Vol. 7, №1, 2023 ISSN 2520-6133

KHAZAR JOURNAL OF SCIENCE AND TECHNOLOGY (KJSAT)

[1] Also H. Issakhanly, H. Isakhanly, H. Isakhanli, H.A.Isayev, G.A.Isayev, or G.A.Isaev due to differences in transliteration. I use Hamlet Isayev in mathematics and science fields and Hamlet Isakhanli/Isaxanli in humanities.

Khazar University
41 Mehseti str., Baku AZ1096
Republic of Azerbaijan
Tel: (99412) 4217927
Website: **www.kjsat.com**

KHAZAR UNIVERSITY PRESS

Khazar Journal of Science and Technology
Khazar University Press 2022
Volume 7 №1 2023, 5-13
DOI: 10.5782/2520-613.202.7.1.5

The Properties and Clinical use of Polyene Antibiotics

Turkan Pashazade
Azerbaijan, Institute of Botany, Azerbaijan National of Sciences
turkan303@mail.ru

Abstract

Model membranes' similarity to the biological cell membrane and their simple structure with controlled composition and properties. They are formed from natural and synthetic lipids, and these membranes are an interesting object for scientific investigations. We studied the ultraviolet absorption spectrum of the complex formed by polyene antibiotics at different concentrations separately and stable with cholesterol. We also study the effects of the antibiotic concentration and channel-expression and the dynamics of channels, which are affected by pH in lipid membranes. We represent and define the behavior of such antibiotic-generated-channels over a wide range of pH values and ionic strength. Our information demonstrates the pH-induced modulation of AmB channels selectivity, stability of the open state, and conductance. This work might be of widespread importance for comprehending molecular aspects regarding fundamental properties of AmB, such as activity, toxicity, and formation of ion channels, and provide a more suitable familiarity. Learning mechanism help to develop a more unassailable AMB formulation for treatment.

Keywords: Amphotericin B ion channel, Sterol, Polyene antibiotic, Bilayer membrane, Membrane structure.

Introduction

The polyene antibiotics - nystatin and amphotericin B are known to increase the ion and nonelectrolyte permeability of sterol-containing biological and artificial membranes (Figure 1). Polyene antibiotics (PA) amphotericin B and nystatin are principally known as major antifungal drugs as well as one of the first model systems for trans-membrane ionic channel structures (Samedova et al., 2018). The effects of polyene antibiotics on thin lipid membranes are consistent with their action on

biological membranes. The biological effect of PA is the formation in the membranes of structural ion channels which permeable for ions and organic substrates. The PA have high affinity to biological membranes, which there are sterols of definite structure. Nystatin and amphotericin B create aqueous pores in thin lipid membranes; the effective radius of these pores is approximately 4 Å. There is a marked correlation between the permeability of a nystatin- or amphotericin B-treated membrane to water and small hydrophilic solutes and the permeability of the human red cell membrane to these same particles. Amphotericin B or nystatin may interact with membrane-bound sterols to produce multi molecular complexes which greatly enhance the permeability of such membranes for anions, and, to a lesser degree, cations. Although both nystatin and amphotericin B greatly increased the conductance of cholesterol-containing membranes, they also expanded cation conductance to a considerable degree.

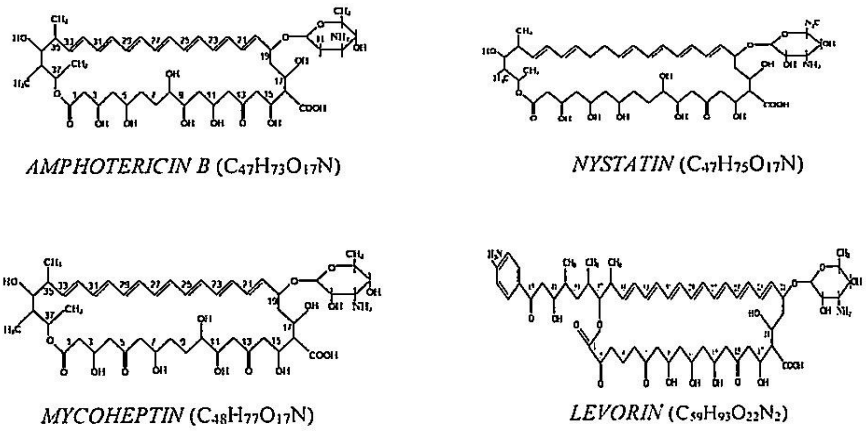


Figure 1. Chemical structure of the polyene antibiotics.

