ORIGINAL PAPERS

THE BIOLOGICAL SUBSTANTIATION OF APPLICATION OF ANTIMICROBIAL PROPERTIES OF *RHEUM RHAPONTICUM* IN PLANT PROTECTION

Alla Gladcaia, Leonid Volosciuc

Keyworlds: *Rheum rhaponticum L., rootextract, plant phytopathogens Fusarium sporotrichiella, Erwinia amylovora, Pseudomonas syringae, Sphaerotheca fuliginea, Cucurbitaceae*

INTRODUCTION

The ability of higher plants to produce and accumulate biologically active products of secondary metabolism, worked out as a result of co-evolution of the species, it is one of the mechanisms to ensure their protection against harmful organisms and can be used in the creation of new plant protection products. Phenolic substances play an important role in plant metabolism. They are involved in respiration, photosynthesis, are responsible for the stimulation or inhibition of growth and development (plant hormones), metabolites and cell protection against plants oxidation, bacteria and fungi damaging etc. It is assumed that they are playing role in the immunity formation [8].The substances of phenolic nature are usually referred aromatic compounds that contain a benzene nucleus with one or more hydroxyl groups. For example, emodin – 1 – O – β – D - glucopyranoside (pesticide, antifidant, antiseptic), which is identified in the rhizomes of *Rheum*.

Recently have suggested that the ability of some higher plants to resist to fungal pathogens is dependent on the emodine content, along with other chemicals in their tissues.Plant survival often depends on their resistance to disease [5], so the plant secondary metabolite emodin found in the extract of rhubarb root, can directly influence plant resistance to diseases caused by plant pathogens.Furthermore, since emodin inhibits soil microorganisms that can compete with the plants for the inorganic substances, emodin may indirectly improve plant competitive advantage in certain circumstances [6].It has been proven that emodin can damage the bacterial membrane, it inhibits bacteria protein synthesis and inhibits the activity of succinate dehydrogenase (LDH) and malate dehydrogenase (MDH) in the regulation of oxidative metabolism of the bacteria respiratory [9].

Antifungal properties of rhubarb (*Rheum*)roots are used in plant protection. Thefungicidal activity was confirmed to be of 20% extract of *Rheum emodi* against the pathogen *Fusarium solani*of eggplant wilting in laboratory experiments. Antifungal properties are explained by the presence of emodine content in *Rheum emodi* [1].The alcoholic extract of the rhubarb roots was tested against spore germination of *Alternaria solani, Heliminthosporium penniseti* and *Curvularia palliscens*. Eman S. H. Farrag [4] has shown that aqueous extracts of Rheum (3%) completely inhibit spore germination and mycelial growth of *Fusarium oxysporum* and *F. solani* and increase the germinating ability of cucumber seeds to 78.75%, respectively. Further, they reduced the level of plantsinfection to 15.56%, respectively, compared with the control. Aloe-emodin, isolated from the *Rheum emodi* roots, demonstrated activity against *Aspergillus niger* and *Rhizopus oryzae* with diameters of zones of inhibition 9.8 and 9.11 mm [2].

This determined the need to study the biological activity of *Rheum*extracts to suppress the development of pathogenic organisms. The objectives of the present work were to:

1. Anatomical and histological examination of the *Rheum* roots for determining the location of anthracene derivatives (emodin).
2. Investigation components of ethanolic extracts from the roots of *Rheum* by using HPLC, Agilent 1100, detector DAD.
3. Determination of the biological effectiveness of *Rheum* extracts for controlling the phytopathogenic agents of agricultural plants.

MATERIAL AND METHODS

As a source for the active substances with fungistatic action, the roots of *Rheum rhaponticum* L. were chosen. It is caused by the following criteria:

• plants are environmentally safe for human health, as this is well-known medicinal and nutritional plants that contain useful biologically active substances;

• the *Rheum rhaponticum* (f. *Polygonaceae*) roots contained 3.9 mg/g anthracene derivative substances, that is 3 times more than in the *Rumex acetosa* (1,2 mg / g)roots, and 8 times more than in leaves of *Reynoutria japonica* Houtt (0,5 mg / d), belonging to the same family.

• because of the simple agricultural technology and the minimum cost of protection against diseases and pests, rhubarb can be considered as an economical stuff.

Test objects: *Fusarium sporotrichiella, Sclerotinia sclerotiorum, Sphaerotheca fuliginea, Erwinia amylovora, Pseudomonas syringae.*

Methods of research:

Prepared microscopic preparations "Roots *Rheum*», were investigated by light microscopy using a histochemical reaction with 33% aqueous sodium hydroxide solution to detect the location of the phenolic substances (emodin).

