Substances for biological protection, regulation of growth and development of agricultural crops based on secondary plant metabolites

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Abstract. Plants are a promising source of a wide range of biologically active substances as biopesticides and growth stimulants in organic agriculture. Screening of the antimicrobial activity of ethanol extracts of air-dry aboveground biomass of 22 plant species of the family Asteraceae, typical representatives of the flora of the Republic of Tatarstan, was carried out. The antibacterial activity of greater knapweed (Centaurea scabiosa L.), milk thistle (Silybum marianum (L.) Gaertn), scentless mayweed (Tripleurospermum inodorum (L.) Sch. Bip.), marsh cudweed (Gnaphalium uliginosum L.) against test strains of microorganisms – Clavibacter michiganensis VKM Ac-1404 (phytopathogenic gram-positive bacterium) and Alternaria solani St108 (phytopathogenic fungi) was established. The highest indices of inhibitory activity were found in the case of marsh cudweed - 0.0063%. The efficiency of pre-sowing treatment of seeds and foliar treatment of plants with an aqueous solution the potassium salts of terpene acids of Siberian cedar pine resin on the yield of winter wheat variety Kazanskaya-560 on gray forest soil at the Republic of Tatarstan was revealed. The yield increase was 3.7-3.9% to control. The grain size indicator (1000 grains weight) varied in the range of 36-38 g with the highest values in the case of pre-sowing treatment.

1. Introduction

According to the forecasts of demographers, the growth of the world’s population is exponential, and by 2050 it will reach 9.8 billion people. To meet the growing needs of people for food, it is necessary to increase their production. However, limited land and water resources, climate change and increased frequency of extreme events can pose a significant threat to achieving the goal of sustainable agriculture [1]. Plants, due to immobility, are exposed to a wide range of abiotic and biotic influences, including contact with pathogenic and opportunistic microorganisms, which have a complex stimulating or inhibitory effect on the general health of plants and crop yields. An important factor limiting the productivity of agricultural plants growing mainly in monoculture, as well as the safety of the resulting crop, is the defeat of phytopathogenic microorganisms [2,3].

Phytopathogenic microorganisms with multiple resistance to pesticide preparations are a threat to the development of the agro-industrial complex on a global scale. The widespread introduction of multicomponent drugs negatively affects the ecological state of soil and aquatic ecosystems and causes deterioration in the health of farm animals and humans [4]. The priority direction of scientific research in this area is the search for new antimicrobial agents that have a wide spectrum of
action and do not cause the formation of resistant strains. Higher plants are a source of natural biologically active substances of secondary origin, many of which are actively used in the pharmaceutical, cosmetic, agricultural and food industries [5-7].

The ability of plants to biosynthesize various substances of specialized metabolism is their unique feature. The most numerous groups of secondary metabolites are: isoprenoids, phenolic compounds and alkaloids. Each of these groups is subdivided into numerous subgroups. In addition to these main groups, minor classes of secondary compounds of plant origin are distinguished: cyanogenic glycosides, sulfur-containing glycosides (thioglycosides), plant amines, non-protein amino acids, polyacetylenes, thiophenes, etc. Secondary substances are usually present in cells and tissues in smaller amounts than substances primary exchange [8]. To date, about 20–30 thousand plant species have been investigated for the presence of secondary metabolites — approximately 10–15% of the entire flora of the Earth [9].

Secondary plant metabolites in most cases act as protectors against pathogenic microorganisms, animal pests, and chemicals [10]. Most of the protective metabolites function due to their antimicrobial activity, and a high concentration of compounds (for example, camalexin, scopoletin, daphnetin, etc.) can suppress or even kill microbes in vitro [11]. On the basis of [12], among the substances of secondary origin, the most active and studied phytochemicals, both individually and in the composition of plant extracts or essential oils, were polyphenols and terpenes. In this case, the number and position of phenolic hydroxyl groups, double bonds, delocalized electrons, and conjugation with sugars in the case of flavonoids, seem to be of decisive importance for the antimicrobial ability.

Phytoantimicrobials may play a key role in combating antimicrobial resistance due to their ability to target the main determinants of microbial drug resistance, including cell membranes, ATP-dependent efflux pumps, cell communications, and biofilms [13].

