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Research on the functional activity of polyene antibiotics in different crops

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Abstract: One way to preserve the purity of ecosystems is the use of polyene antibiotics (PA) with specificity and selectivity of their action on viral and fungi plant cells. In the process of our prolonged scientific activity on the base of polyene antibiotics was discovered a new membrane-active preparation whose basic action is connected with its ability to quickly, high selectivity, and efficiency to destroy simultaneously viral and fungi infections of plants. The most effective of the studied PA were amphotericin B and levorin A_2, produced respectively by soil microorganisms *Actinomyces nodosus* and *Actinomyces levoris*. A theoretical analysis of practical aspects of the use of PA is presented to develop an ecological model of environmental protection from carriers of infection. The relationship between the structure of antibiotics and their function in membranes has been established. The physicochemical properties and biological role of dimethyl sulfoxide (DMSO) in combination with PA were studied for the first time. It was found that the use of amphotericin B and levorin A_2 in the DMSO complex enhances the biological activity of the initial antibiotics. It was found that the studied antibiotics have a steep dependence of conductivity on their concentration, which made it possible to identify the effective concentrations of each of them in the formation of ion channels. The conducted research allowed to theoretically substantiate and present practical recommendations for the targeted synthesis of PA and their derivatives with specified properties. For example, alkylation of the polar part of PA molecules increases the biological activity and selectivity of their action on cell membranes. As a result of the conducted research, for the first time it was possible to identify a new compound Infanvir, which has the ability to effectively and selectively suppress the growth of pathogenic viral infections in plant cells. A Eurasian patent has been obtained for the developed drug.

Keywords: *crops, fungi infection, INFANVIR, polyene antibiotics, viral infection*

INTRODUCTION

Vegetable and fruit crops are a valuable source of biologically active compounds. Their protection from pathogenic microorganisms is an important problem for agriculture. The social aspect of maintaining the purity of ecosystems involves the creation of effective ways to combat pathogenic microorganisms. Currently existing protective equipment is not able to completely prevent the spread of viral and fungal infections. Recently, intensive work has been carried out in many countries to create drugs that can selectively affect the cells of pathogenic microorganisms and effectively suppress their growth [Ibragimova, 2010; Huseynova et al., 2012].

Generally, plant diseases caused by the three main pathogenic microbes: fungus, bacteria and virus. Here are a few examples of common signs and symptoms of fungal, bacterial and viral plant diseases. Fungal disease signs are leaf rust, stem rust, sclerotinia, powdery mildew. Bacterial disease signs are bacterial ooze, water-soaked lesions, bacterial streaming in water from a cut stem. Viral disease signs are none because the viruses themselves cannot be seen. Fungal disease symptoms are anthracnose, phytophthora, leaf spot, chlorosis. Bacterial disease symptoms are leaf spot with yellow halo, fruit spot, cancer, crown gal. Viral disease symptoms are mosaic leaf pattern, crinkled leaves, yellow leaves, plant stunting. There is a lot of overlap between fungal, bacterial and viral disease symptoms [Alekseeva, Ivanova, 2015].

The plant diseases are attempted to control by the use of antibiotics by plant pathologists all over the world since the discovery of penicillin. Although there are about 900 kind of antibiotics of which the chemical structures have been studied, only more than 10 types of antibiotics possessed practical application towards agriculture. The reason may be due to the instable nature of antibiotic, high cost, high toxicity on warm-

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blooded animal and toxic effects on plants. Agricultural antibiotics are usually formulated as powders with 17% to 20% active ingredient [Manyi-Loh et al., 2018].

The powder formulation is dissolved or suspended in water to get concentrations of 50 to 300 ppm with its suitable application as a fine mist to the target plant parts. Due to its expensive in nature, antibiotics are used primarily on high-valued fruit and vegetable crops and ornamental plants production where their cost of use can be regained. The widely used known antibiotics available belongs to actinomycetes and few are of fungi and bacteria origin [Abhilash et al., 2016].