Methods of preparing of rhubarb root extract: shredded in powder rhubarb roots were extracted with ethanol. Determination of the concentration of emodine content was carried out on the spectrophotometer. The research of the component composition of ethanol extracts from *Rheum rhaponticum* L. rootswas made by using HPLC, Agilent 1100, detector DAD.

Determination of the influence of the *Rheum* roots extract in the control of plant pathogens *Fusarium sporotrichiella, Sclerotinia sclerotiorum, Erwinia amylovora* and *Pseudomonas syringae*was carried out on nutrient mediums. The sterile agar was placed in petri dishes with a suspension of the pathogen. In the center of the cup was placed a sterile paper disc impregnated with *Rheum*extract. In control paper discs were not treated. After germination of the pathogen on nutrient mediums, the diameters of pathogen inhibition zones by extract were measured.

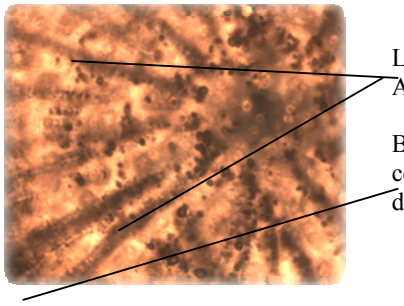
Evaluation of the *Rheum*extracts effectiveness was held against *Sphaerotheca fuliginea* of the f. *Cucurbitaceae* vegetable crops under the conditions of small plots. As the standard preparation was used REKOL. Treatments of plants by *Rheum*extracts in various concentrations were carried out every 7 days during two months.

RESULTS AND DISCUSSIONS

In the investigation of anatomic signs was determined that the roots in a cross section are composed of tissues of the central cylinder and the primary cortex, periderm (cork) is on the surface. Deeper, after the cork, the primary root cortex parenchyma, is located individual cells of which are colored red, due to the presence of anthracene derivatives (Fig. 1).

Thus, the peculiarities of localization anthracene derivatives were revealed - the main parenchyma secondary cortex and parenchyma medullary rays (established by histochemical reaction with alkali). We have developed a technological scheme of obtaining ethanol-water extract of *Rheum*: Gathering vegetative raw materials - drying vegetable raw materials - dry crushing vegetative raw materials - the ratio of dry matter to ethanol 1: 8 - extraction (t^0 = 80°C, t = 5-6 h) - filtration - concentration until dry substance (t = 60°C) - dilution of dry extract by 20% ethanol.

Comparative investigations of the component composition aqueous-alcoholic of *Rheum rhaponticum* L. roots extract were performed by HPLC, Agilent 1100, detector DAD. Detection of anthracene derivatives was performed at the analytical wavelength λ max 437 nm (Figure 2).



Localization of anthracene derivatives:
A) parenchyma of medullary rays

B) the parenchyma of the root bark isolated, cells are colored red, due to the presence of anthracene derivatives.

Fig.1. Rhubarb root (× 10)

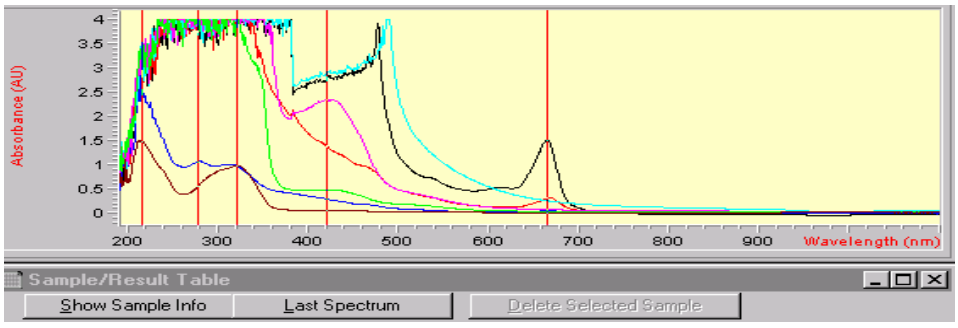


Fig. 2. The peaks of wavelength 437 nm *R. rhaponticum* L alcoholic extracts proves the presence of emodine content

It is proved that the dominant component of the *Rheum emodin* aqueous-alcoholic extracts is that whichdetermines the color red extract. Emodine content concentration was determined with a spectrophotometer.

When determining the effect of phenolic compounds from the rhubarb root extract against pathogen *Erwiniaamylovora* by usingagar diffusion method with paper filters, has been noted the sensitivity of this pathogen to extract. The diameter of the resulting sterile inhibition zone of pathogen growth on the average value is 4.3 mm (Fig. 3). In a similar experiment with the pathogen *Pseudomonas syringae* diameter of suppressing pathogen growth zone was 3.8 mm (Fig. 4).