Additionally, the effect of amphotericin B on the electrical properties of the membranes occurred over a moderately narrow concentration range. In this scale, there was no detectable effect on membrane stability. Possibly the many hydroxyl groups in nystatin and amphotericin B are capable for anion selectivity. Nystatin and amphotericin B induce a cation-selective conductance when added to one side of a lipid bilayer membrane and an anion-selective conductance when added to both sides. The concentrations of antibiotic required for the one-sided action are comparable to those employed on plasma membranes and are considerably larger than those required for the two-sided action. The one-sided action of the polyene antibiotic amphotericin B on phospholipid bilayer membranes formed from phosphatidylcholines and sterols have been investigated. The properties of ion channels formed in membranes by polyene antibiotics with various chemical

structure of hydrophilic and hydrophobic chain of molecules are investigated. Small differences in a hydrophilic chain with the changed number of hydroxyl and carbonyl groups significantly influence on the size of conductivity and selectivity of the channel. The more number of double bounds in a hydrophobic part of polyene molecules leads to the higher biological activity of antibiotics. Measurement anion - cationic selectivity of the channels formed by polyenes showed that anionic selectivity, as well as conduction of channels, decreases among antibiotics: amphotericin B – nystatin. Research of physical and chemical properties of the single ion channels on the bilayer lipid membranes in the presence of polyene antibiotics makes possible to create theoretically reasonable recommendation to purposeful synthesis of new antibiotics with the known properties of molecule.

Materials and methods

Lipid membranes are to be used to study the effect of PA on membranes at the molecular level. Bimolecular lipid membranes (BLM) are made of phospholipids present in the brain tissues of large and small horned animals. It is one of the methods of reflecting the formation of lipid membranes by inserting phospholipids in the hollow part of a glass made of Teflon material. This method showed that PA is highly sensitive to sterols in the membranes. Polyenes interact with sterols to form molecular-sized ion channels in membranes, and the physicochemical properties of these channels have been studied by the patch-clamp method. (Samedova et al., 2018).

Membrane leaching of the antibiotic by the perfusion method and the reduction of membrane permeability has observed by the kinetic relaxation method. The purposeful synthesis of polyene antibiotics and the study of their physicochemical properties in membranes opens a wide way to define theoretical ways and create new antibiotics. All experiments were performed at a room temperature 23◦C. The stock AmB solution was obtained by dissolving AmB in DMSO. The conclusive pH was calibrated with a pH mini-electrode. Changes in pH were conducted by addition of small volumes of concentrated solutions of either HCl or KOH. The analysis was made using pClamp 8.2.

Results and disscussions

When amphotericin B enters one side of the membrane, it causes the formation of ion channels in the membrane (Figure 2.)

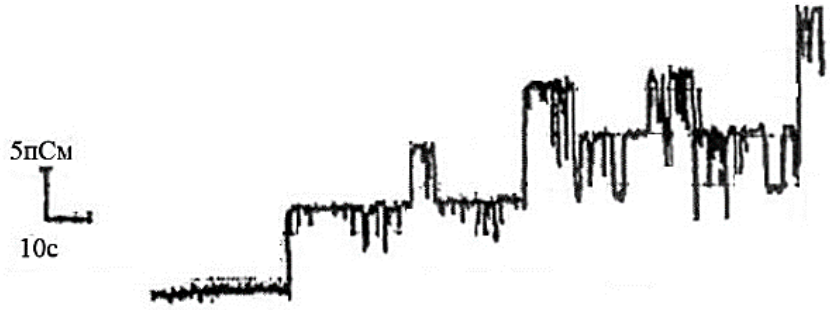


Figure 2. Dynamics of operation of single ion channels formed on one side.

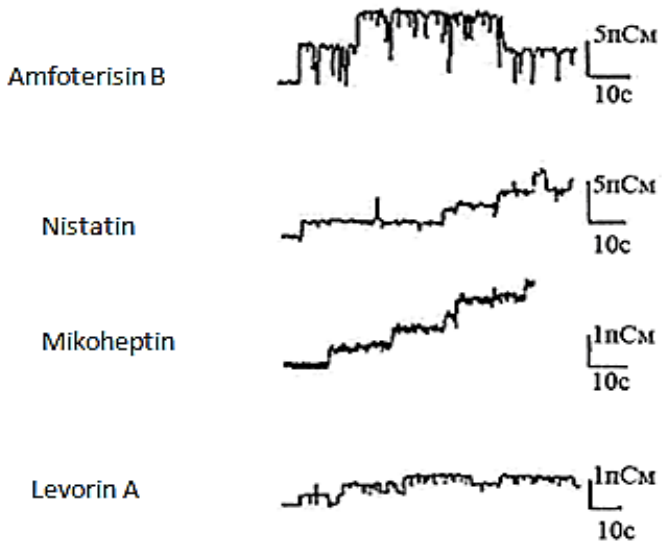


Figure 3. Single ion channels in lipid membranes in the presence of PA.