Polyene macrolide antibiotics are of particular interest. The composition of the drug INFANVIR contains two components that are not identical in their chemical structure and physico-chemical properties. Studies have shown that polyene antibiotics (PA) have important properties - to inactivate some viruses, prevent their penetration into the cell and inhibit their reproduction. Moreover, water-soluble derivatives of PA - amphotericin B, levorin and mycoheptin, when administered together with inactivated antiviral drugs, can stimulate specific immunogenesis. Recently, there have been real prospects for expanding the scope of application of PAs to combat viral and fungal infections of plants [Ibragimova et al., 2014; Ibragimova, 2010].

MATERIAL AND METHODS

INFANVIR which we got Eurasian Patent No. 022438 is a yellow amorphous powder without an accurate melting point. It is highly soluble in dimethyl sulfoxide (DMSO), dimethylformamide and pyridine, slightly soluble in lower alcohols. Solubility in alcohols increases in the presence of 20-30% water. Insoluble in chloroform, anhydrous acetone, diethyl and petroleum ether. INFANVIR has amphoteric properties, ionizing, forms a cation in an acidic environment, and an anion in an alkaline environment [Eurasian Patent No. 022438]. According to its chemical nature, INFANVIR belongs to heptaene PAs. In a complex with DMSO, it is a dark yellow liquid with a bitter taste and a specific odor. INFANVIR is one of the components of the complex antibiotic mixture levorin.

The drug INFANVIR is obtained by dissolving 1 g of a powder of the active ingredient with a biological activity of 25,000 units / mg in 100 ml of DMSO [Yu Z. et al., 2016]. After thorough mixing, the composition is kept for a day at room temperature. The liquid is then filtered and stored in a dark, cool place. The result is a stock solution of infanvir ready for use. The indicated concentration of INFANVIR is threshold, since above this concentration the active ingredient precipitates when diluted with water. The usage of the drug with such a ratio of components is highly effective. 1 l solution of INFANVIR diluted in 100-200 liters of water and then infected surface of vegetable crops sprayed with this solution and their root system is processed [Ibragimova, 2010]. The biological activity of INFANVIR was determined by the lipid bilayer membrane (BLM) method. BLM was obtained from phospholipids isolated from the white matter of bovine brain by applying a drop of phospholipids to a hole in a Teflon cell. Total phospholipids were purified from neutral lipids by acetone washing and stored at 0°C at a concentration of 20 mg/ml in chloroform-methanol solution in a volume ratio (2:1). The integral conductivity of membranes was studied depending on the antibiotic concentration in the potential fixation mode. At a certain concentration of the antibiotic, the maximum conductivity of the membranes is achieved, which is taken as the main component in the preparation of the drug.

Test report for the biologically active drug "INFANVIR" based on the company "AGRI BIO ECOTECH" (Absheron, Azerbaijan) and based on the company "REAL PLUS". In order to find an effective drug against pathogenic microorganisms of vegetable crops, experiments were carried out in greenhouses, as well as in open soils of the company "AGRI BIO ECOTECH" and "REAL PLUS", where cucumbers, tomatoes, eggplants and peppers are grown. A hundred plants are used for each crops and ten of them are control plants in each crops.

On the basis of the company "AGRI BIO ECOTEH" a test was carried out of the biologically active drug "INFANVIR", developed at the Department of Soil Science of Baku State University. The drug "INFANVIR" is created on the basis of polyene antibiotics (due to patent reasons, the composition of the drug "INFANVIR" is not disclosed). The mechanism of action of this class of compounds is based on their binding to the cytoplasmic membranes of cells, the formation of ion channels in them, which ultimately leads to cell lysis. To date, not a single drug has been found that would have the ability to stop and completely suppress the development of infections in plants. From this point of view, the relevance of the ongoing research does not raise any doubts. Studies on the basis of "AGRI BIO ECOTEH" in greenhouses, as well as in open ground, showed the high effectiveness of the drug on pathogenic microorganisms. Treatment of plants, as well as soil affected by viral and fungal

infections, by spraying infected areas with INFANVIR solution at the rate of 100 ml of the original solution dissolved in 10 liters of water at 15-35° C leads to the complete destruction of viral and fungal infections.