These results correlate with the work of Zhou Lei, Yun Bao-Yi, Wang Ye-Ju, Xie Ming-Jie; (2011) who pruved that emodin may cause the damage of bacterial membranes, inhibition of activity of bacteria protein synthesisand succinate dehydrogenase (LDH) and malate dehydrogenase (MDH), at regulation of oxidative metabolism of the respiratory bacteria.

When determining the effect of phenolic compounds of rhubarb extract against pathogenic fungus *F. sporotrichiella* by the agar diffusion method using paper filters, high sensitivity ofthis pathogen has been noted to the extract.There wasformedsterile zone of suppression of pathogen growth equal to 22.4 mm, whereas in the control the pathogen growth was observed throughout the surface of agar plates (Fig. 5). The extract from the root of the genus *Rheum* plant did not affect the development of *Sclerotinia sclerotiorum* (Fig. 6).

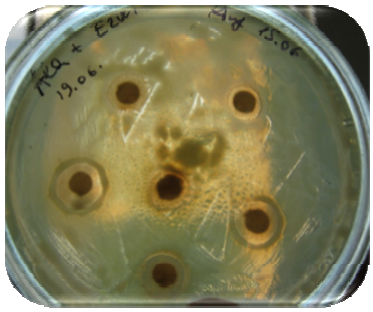


Fig. 3. Inhibition zone diameter of the pathogen *Erwinia amylovora* growth

Fig. 4. Inhibition zone diameter of the pathogen *Pseudomonas syringae*growth

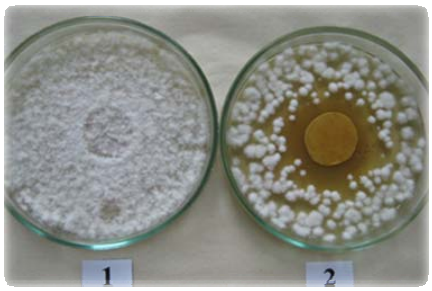


Fig. 5. Effect of the *Rheum* extract on the pathogen *F. Sporotrichiella*: 1 - control 2 – experience

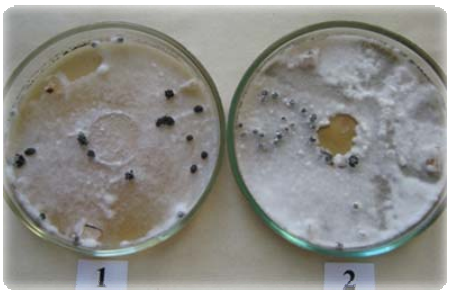


Fig. 6. Effect of the *Rheum*extract on the pathogen *Sclerotinia sclerotiorum*: 1 - control 2 - experience

These results correlate with the Eman S. H. Farrag [4] research, who proved that extracts of *Rheum* (3%) completely inhibit spore germination and mycelial growth *Fusarium oxysporum* and *F. solani* and increase the germinating ability of cucumber seeds to 78.75%, respectively. Further, they reduced the infestation of plants to 15.56%, respectively, compared with the control.The antimicrobial activity of the *Rheum emodi* extract was examined by Babu KS et al [1]. The compounds were tested against Gram-positive bacteria *Bacillus subtilis, Bacillus sphaericus, Staphylococcus aureus* and Gram-negative bacteria *Klebsiella aerogenes, Chromobacterium violaceum, Pseudomonas aeruginosa*. Diameter of inhibition zones of 12-14 mm was tested at concentrations of 30 and 100 ug / ml. Emodin, isolated from *Rheum emodi* roots, demonstrated antifungal activity against *Aspergillus niger*and *Rhizopus oryzae* with diameters of inhibition zones 8-9 and 9-11 mm for test concentrations of 100 and 150 mg / ml, respectively.

It was also determined that emodin is effective against the germination of 17 tested fungi species,inclusive of seven *Alternaria* species, and three *Fusarium*species (Singh et al., 1992). Not less than 50% inhibition of fungal species spore was achieved by emodine content at concentration of 500 ug / ml, and maximal inhibition (up to 100% for some species) observed by emodine content at concentration of 2000 pg / mL [7].

On the example of investigations of the fungicidal *Rheum* extracts biological efficiency to control *Sphaerotheca fuliginea* on *Cucurbita pepo*. We found the dynamics of the effectiveness of the extract from 16% - 26% after the first weekly treatments up to - 59.2% after a month of regular treatment (Figure 7.).