To analyze the properties of ion channels depending on the structure of the lactone ring of polyene molecules, studies were conducted in the form of comparing the properties of amphotericin B, nystatin, and mycoheptin channels in lipid membranes obtained from a mixture of phospholipids and cholesterol. (Figure 3). The polyene heptaene chain is the same for amphotericin B and mycoheptin, a double bond in the middle of the polyene chain in the nystatin molecule is hydrogenated (tetraene). The hydrophilic chain of the lactone ring is different for all three antibiotics: nystatin and amphotericin. The support of hydrophilic groups is the same, only the locations are different. In mycoheptin, in addition, one hydroxyl group is replaced by one carbonyl group. The amphotericin channel has the highest conductivity (6.5 pS). The nystatin channel has a lower conductivity (2 pS). The mycoheptin channel exhibits minimal

conductivity (0.5 pS). The permeability of the levorin channel (0.2-0.3 pS cm) is even lower.

One of the parameters characterizing the biological effectiveness of PA is the induced permeability level of antibiotics, the permeability level measured in BLM, and the constant time of antibiotic flushing. Studies of alkyl derivatives of amphotericin and levorin have shown that the biological activity of antibiotics decreases as the length of the alkyl chain increases. When a methamphosin molecule is inserted into one side of the membrane, discrete conductive single ion channels are formed, and methamphosine is the only derivative that increases the conductivity when it is on one side of the membrane.

We also examined at the single-channel level the effects of pH changes on the biophysical properties of AmB channels inserted in bilayer lipid membranes. Our information showed the pH-induced modulation of AmB channels open probability, and conductance.Although the ion selectivity did not change. The acidity greatly decreased the open probability of the channel. Also this information suggests that presumably an H+ on the regulatory site is available from the extracellular (cis) side of the channel where pH changes.(Figures 4 and 5)

The pH changes even changed the single-channel conductance of AmB oligomers. Our information indicates that the single channel conductance of AmB channels value is larger at pH 7 and pH 8, showing a drop at acid pH values. Furthermore, these data point out that probably an H+ on the regulatory site is available from the extracellular (cis) side of the channel where pH changes. The pH changes also altered the single-channel conductance of AmB oligomers. Our data show that the single channel conductance of AmB channels value is larger at pH 7 and pH 8, showing a drop at acid pH values (Figure 4).

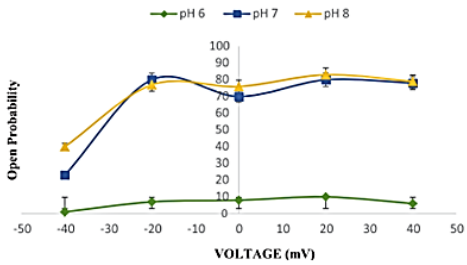


Fig. 4

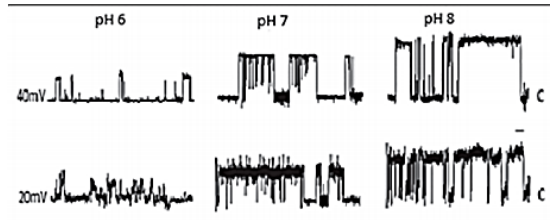


Fig. 5

Figure 4 and 5. Typical current recordings of single ion channels formed by amphotericin B in phosphorylcholine bilayer membranes measured at different potential and channel open probability at different voltages

The capacitance of the bilayers was smaller in a solution of lower pH than in a solution of medium pH. Since phosphorylcholine has a zwitterionic group, the polar groups would be positively charged at lower pH values, and this would lead to a mechanical constriction of the AmB pore displayed by a drop in its conductance at pH = 6.In contrary to open probability and conductance the reversal potential and ion selectivity did not change by pH. The reversalpotential analysis indicated that Amb in these pH was cation channel. At pH range from 6 to 8 the carboxyl group of AmB is deprotonated, so that the selectivity of channels is influenced mostly by the electric profile within the conductive pore, directing to a cation-selective behavior. We discovered that AmB channels become even more cationic selective in the acidic pH and can even change to anionic in the alkaline pH. In the finale, our data reveals that when pH changes from basic values (pH = 8) to acidic (pH = 6), the visible decrease happened in the conductance and open probability. This data is useful to sufficiently comprehend the mechanism of action of AmB and the toxic side effects manifestations.

For a more reasonable understanding of how amphotericin molecules interact with the lipid membrane, we carried out electrophysiology investigations at the single-molecule level and aimed to quantify how pH differences of the buffer solution, which are predicted to change the ionization state of AmB's polar head, change the stability of AmB-lipids complexes, it is noticed that low pH values facilitate more prolonged residence times of the AmB channel in its open state, whereas at neutral and alkaline pH's rapid flickering occasions between open and closed forms ensue (Figure 6), (Pashazade, 2022).