The effect of the drug "INFANVIR" on plant objects (*Prunus persica* (L.) Batsch) in conditions of plant disease with tobacco mosaic virus are also studied in the plantation in Guba district (Azerbaijan). The author of the drug drew attention to the fact that the drug "INFANVIR" has the ability to completely suppress the growth of the tobacco mosaic virus (Tobacco mosaic virus). It should be noted that after treatment with INFANVIR, infected plants are not only cured, but complete plant regeneration occurs. Eurasian patent has been obtained for the developed drug.

RESULTS

Almost half of the detected infectious plant diseases are viral [Baghirova et al., 2020]. To obtain biologically active compounds, soil actinomycetes are used that are capable of synthesizing antibiotic substances that have a specific effect on pathogenic microorganisms. Soil actinomycetes, synthesizing most antibiotics, play a key role in enhancing soil fertility. Antibiotics have several valuable advantages in the fight against phytopathogenic microorganisms in comparison with other substances. They easily penetrate the organs and tissues of plants, have an antibacterial effect and are relatively slowly inactivated in them. The use of antibiotic preparations in crop production gives a significant economic effect. They are widely used in crop production due to the negative consequences of the use of pesticides. Antibiotics have a selective action and, suppressing the development of phytopathogenic viruses and fungi, are practically harmless to plants. Numerous experimental studies have shown that most of the antibiotics used penetrate well into plant tissues through roots, stems, and leaf surfaces, and are absorbed into seeds (Fig. 1).

Amphoteric antibiotics penetrate especially quickly into plant tissues. There are various ways to introduce antibiotics into plant tissues. The most widely used methods are spraying or pollinating the aerial parts of the plant, soaking the seeds, and direct tillage. This treatment method gives good results in the fight against diseases, the pathogens of which develop on the surface and in plant tissues (Fig. 2).

The drug "INFANVIR" was created based on membrane-active macrolide antibiotics. The mechanism of action of this class of compounds is the basis of their formation of molecular-sized structural channels in cell membranes, selectively passing through ions and organic compounds. The biological effect of PA is connected with change of permeability of lipid and cell membranes for ions and organic substances. For determination of biological activity of antibiotic dependence of membrane conductivity in membranes on concentration INFANVIR was studied. [Ibrahimova, 2010] You can see in figure 3, that maximum activity of an antibiotic is observed at concentration 10^{-6}-10^{-5} M.

Membranes were formed of mix phospholipid – cholesterol 20:1 in the water salt solutions containing 10^{-1} M of KCl at pH=6.5, t=220° C. Potential on a membrane of +100 mV (from the antibiotic side).

Proceeding from these data concentration of an antibiotic which corresponds to its maximum biological activity is calculated. At small 10^{-1} M of INFANVIR

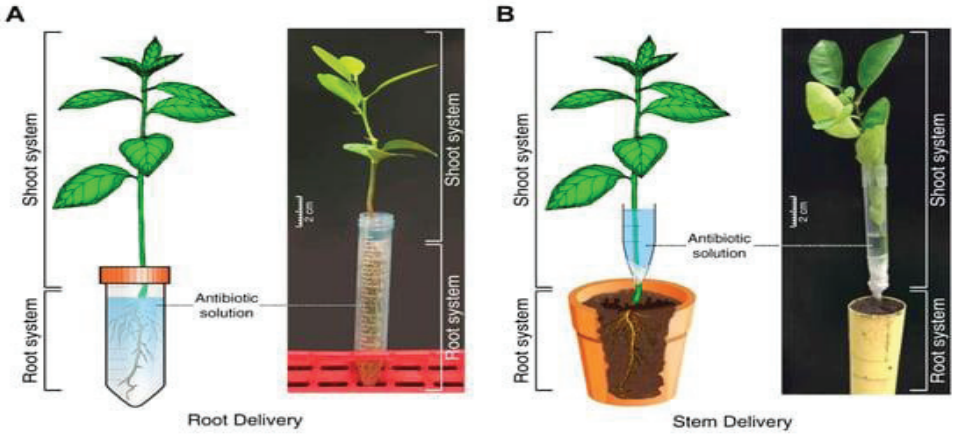

Figure 1. Delivery of antibiotics into citrus seedlings. Root drench (A) and stem delivery (B). [Fuad Al Rimawi et al., 2019]