The volume of the world market for vegetable raw materials is constantly growing and, according to forecasts, by the end of 2023 will reach 111 billion dollars, the compound annual growth rate will be ~ 7.2% during 2017-2023 [14]. The market value of the secondary plant metabolites used in the national economy in the form of essential oils by 2022 worldwide will reach $ 27 billion. The increase in growth rates is explained by a change in consumer preference trends towards drugs of natural origin [15]. However, it should be taken into account that the prevalence and diversity of plant resources is determined by the soil and climatic conditions of the region. In this regard, special attention should be paid to the study of the biological activity of the flora, typical for the Russian Federation, in order to create a database of promising plants.

The need to create sustainable systems of farming and ecological farming, as well as to increase the antibiotic resistance of pathogenic microorganisms to plant protection products, made it necessary to search for alternative methods of combating phytopathogens. In this regard, an urgent task is to search for compounds of plant origin as a potential source of active elements in the formulation of new environmentally friendly and effective drugs.

2. Materials and methods

Screening of plants of the family Asteraceae growing in the Republic of Tatarstan was carried out according to indicators of antibacterial activity. We studied 22 plant species – British elecampane (Pantenema britannicum L.), sneezeweed (Achillea ptarmica L.), common yarrow (Achillea millefolium L.), brown knapweed (Centaurea jacea L.), blue cornflower (Centaurea cyanus L.), greater knapweed (Centaurea scabiosa L.), three-lobe beggartick (Bidens tripartita L.), creeping thistle (Cirsium arvense (L.) Scop), spiny plumeless thistle (Carduus acanthoides L.), milk thistle (Silybum marianum (L.) Gaertn), scentless mayweed (Tripleurospermum inodorum (L.) Sch.Bip.), Canadian horseweed (Erigeron canadensis L.), globe thistle (Echinops ritro var.tenuifolius DC.), creeping thistle (Cirsium arvense (L.) Scop), field milk thistle (Sonchus arvensis L.), greater burdock (Arctium lappa L.), nodding thistle (Carduus thoeimeri Weinm.), Mexican Marygold (Tagetes erecta L.), Canadian goldenrod (Solidago canadensis L.), bristly hawkbit (Leontodon hispidus L.), marsh cudweed (Gnaphalium uliginosum L.).
To prepare the extracts, there were used air-dry aboveground biomass collected during the flowering period. Extraction was done with 70% ethanol. The ratio of the weight of the sample of plant biomass to the volume of the extractant was 1:10. Then the mixture was stirred on a magnetic stirrer at a temperature of 45°C for 2 hours and filtered on a paper filter. Additionally, centrifugation was performed (5000 rpm, 15 min).

Potassium salts of terpene acids of Siberian cedar pine resin *Pinus sibirica* Du Tour (Altai Territory, Russian Federation) were used as plant growth regulators. Potassium salts were obtained as described in [16]. The studies were carried out on the basis of the experimental fields of the Federal Research Center "Kazan Scientific Center of the Russian Academy of Sciences" in the Republic of Tatarstan (Russian Federation). The experiments were repeated triplicate. The shape of the plots is rectangular, the area is 10 m².

The studies were carried out on winter wheat variety Kazanskaya 560. The placement of variants within the repetitions is systematic. Pre-sowing seed treatment was carried out by spraying the grains with simultaneous mixing with a working solution at a concentration of 0.1% at a consumption of 0.1 l/t of seeds. Foliar treatment was carried out twice – in the autumn (in the tillering phase) and in the summer (in the flowering phase). For spraying, a knapsack sprayer was used, the consumption of the working solution was 300 l/ha.

Evaluation of the antimicrobial activity of the compounds was carried out by determining the minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC) and minimum fungicidal concentrations (MFC) by the method of serial dilutions [17].

The phytopathogenic bacterium *Clavibacter michiganensis VKM Ac-1404* (representative of Gram + microorganisms) and the phytopathogenic fungi *Alternaria solani* St108, widespread pathogens parasitizing plants, were used as test strains of microorganisms. Microorganisms were cultured in standard sterile nutrient broths. The concentration of bacteria was determined using a DEN-1B densitometer (Biosan, Latvia) according to standard protocols. Norfloxacin (Sigma-Aldrich Co., USA), ketoconazole (Sigma-Aldrich Co., USA), chloramphenicol (Kazan pharmaceutical plant, Russia) and difenoconazole (Score250 EC, Syngenta, USA).