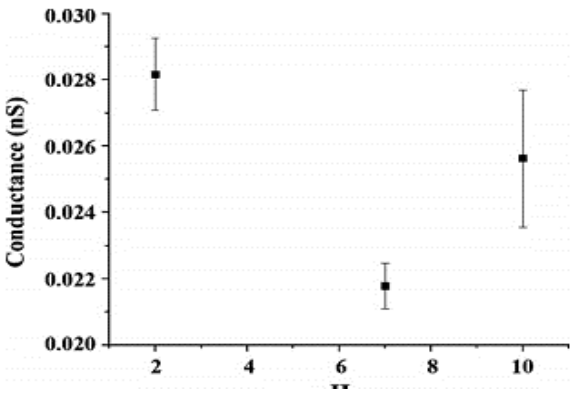


Figure 6. Typical current recordings of single ion channels formed by amphotericin B in phosphorylcholine bilayer membranes measured at different potential and pH dependence of the single-channel conductance of AmB oligomers.

The conductance for pH 7 and 8 were 500 and 560 pS. By reduction of cis pH to 6, the single-channel conductance decreased to 250 pS. As seen in this figure the reversal potential in the pH of 6, 7, and 8 were 35, 36, and 40 mV. The calculation of ionic selectivity founded on the middle values of related reversal potentials indicated a highly cation-selective channel. By changing the pH between 6-8 the ion selectivity did not significantly change. The effect of voltage on the channel activity was analyzed by measuring the channel open probability as a function of voltage in asymmetrical K+ conditions (200 mM K cis/50 mM K trans. As seen the channel open probability increased at potentials above −40 mV in the pH of 7 and 8. By reduction of cis pH to 6, the open probability significantly decreased in all voltages (Pashazade, 2022)

Clinical use of polyene antibiotics.

uses for polyenes that appear in the World Health Organization model list of essential medicines: 22nd list 2021. We then address their current clinical use and some important clinical advances. In its several formulations, AmB is used to treat coccidioidomycosis via intrathecal route, mucocutaneous leishmaniasis, invasive aspergillosis, blastomycosis, candidiasis, coccidioidomycosis, cryptococcal meningitis in patients with HIV infection, cryptococcosis, severe fungal infection of central nervous system, severe fungal infection of lung, histoplasmosis, histoplasmosis in patients with HIV infection, pulmonary cryptococcosis in patients with HIV infection, infection by Basidiobolus, mucormycosis, sporotrichosis, and urinary tract mycosis. Nys is used to treat candidal vulvovaginitis, candidiasis of skin, cutaneous and mucocutaneous infection, and non-esophageal gastrointestinal candidiasis. Natamycin is used to treat blepharitis, fungal conjunctivitis, and fungal keratitis. In addition to polyenes' use as antimycotics and antiparasitics, experimental trials of other therapeutic applications have been reported. (Pashazade, 2020)

Conclusion

In significance, we conclude that:

The high antifungal activity and low resistance incidence to polyene treatment are the main reasons why they are still being studied. Among polyene antibiotics, amphotericin B, levorin and nystatin have the strongest membrane effect. Amphotericin B channels are selective for anions, but levorin channels ideally transport cations through membranes. This difference depends on the number of carbonyl and carboxyl groups present in the hydrophilic chain of antibiotics.

Amphotericin B and its N-methyl derivative form unilateral conduction channels through the membrane and allow the transmembrane transfer of ions into the cell. It has been shown that the longer the aquifers of amphotericin B and levorin, the longer the molecules remain in the membrane.

In acidic solutions, the reversal potential of AmB channels carries a negative value, so that AmB channels become anion-selective When raising the pH value of the bathing solution AmB channels display a cationic selective behavior. AmB oligomers are mostly anionic-selective in low-pH solutions and cationic-selective at neutral and alkaline solutions. Acidity changes change the single-channel conductance of AmB oligomers. Extracellular acidity can reduce AmB activity.

References

Asandei, A, & Luchian, T. (2008). Ion selectivity, transport properties and dynamics of amphotericin B channels studied over a wide range of acidity changes. Colloids Surf B Biointerfaces, 67:99-106.

Belkherroubi-Sari, L., Boucherit Otmani, Z., Chéron, M., Boucherit, K., Benyoucef, M., & Belbraouet, S. (2008). Modulation of the polyene antibiotic amphotericin B selective toxicity by pH change of the stock solutions. Afr J Microbiol Res; 2:242-6.

Bolard, J., Legrand, P., Heitz, F., & Cybulska, B. (1991). One-side action of amphotericin B on cholesterol-containing membranes is determined by its self-association in the medium, Biochemistry, 30: 5707.

Cavassin, F.B., Luiz, Bau´-Carneiro, J., Vilas-Boas. R.R., & Queiroz-Telles, F. (2021). Sixty years of Amphotericin B: An Overview of the Main Antifungal Agent Used to Treat Invasive Fungal Infections. Infect. Dis. Ther., p.1-33.

Chiriac, R., & Luchian, T. (2007). pH modulation of transport properties of alamethicin oligomers inserted in zwitterionic-based artificial lipid membranes, Biophys. Chem. 130-139.

Cotero, B.V., Rebolledo-Antúnez, S., & Ortega-Blake, I. (1998). On the role of sterol in the formation of the amphotericin B channel. Biochim Biophys Acta; 1375:43-51.