Figure 2. The effect of the drug "INFANVIR" on plant objects (peach) in conditions of plant disease with tobacco mosaic virus: 1- the initial period, 2- plants before treatment with the drug and 3- the same plants after treatment with the drug. Conditions of the experiment behavior – 100 ml of "INFANVIR" +200 ml of H_2O into the soil,irrigation of leaves 100 ml of "INFANVIR" + 1 l. H_2O.

concentration on single ionic channel with the low conductivity which size about 0.3-0.5 pSm are formed in membranes. Research showed that INFANVIR the forming molecular complex at interaction with cytoplasmatic membranes, promotes suppression of virus and fungoid infections of vegetable and other types of crops. Biological activity of PA sharply increasese at dissolution in DMSO. PA in DMSO solution are about 10 times more effective in comparison with initial water-soluble forms of antibiotics. INFANVIR contains DMSO and an active component that allows to use it at treatment of viral and fungoid diseases of vegetable crops in the structure. INFANVIR possesses ability to interact with envelops of virus particles and membranes of fungi cell. There is the lysis of cells as result of such interaction. Studies conducted in greenhouses, as well as in open fields, of the preparation "INFANVIR" showed high effectiveness of its effect on pathogenic microorganisms. The initial solution of 100 ml of preparation of viral (Tobacco mosaic virus) and fungal infected plants and soil was dissolved in 10 liters of water (temperature -15-35°). As a result, this operation led to the complete destruction of viral and fungal infections.

The effect of INFANVIR to bacterial disease in cucumbers, tomatoes, eggplants and peppers shows that this drug is highly effective in 60 and 120 minutes exposition time against bacterial infections such as *Canditatus Phytoplasma solani*, *Pseudomonas syringae* pv. *tomato*, *Ralstonia solanacearum*, *Septoria* spp., *Cercospora* spp., *Sclerotinica sclerotiorum*, *Ralstonia salonacearum*, *Phytophthora capsici*, *Leveillula taurica*.

It has been shown that drug users can completely suppress the growth of the tobacco mosaic virus, tomato mosaic virus, cucumber mosaic virus and alfalfa mosaic virus. In particular, it should be noted that after treatment with "INFANVIR," the infected plants are not only cured but also complete recovery of the plants occurs. In addition, vegetable plants treated with the drug had twice the yield of control plants and also disease spots number decrease twice (Fig.4). It should be noted that antibiotics have a short active period on plants, usually less than a week, and significant residues have not been detected on harvested fruits and vegetables. The results demonstrated that the antiviral effects of INFANVIR were as effective as those of the commercial agent

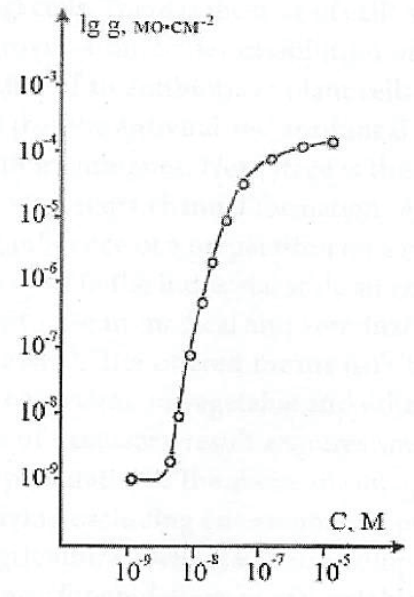

Figure 3. Dependence of conductivity of lipid membranes on INFANVIR concentration.

