Other fungal colonies 31	103
L. decidua	*A. pullulans* 58 *Alternaria* sp. 32 *Trichoderma* sp. 5 *M. mucedo* 4 *Fusarium sporotrichioides* Sherb.3 *N. sitophila* 1 *Penicillium* sp. 1 Other fungal colonies 3	107
P. sylvestris	*A. pullulans* 42 *Alternaria* sp. 42 *F. sporotrichioides* 16 *Rhyzopus stolonifer* (Ehrenb.) Vuill. 6 Other fungal colonies 1	110
LSD_{05}	*Alternaria* sp. 6.8 *A. pullulans* 5.5	6.3

The amount of *A. pullulans* and *Alternaria* sp. increased, the germination of colonies without sporulation of fungi decreased, *Fusarium* fungi appeared in variants with pine needles. Germination of fungi of the genus Mucor was also observed. Pine needles provoked the release of the largest amount of *F. sporotrichioides*.

The average length of seedlings in the variants with soaking the seeds in the solutions of the needles was less than in the control (Fig. 3). The reduction in plant growth with the use of pine was 26.7% compared to the control and was greater than in the variant with soaking in a solution of larch needles (12%) on the 7th day. This fact may be explained by the greater isolation of Fusarium fungi for soaking the seeds in an aqueous solution of pine needles. Measurement of the length of wheat seedlings on the 14th day demonstrated the preservation of the dominance of the indicator in the control. The picture of the negative impact has hardly changed. The high percentage of isolation of *Fusarium* fungi by treatment with a solution of pine needles suspended the development of seedlings more significantly at this date of accounting – 33% compared to the control. The use of larch suspended germination by 7%.

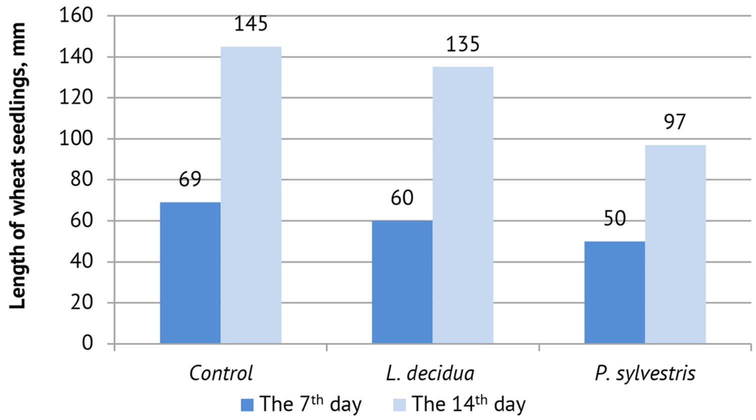

***Figure 3**. The effect of aqueous solutions of needles on the development of wheat seedlings (LSD_{05}7=5.3; LSD_{05}14=4.9)*

The antimicrobial action of essential oils of the *Pinaceae* family has been proven by various authors. Pine essential oil was very active only against bacteria, but not against fungi (Lis-Balchin *et al.*, 1998). Essential oil (at a concentration of 2%) from North American pine is able to inhibit the growth of two species: *F. culmorum* and *F. solani*. Fungicidal activity against *F. poae* was observed at 5% concentration of pine oil (Krauze-Baranowska *et al.*, 2002).

Pine needles and bark contain a lot of essential oil, so they are used in medicine (Baldan *et al.*, 2017). Studies of the antimicrobial action of larch bark and wood have shown their high effectiveness against *Aspergillus flavus*, *A. niger* and *Penicillium funiculosum*. (Salem *et al.*, 2016).

The obtained results showed the effect of plant extracts on the entire mycobiota of winter wheat seeds, unlike other studies that demonstrated only a decrease in its individual representatives (Arshad Ali *et al.*, 2015 Ahmad *et al.*, 2016).

CONCLUSIONS

Soaking the seeds in aqueous solutions of *C. sinensis*, *C. limon*, *Z. officinale* (as well as its juice), *L. decidua* and *P. sylvestris* led to significant changes in the mycobiota of winter wheat seeds grown in the North-East of Ukraine. The dominance of *Alternaria* sp. in the mycocomplex from winter wheat seeds on control was established. Two genera (*Penicillium* sp. and *Trichoderma* sp.) and five species (*Cladosporium herbarum* (Pers.: Fr.) Link, *Neurospora sitophila* Shear, *Mucor mucedo* L., *Nigrospora oryzae* (Berkeley et Broome) Petch., and *Aureobasidium pullulans* (de Bary) G. Arnaud) were identified here as well.

Solutions of citrus juice increased the number of fungal colonies, did not affect seed germination and significantly lessened the length of seedlings on the 7th day. They reduced the number of *Alternaria* sp., *Penicillium* sp. occupied a dominant position in the seed mycocomplex. *C. limon* had the greatest effect on the length of seedlings, because the maximum amount of *Penicillium sp*. sprouted. These fungi are able to inhibit plant development. Ginger juice and 40% aqueous solution had a citrus-like effect on seed mycobiota, except that they further expanded the range of fungal species. The most unexpected changes in the mycocomplex were caused by soaking the seeds in aqueous solutions of pine and larch needles: the appearance of *F. sporotrichioides*, *Mucor* fungi, an increase in the number of *A. pullulans* and *Alternaria* sp.

These plants cannot be used to regulate the mycobiota of wheat seeds because they adversely affect plant growth. *C. limon* and *Z. officinale* are effective reducers of *Alternaria* sp.

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Застосування рослин для регулювання мікобіоти насіння пшениці озимої

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Анотація. Рослини, маючи широкий набір різних речовин, які є природними для екосистем, можуть бути ефективними біофунгіцидами. Більшість наукових досліджень продемонстрували ефективність екстрактів рослин проти певних фітопатогенних видів/родів в умовах in vitro. Ці результати не дають відповіді на те, як певні речовини будуть впливати на системи мікроорганізмів. Було поставлено за мету вивчити вплив рослин на виділення представників мікобіоти насіння пшениці озимої, особливості його проростання та розвиток проростків. Дослідили мікокомплекс насіння врожаїв 2017 та 2019 рр. з Північного Сходу України. Насіння було витримано у водних розчинах рослин та розкладено на картопляно-глюкозний агар. Оцінили вплив *Citrus sinensis* L., *Citrus limon* L., *Zingiber officinale* Roscoe, Larix decidua Mill. та Pinus sylvestris L. Всі рослинні розчини змінили кількісний та якісний склад мікобіоти насіння. Було встановлено негативну дію замочування насіння на розвиток проростків пшениці, що не дозволить їх застосувати для регулювання насіннєвого мікокомплексу у подальшому. *C. limon* (67 %) та *Z. officinale* (у середньому 52 %) найефективніше знизили чисельність домінуючих *Alternaria* sp. Обробка насіння збільшила кількість грибних колоній порівняно з контролем, за винятком 40 %-го розчину імбиру, та за застосування імбиру розширила спектр грибів, а за використання цитрусових та хвої – його звузила. Рослинні розчини змінили домінування *Alternaria* sp. у мікобіоті насіння на превалювання *Penicillium* sp. та *Aureobasidium pullulans* (de Bary) G. Arnaud. Найістотніших змін мікобіота насіння пшениці набула під дією *L. decidua* та P. *sylvestris*

Ключові слова: біофунгіциди, цитрусові, імбир, хвоя, протруєння насіння, грибний комплекс