Huang, W., Zhang, Z., Han, X., Tang, J., Wang, J., Dong, S., & Wang, E. (2002). Ion channel behavior of amphotericin B in sterol free and cholesterol or ergosterol containing supported phosphatidylcholine bilayer model membranes investigated by electrochemistry, Biophys. J. 83: 3245.

Jakl, M., Straka, M., Jaklová Dytrtová, J., & Roithová, J. (2014). Formation and stability of calcium complexes of dimethyl sulfoxide in water. International Journal of Mass Spectrometry, Vol. 360, p. 8-14.

Kamiński, D.M. (2014). Recent progress in the study of the interactions of amphotericin B with cholesterol and ergosterol in lipid environments. European Biophysics Journal, *43 (*10-11), p. 453-467.

Lee, Y., Pincus, Ph.A., & Hyeon, Ch. (2016). Effects of Dimethyl Sulfoxide on Surface Water near Phospholipid Bilayers. Biophysical Journal. 111 (11), p. 2481-2491.

Pashazade T.C. (2022). Study of physical and chemical properties of ion channels created by polyene antibiotics in lipid membranes. Advances in Biology & Earth Sciences, 7(2); 1 - 7.

Pashazade, T. C. (2018). Some experiment on biomolecular lipid membranes. Symposium dedicated to the 120th anniversary of the Institute of Botany of ANAS and Academician VI Ulyanishev of the Azerbaijan Botanical Society. December 25, p.82.

Pashazade, T. C. (2019). The action of nystatin and amphotericin B on the thin lipid membranes. Action of nystatin and amphotericin B on lipid bilayer membranes. VI Biophysical Congress, Russia, Sochi. Vol 1, p. 178-179.

Pashazade, T. C. (2020). Pharmacology of amphotericin B. Eurasian Scientific Congress. I-st International Scientific and Practical Conference, Barcelona, Spain, p. 33-38.

Pashazade, T.C., & Gasimov, X.M. (2021). Investigation of the interaction of polyene antibiotics with cholesterol, Journal of Life Sciences & Biomedicine, 3(76), No 1, p. 77-83.

Pinisetty, R., Alapati, R.V., & Devireddy, A. (2012). Molecular Dynamics Study of DMPC Lipid Bilayers Interacting with Dimethyl sulfoxide–Water Mixtures. The Journal of Membrane Biology, 245 (12), p. 807-814.

Samedova, A. A., Tagi-zade, T.P., & Kasumov, Kh.M. (2018). Dependence of ion channel properties formed by polyene antibiotics molecules on the lactone ring structure. J Bioorganic Chemistry. Vol 44, p. 337–345.

Khazar Journal of Science and Technology Volume 7 №1 2023, 14-19
Khazar University Press 2022 DOI: 10.5782/2520-613.202.7.1.14

PREVENTION OF NECROBACTERIOSIS IN HUGE HORNS ANIMALS

Huseynov Tahir Talib
Lankaran State University, doctoral student
Email: huseynov tahir1967@gmail.com

Abstract

Necrobacteriosis is widespread among the huge horns animals in the republic. The causative agents of Fusobacterium necroforum necrobacteriosis are usually harmless microorganisms which live in the usual case, in the gastrointestinal tract of ruminant animals. It is a violation of the basic calcium metabolism in the body, which turns them into the causative agents of severe pathologies of the bone tissue, muscles and internal organs of the animal. The starting mechanism of necrobacteriosis occurs after the loss of the skin's natural impermeability. This happens during mechanical damage to the skin and nails of animals, as well as in violation of mineral and vitamin metabolism in the body.

At this stage, factororamill is defined as an infectious disease, necrobacteriosis requires a non-standard approach to fight against brucellosis, tuberculosis, leukemia and a number of other diseases. At first glance, vaccinating all farm animals with quality vaccines seems to be the solution/ OR: At the first glance, the solution seems to be vaccinating all farm animals with quality vaccines. However, the perpetrators of these diseases are observed living inside the animal`s body. The animal's immune system reacts weakly to the presence of the causative agent of this disease - in the process of evolution, the pathogen is genetically close to the host's body, and in this case, the vaccine does not give/show/come with the desired effect. Only when a stressful situation arises - low-quality diet, care, delivery, transportation, etc. When the immune balance between the host organism and the pathogen is disturbed, the clinical signs of the disease manifest themselves. Therefore, the prevention of diseases from this group should be focused on prevention, elimination of stressful situations and the identification of latent carriers of the pathogen in animals. An example is the proper prevention and even complete prevention of the spread of necrobacteriosis in animals contracted/contaminated with factorial infectious disease through proper nutrition and veterinary care.