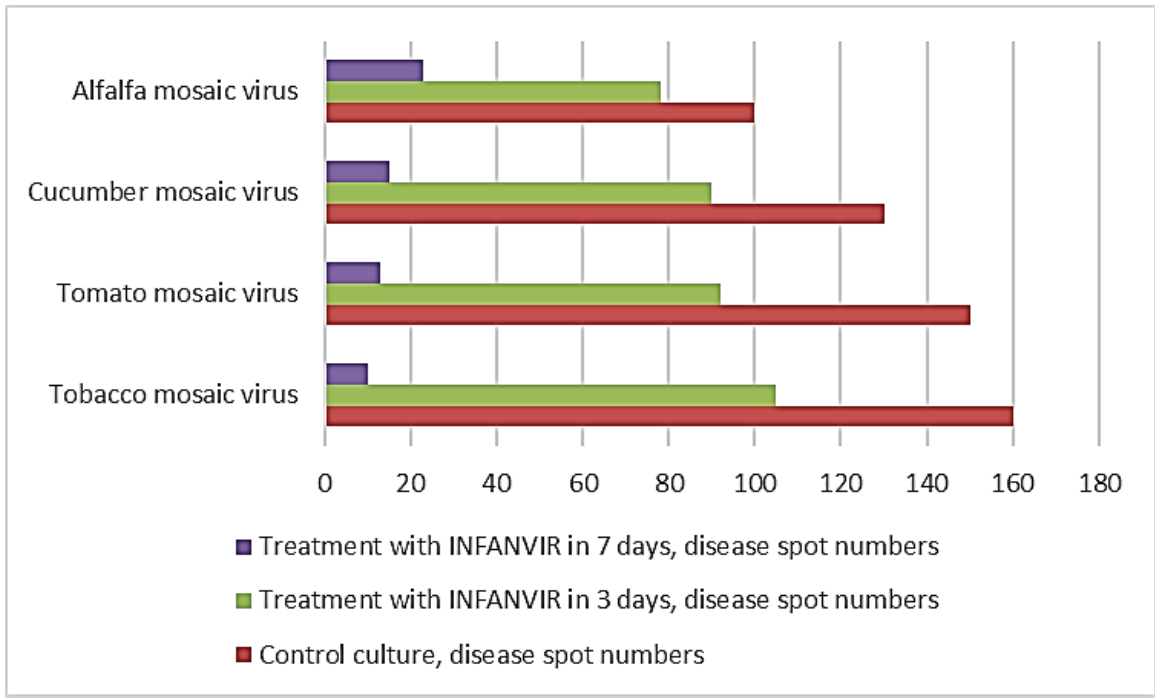

Figure 4. Virus diseases spot numbers and treatments dependence.

lentinan, in either the protective effect, inactivation effect or curative effect and also the results demonstrate that INFANVIR have potential as eco-friendly and safe strategies to control tobacco mosaic viruses in the future [Ibrahimova et al., 2014].

The use of antibiotics in plant protection is not as high as in human and veterinary medicine, it is important to consider its potential impact on the phytobiome. There is a risk of unintended consequences, such as the development of antibiotic resistance, which should not be ignored.

It is important to note that the risk of antibiotic resistance associated with the use of antibiotics in plant protection cannot be ruled out due to the lack of data. Therefore, it is crucial to adopt strong antimicrobial stewardship practices to mitigate this risk, as suggested by Miller et al. [2022]. It is also necessary to develop practical and achievable surveillance programs that collect quantitative data on the use and sales of antibiotics, as well as the crops and area of their application. This will aid in better understanding the situation and assessing the risks of antibiotic resistance selection and spread. Accurate data on the amounts of antibiotics used in different crops are an essential factor in identifying and quantifying the related risk of developing antibiotic resistance.

Antibiotics became widespread in crop production when the adverse effects of the use of pesticides became apparent, which, along with the suppression of phytopathogenic microflora, poison beneficial species of fish, birds and animals that feed on pollinated plants. Antibiotics have selectivity of action and, suppressing the development of phytopathogenic bacteria and fungi, are practically harmless to plants and animals. Studying the selective permeability of levorin channels for ions and organic compounds is an important aspect for the protection of agroecosystems.

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Müxtəlif bitkilərdə polien antibiotiklərinin funksional aktivliyinin tədqiqi