The results obtained indicate that the action of the extract increases gradually, and that it correlates with the results, showing that antibiotic compounds, such as phenols and polyphenols, play an important role in non-specific resistance to filamentous fungus. [10].

Rhubarb extract inhibits spore germination and activates the immune response with broad spectrum of action. Locally induced protection mechanisms that restrict pathogen infection, are characterized by a complex of hypersensitivity reactions (HR), which causes necrosis and cell death (Figs. 8, 9).

A ring of dead tissue around developing destruction makes it immune to subsequent infection (localized acquired resistance). [3]

This action of the extract is especially important for the plant protection during the growing period from reinfestation by *Sphaerotheca fuliginea*. These results allow us to conclude that the *Rheum* extract is a good preventive and the moderate therapeutic effect to control *Sphaerotheca fuliginea*.

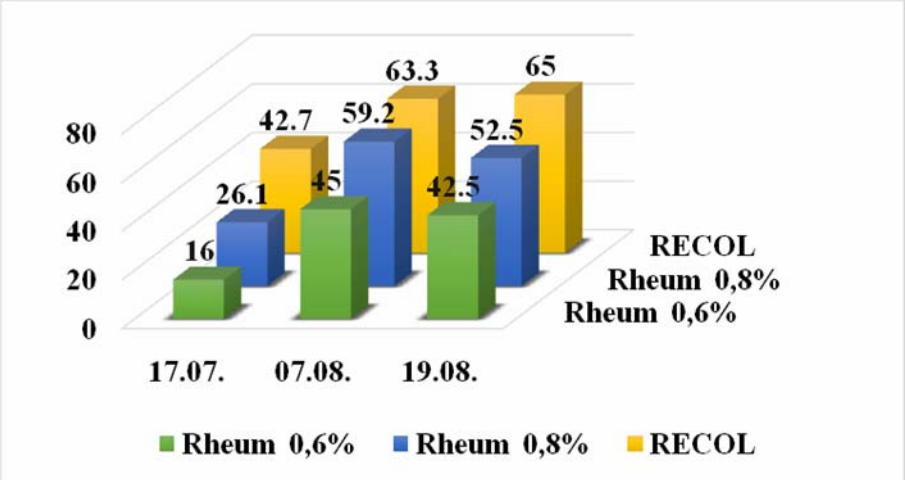


Fig. 7. Dynamics of the efficiency (%) of the *Rheum* root extract in protection of *Cucurbita pepo* from *Sphaerotheca fuliginea*

Fig. 8. The appearance of symptoms *Sphaerotheca fuliginea* on *Cucurbita pepo*

Fig. 9. Necrosis and cell death, limiting the development of the pathogen

CONCLUSIONS

Carrying out of the anatomical and histological analysis of the *Rheum* roots by using quantum microscopy and histochemical reaction with 33% sodium hydroxide solution is a reliable method to identify the place of localization the anthracene derivatives in the main parenchyma secondary cortex and in the parenchyma of the medullary rays.

It was determined the lower limit of the the*Rheum* roots anthracene derivatives content not less than 4.0%.

It is proved that the *Rheum* extracthas an inhibitory effect on the pathogen *Fusarium sporotrichiella* (sterile zone of inhibition of the pathogen growth in the average value 22.4 mm) and does not affect the development of *Sclerotiniasclerotiorum*. It was noted the sensitivity of *Erwiniaamylovora* Burrill to the extract. The diameter of the resulting sterile pathogen growth inhibition zone on the average value is 4.3 mm. The average diameter of *Pseudomonas syringae* growth inhibition zone was 3.8 mm.

It has been proven that the application of the *Rheum* extract for controlling *Sphaerothecafuliginea* is a good preventive and the moderate therapeutic effect, increasing in accordance with the consistent, regular treatment, which is especially important for the plantprotection against reinfestationby *Sphaerothecafuliginea* throughout the growing period.

ABSTRACT

The most of plant secondary metabolites protect plants against pathogens. Polyphenols and their derivatives play an important role in protection of plants against the fungal diseases. We studied the *Rheum rhaponticum L.* (*Polygonaceae)* alcoholic extract in order to reveal the anthraquinone derivatives which induce resistance to plant phytopathogens and act as an antimicrobial agent with fungicidal activity. An alcoholic extract of *Rheum rhaponticum* L. was prepared and analyzed using chromatography.

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AUTHORS' ADDRESS

GLADCAIA ALLA, VOLOSCIUC LEONID - Institute of Genetics, Physiology and Plant Protection of Academy of Sciences of Moldova, Chisinau, Republic of Moldova.f.: (+373 22) 77-04-47, E-mail: asm_igfpp@yahoo.com