During the epizootiological, clinical and pathoanatomical examinations carried out on farms, the spontaneous occurrence of these diseases in huge horned animals was studied by examining the materials taken from the nails suspected of necrobacteriosis. The pathogenicity of cultures of Fusobacterium necrophorum isolated as a result of bacteriological examination was determined on various laboratory animals, rabbits and white mice. The sensitivity of these agents to drugs was determined and their effectiveness was studied in the treatment of necrobacteriosis in laboratory experiments.

Keywords: huge horns animals, disease, treatment, necrobacteriosis, nutrient, environment.

Introduction

It is obvious that the state pays more and more attention to the development of agriculture in Azerbaijan. Undoubtedly, the success achieved is based on consistent and well-thought-out policy, courageous and selfless activity. As a result of successful agrarian policy, large-scale work has been carried out in recent years to accelerate the development of agriculture in our country, and ongoing projects give reason to believe that in the near future our country will become a major exporter in the region.

There is new farmer pastoral farming in the country. However, most of them do not meet the requirements for pastoral farming, or rather, there are no bio-protection, sanitary checkpoints, veterinary laboratories and so on. Therefore, the import of infectious agents from these farms and their spread to the external environment is not prevented. Therefore, a number of infectious diseases, including necrobacteriosis of cattle, spread and cause serious damage to pastoral farming (Alasgarov, 2006; Aliyev et al., 2010; Aliyev et al., 2013; Gadimov et al., 1990).

Drugs are used for the treatment and prevention of this disease blindly, that is applied without studying the susceptibility of pathogens to them, which in most cases does not work.

Of course, it is important to apply preventive measures against infectious diseases and prevent diseases.

However, in the event of a disease, the correct diagnosis of the disease, in short, the identification of the etiological factor, ensures the realization of the possibility of proper treatment of the disease in the future (Alasgarov, 2006; Aliyev et al., 2013).

In view of the above, we aimed to study the effectiveness of drugs used to facilitate the diagnosis and treatment of these diseases.

Cattle necrobacteriosis is a disease that causes/leads to great damage to livestock, so the prevention of this disease is of great importance.

The causative agent of necrobacteriosis is a serious anaerobic microorganism Fusobacterium necrophorum. It is a gram-negative and highly polymorphic microorganism that does not form spores or capsules.

Our goal was to study the causes of cattle necrobacteriosis, which is widespread in large dairy farms in the city of Lankaran, and to develop proposals for its prevention.

Materials and methods

The research was conducted at the Department of Veterinary and Agrarian Disciplines of Lankaran State University and identified on the basis of monitoring on unhealthy farms. Anamnestic data conducted on farms, pathological materials taken for necrobacteriosis during clinical and epizootiological examinations were involved in bacteriological examination in the laboratory of the department. The pathogenicity of the pathogens isolated from these materials, Fusobacterium necrophorum, was studied in experiments on white mice, and their susceptibility to drugs was studied by sequential dilution of discs and drugs approved for veterinary laboratories. As a result, the therapeutic efficacy of these microbial-susceptible drugs was tested on farm animals on spontaneously sick animals (Bessarabov et al., 2007; Belyaev & Belyaeva, 2013; Volkova, 2009).

Results and disscussion

In the farms we researched, the causative agent of necrobacteriosis, Fusobacterium necrophorum, was isolated from sick animals, and through taking advantage of laboratory tests, the presence of this causative agent as the main cause of nail diseases among cattle was confirmed..

However, when considering the specific gravity of necrobacteriosis during the observation period, it appears that in the nosological profile there is a gradual increase in this disease: as it varies from year to year from 2.7% in 2019 (26 cases) to 3 in 2020, reached 6% (31 cases).

The incidence of necrobacteriosis in huge horn animals is due to insufficient supply of vitamins to animals on farms in the spring (April, May), which ultimately leads to a decrease in the body's "strength" and the impact of pathogenic factors. The second crucial factor is the change in the temperature-humidity regime of the microclimate. High humidity, combined with sharp temperature fluctuations, creates optimal conditions for the intensification of the pathogenicity of Fusobacterium necrophorum and the development of the infectious process.

Necrobacteriosis of huge horn animals was also observed in/during the warm months of the year (July, October). In July, the disease in animals is caused by hot and dry weather, which in turn weakens the protective properties of the epidermal membrane of the nails, allowing pathogens to enter through them.

Samples taken from 10 cows, 6 to 8-month-old calves and 12 from dairy cows showed that signs of disease during the study in 10% sterile ram or bull blood, 0.4% glucose solution in Marten broth, 5-10% sterile ram or bull blood, 2% glucose, 0.2% cystine with the addition of semi-liquid Muromtsev, as well as Kita-Tarossi was cultivated in food environment. 1% glucose, 10-20% serum agar and glucose-blood agar was used in the research. The results of laboratory tests showed that in 72% of broth cultures, intense nausea, and in 75% of semi-liquid and solid nutrient media, ash colonies were formed. During microscopy (smearing of smears by Gram and Muromtsev methods), Fusobacterium necrophorum was found in the form of thin filaments and cocci, typical of microorganisms.