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Ekosistemləri təmiz saxlamağın bir yolu bitkilərdə virus və göbələk hüceyrələrinə qarşı spesifikliyi və seçiciliyi ilə seçilən polien antibiotiklərinin (PA) istifadəsidir. Polien antibiotiklərinə əsaslanan uzunmüddətli elmi fəaliyyətimiz zamanı əsas təsiri bitkilərin virus və göbələk infeksiyalarını eyni vaxtda məhv etmək qabiliyyəti olan yüksək seçiciliyi və effektivliyi ilə əlaqəli yeni bir membrano-aktiv dərman kəşf edildi. Tədqiq olunan PA-dan ən təsirli olanı torpaq mikroorqanizmləri *Actinomyces nodosus* və *Actinomyces levoris* tərəfindən istehsal olunan amfoterisin B və levorin A_2 idi. Ətraf mühitin infeksiya daşıyıcılarından qorunmasının ekoloji modelini hazırlamaq üçün PA-dan istifadənin praktiki aspektlərinin nəzəri təhlili təqdim olunur. Antibiotiklərin quruluşu ilə onların membranlardakı funksiyaları arasında əlaqə qurulur. Dimetilsulfoksidin (DMSO) fiziki-kimyəvi xüsusiyyətləri və bioloji rolu PA ilə birlikdə öyrənilmişdir. DMSO kompleksi ilə birlikdə amfoterisin B və levorin A_2 istifadəsinin ilkin antibiotiklərin bioloji aktivliyini artırdığı aşkar edilmişdir. Tədqiq olunan antibiotiklərin keçiriciliyinin onların konsentrasiyasından kəskin asılılığına malik olduğu, ion kanallarının əmələ gəlməsində hər birinin təsirli konsentrasiyalarını təyin etməyə imkan verdiyi aşkar edilmişdir. Aparılan tədqiqatlar PA-ların və müəyyən xüsusiyyətlərə malik törəmələrinin məqsədyönlü sintezi üçün nəzəri cəhətdən əsaslandırmağa və praktiki tövsiyələr verməyə imkan verdi. Məsələn, PA molekullarının qütb hissəsinin alkilləşməsi bioloji aktivliyi və hüceyrə

membranlarına təsirinin seçiciliyini artırır. Aparılan tədqiqatlar nəticəsində ilk dəfə bitki hüceyrələrində patogen virus infeksiyalarının böyüməsini effektiv və seçici şəkildə dayandırmaq qabiliyyətinə malik olan yeni İNFANVİR birləşməsini müəyyən etmək mümkün oldu. Hazırlanmış dərman üçün Avrasiya patenti alındı.

Açar sözlər: *kənd təsərrüfatı bitkiləri, göbələk infeksiyası, INFANVIR, polien antibiotiklər, viral infeksiya*

Исследование функциональной активности полиеновых антибиотиков в различных сельскохозяйственных культурах.

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Одним из способов сохранения чистоты экосистем является использование полиеновых антибиотиков (ПА), обладающих специфичностью и избирательностью действия на вирусные и грибковые клетки растений. В процессе нашей длительной научной деятельности на основе полиеновых антибиотиков был открыт новый мембраноактивный препарат, основное действие которого связано с его способностью быстро, с высокой избирательностью и эффективностью уничтожать одновременно вирусные и грибковые инфекции растений. Наиболее эффективными из изученных препаратов были амфотерицин В и леворин A_2, продуцируемые соответственно почвенными микроорганизмами *Actinomyces nodosus* и *Actinomyces levoris*. Представлен теоретический анализ практических аспектов применения ПА для разработки экологической модели защиты окружающей среды от переносчиков инфекции. Установлена взаимосвязь между структурой и функцией ПАв мембранах. Впервые были изучены физико-химические свойства и биологическая роль диметилсульфоксида (ДМСО) в сочетании с ПА. Было обнаружено, что использование амфотерицина В и леворина A_2 в комплексе с ДМСО повышает биологическую активность исходных антибиотиков. Было обнаружено, что изучаемые антибиотики имеют резкую зависимость проводимости от их концентрации, что позволило определить эффективные концентрации каждого из них при формировании ионных каналов. Проведенные исследования позволили теоретически обосновать и представить практические рекомендации по целенаправленному синтезу роизводных ПАс заданными свойствами. Например, алкилирование полярной части молекул ПА повышает биологическую активность и избирательность их воздействия на клеточные мембраны. В результате проведенных исследований впервые удалось идентифицировать новое соединение Инфанвир, которое обладает способностью эффективно и избирательно подавлять рост патогенных вирусных инфекций в клетках растений. На разработанный препарат получен евразийский патент.

Ключевые слова: *сельскохозяйственные культуры, грибковая инфекция, ИНФАНВИР, полиеновые антибиотики, вирусная инфекция*