3. Results and discussion

3.1. Antimicrobial activity of plant extracts

The antimicrobial activity of ethanol extracts of Asteraceae plants against *Clavibacter michiganensis VKM Ac-1404* and *Alternaria solani* St108 varied in a wide concentration range from 0.0125 to 0.25% in the case of minimal inhibitory concentrations (MIC) and from 0.015 to 0.375% in the case of minimal bactericidal (MBC) and minimum fungicidal concentrations (MFC) – 0.0125-0.25%. The highest indicators of activity were found in the case of greater knapweed, milk thistle, scentless mayweed and marsh cudweed. The data are presented in Table 1.

### Table 1. Antimicrobial activity of ethanol extracts of some representatives of plants of the family Asteraceae against phytopathogens

| Plant species                        | *Clavibacter michiganensis* | *Alternaria solani* |
|--------------------------------------|-----------------------------|---------------------|
|                                      | MIC, %                      | MBC, %              | MIC, %    | MFC, %   |
| *Centaurea scabiosa* L.              | 0.0625±0.0004               | 0.2500±0.0100       | 0.1250±0.007 | 0.5000±0.010 |
| *Silybum marianum* (L.) Gaertn       | 0.0313±0.0001               | 0.0313±0.0005       | 0.0150±0.002 | 0.0150±0.001 |
| *Tripleurospermum inodorum* (L.) Sch.Bip. | 0.0500±0.0050               | 0.0500±0.0050       | 0.0125±0.001 | 0.0250±0.002 |
| *Gnaphalium uliginosum* L.           | 0.0063±0.0002               | 0.0250±0.0040       | 0.0063±0.006 | 0.0125±0.001 |

a Antimicrobial activity of chloramphenicol MIC 1.9 µg/ml, MBC 1.9 µg/ml.

b Antimicrobial activity of difenoconazole MIC 1.9 µg/ml, MFC 31.3 µg/ml.
The ethanol extract of marsh cudweed had high rates of activity against both the bacterial pathogen and the phytopathogenic fungi. At the same time, the indicators of the inhibitory activity of the extract were significantly higher than that of the bactericidal and fungicidal.

The antimicrobial activity of ethanol extracts of the studied plants is apparently due to the presence in their composition of components with antibiotic properties – phytol, squalene, β-Amirin, and a number of sesquiterpene lactones [17].

3.2. Growth-stimulating activity of an aqueous solution of potassium salts of terpenic acids of Siberian cedar pine resin

Pre-sowing seed treatment with an aqueous solution of saponified resin had a stimulating effect on the germination of winter wheat: the field germination rate of the treated seeds was 6% higher than in the control.

Pre-sowing treatment and a single autumn foliar treatment of seedlings contributed to an increase in the sugar content in shoots by 8-9%.

The effectiveness of the use of an aqueous solution of salts of the Siberian cedar pine resin on the yield of winter wheat variety Kazanskaya-560 was established. The increase in yield in the case of pre-sowing treatment was 3.7%, foliar – 3.9%.

The grain size indicator (1000 grains weight) varied in the range of 36-38 g with the highest values in the case of pre-sowing treatment.

The mechanism of the growth-stimulating effect of an aqueous solution of potassium salts of terpenic acids of the Siberian cedar pine resin is due to a complex of factors. Earlier, we established the antioxidant activity of the studied solutions of potassium salts of resin in a concentration of 1 mg/ml [16]. An imbalance between free radicals and antioxidants in the cells of living organisms (oxidative stress) leads to a decrease in their metabolic activity. Neutralization of reactive oxygen species using solutions of potassium salts of resin terpenic acids improved growth and development indicators.

Analysis of the phytochemical composition of the Siberian cedar pine resin by the GLC-MS method revealed the presence of α-pinene D-limonene and abietic acid; their total amount was 74.3% of the total number of identified compounds. There is information about the nonspecific antimicrobial action of these compounds due to inhibition of the growth of pathogen cells and / or damage to their cell walls [16].

4. Conclusions

1. Screening of antimicrobial activity of ethanol extracts of air-dry aboveground biomass of 22 plant species of the family Asteraceae, typical representatives of the flora of the Republic of Tatarstan, was carried out.

2. As a result of the research, 4 plant species with antibacterial activity against test strains - phytopathogenic gram-positive bacteria and phytopathogenic fungi – were identified. The highest indices of inhibitory activity were found in the case of marsh cudweed – 0.0063%.

3. The efficiency of using aqueous solutions of potassium salts of terpenic acids of the Siberian cedar pine resin for pre-sowing treatment of seeds and foliar treatment of winter wheat plants when on gray forest soil in the conditions of the Republic of Tatarstan has been shown.

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