Antibodies to Fusobacterium necrophorum were high in blood serum from clinically healthy dairy cattle.

Examination of the susceptibility of isolated cultures of Fusobacterium necrophorum to amoxicillin -150, norsulfazole, trisulfone, sulfodimethoxine and azithronite by standard discs and in the testing of antibiotics by sequential rinsing methods, turned out that they are only sensitive to amoxicillin -150, azitronite, trisulfone, sulfadimethoxine and norsulfazole.

As the causative agent, Subject is severely anaerobic. Pure cultures of Fusobacterium necrophorum were obtained using glucose-blood agar food environment. Then the pathogenicity of the isolated Fusobacterium necrophorum

was tested in various laboratory animals, rabbits and white mice. The isolated agent was diluted 1:10 in saline and injected subcutaneously in a volume of 0.5-1.0 ml into 1/3 of rabbits' ears and 0.2-0.4 ml subcutaneously into the tail area of white mice. The laboratory operator/specialist controlled the animals for 10 days. On the 4th day of the experiments, skin necrosis was observed at the injection sites. However, as a result of experiments, the pathogenicity of these perpetrators was confirmed.

The therapeutic efficacy of drugs sensitive to these pathogens was tested experimentally on rabbits infected with them individually. For this purpose, we divided the experimental rabbits into 2 groups for each culture, provided that there were 5 heads in each group, and 3 rabbits were kept in the control group. 4 different therapeutic agents were used for treatment. All experimental rabbits were infected with Fusobacterium necrophorum cultures by subcutaneous injection of 1 ml (2 billion ml) with 1-day broth cultures. Twelve hours after infection, all rabbits in the control group were treated, except for the rabbits in the control group. Therapeutic animals were treated with therapeutic doses of amoxicillin-150 and azithronite. The effectiveness of the treatment was assessed on the basis of their maintenance and general condition.

In order to prevent necrobacteriosis in farms at risk of infection, new blood samples were taken from the mother herd with high titers of antibodies against Fusobacterium necrophorum in animal blood samples. The blood serum was mixed and filtered through special filters (Zeitz filter) and packed in sterile conditions. After checking the sterility and harmlessness of blood serum, it was used according to a special scheme.

Thus, on the first day, 10 ml of serum was injected intramuscularly into calves, with an interval of 10 days, the same animals were injected intramuscularly again with 10 ml of serum, and on the third day, with an interval of 10 days, 5 ml of serum was injected intramuscularly.

Animals in the control group were vaccinated against necrobacteriosis.

All animals were kept under control for six months, during which no signs of disease were observed in the animals.

References

Alasgarov, Z. A. (2006). The dangerous animal diseases for humans. Baku, Azerneshr, 296 p.

Aliyev, E. A., Azimov, I. M., Valiyev, U. M., & Safiyeva, N.V. (2010). The infectious diseases of horses. Baku, Uniprint, 184 p.

Aliyev, E. A., Azimov, I. M., Valiyev, U. M., & Safiyeva, N.V. (2013). Epizootology and infectious diseases. Baku, Uniprint, 1020 p.

Belyaev, L. I., & Belyaeva, M. M. (2013). The correct approach to the diagnosis and prevention of factorial infectious diseases of animals. Zh. Veterinary. No. 5, 14-15.

Bessarabov, B. F., Voronin, E. S., et al. (2007). Infectious diseases of animals. M: Kolos, 671 p.

Gadimov, R. A., Mammadov, I. B., & Julfayev, S. A. (1990). Special epizootology. Baku, Baku University Publishing House, 500 p.

Volkova, E. A. (2009). Cultural properties of enterobacteria in diagnostic environment. Zh.Veterinary. No. 2, 27-28.

Khazar Journal of Science and Technology Volume 7 №1 2023, 20-31
Khazar University Press 2022 DOI: 10.5782/2520-613.202.7.1.20

S-Genotype Profiles of Azerbaijan Apricot Germplasm

Amina Rakida, Zeynal Akparov
Genetic Resources Institute, Ministry of Science and Education, Baku, Azerbaijan
Correspondence:aminkarakida@mail.ru

Abstract

Apricot is one of the important export products of Azerbaijan. Some studies showed that unproductiveness problem of apricots together with increasing production areas arises because of self-incompatibility. In flowering plants, gametophytic self-incompatibility, controlled by a single locus with several allelic variants, is one of the major problems preventing self-fertilization. Among fruit trees, apricots show a high degree self-incompatibility, especially in Middle-Asian and Iranian-Caucasian eco-geographical groups. In this study, the S-genotypes of a set of 61 Azerbaijan apricot (*Prunus armeniaca* L.) cultivars were determined by polymerase chain reaction (PCR) amplification of their S-RNase intron regions. In addition, the S-genotyping method was extended to the S haplotype-specific F-box (SFB) gene to detect the non-functional SC-haplotype and hence identification of self-compatible apricot cultivars were carried out by using four primer pairs (SRc-F and SRc-R, EM-PC2consFD and EM-PC3consRD, AprSC8-R and PaConsI-F, AprFBC8-F and AprFBC8-R). A total of 9 S-RNase alleles (S_2, S_3, S_6, S_7, S_8, S_{11}, S_{12}, S_{13} and S_c) were determined in the 61 apricot genotypes. As Azerbaijan apricot genotypes are determined to be mostly self-incompatible, the data obtained hereby might be of good use for apricot breeding programs and more practically, for new apricot plantations; thus, pollinator cultivars should be considered when self-incompatible apricot cultivars are being used.

Keywords: alleles, *Prunus armeniaca* L., primers, self-(in)compatibility

Introduction

Apricot is thought to have originated in China, from where it was disseminated to Europe through central Asia and Asia Minor (Faust et al., 1998). According to

Kostina (1969), apricot cultivars are classified into four major eco-geographical groups: central Asian, Irano-Caucasian, European, and Dzhungar-Zailing (Tien-shan area). The central Asian and Irano-Caucasian (encompassing Turkish cultivars) groups show the richest phenotypic variability, while the European group (including cultivars grown in North America, Australia, and South Africa) is said to have the least diversity (Mehlenbacher et al., 1991). Apricot cultivars originating in the eastern Europe cultivar group can be clearly distinguished in their pomological characteristics from other cultivars within the European origin (Faust et al., 1998; Kostina, 1970).

In Rosaceae, many fruit species such as Japanese pear (*Pyrus pyrifolia*), apple (*Malus* × *domestica*), sweet cherry (*Prunus avium*), almond (*Prunus dulcis*) and apricot (*Prunus armeniaca*) exhibit selfincompatibility (SI) and require pollination with pollen from compatible SI genotypes for stable fruit production. Aside from this practical importance, SI of Rosaceae is interesting from an evolutionary point of view, because the common ancestor of Asterid and Rosid is thought to exhibit S-RNase-based gametophytic self-incompatibility (Yilmaz et al., 2016).

Similar to other Prunus L. species, apricots reportedly demonstrate gametophytic self-incompatibility controlled by a single locus with multiple variants, termed S-haplotypes (de Nettancourt, 2001). The Irano-Caucasian apricots were described as predominantly self-incompatible (SI), while most European apricots are self-compatible (SC) (Hala´sz et al., 2005; Kostina, 1970). Cross-incompatibility, resulting in the mutual failure of fruit set between a pair of cultivars, occurs frequently in predominantly SI species. In apricot, the first cross-incompatibility group was described among the North American cultivars, Goldrich, Hargrand, and Lambertin-1 (Egea and Burgos, 1996), while the second group encompassed giant-fruited Hungarian apricots (Szab´o and Ny´eki, 1991).

In new apricot plantations, self-incompatibility is one of the important problems and in order to solve this issue, molecular techniques are being used to determine self-(in) compatibility alleles in apricot cultivars (Burgos et al., 1998; Halasz et al., 2005, 2007; Yilmaz, 2008; Yilmaz et al., 2013). Genetically, SI of Rosaceae is controlled by a single S locus with multiple alleles (Sonneveld et al., 2003). The S-gene product is a ribonuclease enzyme, while the pollen product is an F-box protein (Entani et al., 2003; Romero et al., 2004). The conventional methods to determine self-incompatibility are time consuming and can be affected by environmental factors (Zhang et al., 2003). Even more, molecular markers have been developed in recent years to determine the self-incompatibility of genotypes (Yaegaki et al., 2001). The Sc-haplotype was long suspected to be a pollen-part mutant of the S8-haplotype (Halasz et al., 2007) with a 353-bp insertion in the SFBC gene (Vilanova et al.,

2005). Although most apricot cultivars are self-compatible, self-incompatibility is present in some interesting cultivars (Hormaza et al., 2007). Up to 2010, a total of 20 SI (self-incompatible) alleles and one SC (self-compatible) allele were determined among European eco-geographical group of apricot (Burgos et al., 1998; Halasz et al., 2005, 2007, 2010) and studies undertaken to determine new SI alleles in apricot have been continuing (Halasz et al., 2013).

The aim of this study was to identify S-allele constitution of 61 apricot genotypes from apricot germplasm in Azerbaijan using polymerase chain reaction (PCR) with specific primer pairs.

Materials and Methods

Materials

A total of 61 apricot genotypes distributed in Nakhchivan, Tartar, Goranboy and Agdash regions of Azerbaijan were used in this study (Table 1).

DNA Isolation

Genomic DNA was extracted from full-expanded young apricot leaf samples, using the Cetyltrimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle, 1987).

PCR studies with S-RNase and SFB-specific primers

For first intron region, SRc-R (Vilanova et al., 2005) and SRc-F (Romero et al., 2004) primer pair were used to determine Sc allele, which yielded bands at 353 bp at apricot cultivars (Vilanova et al., 2005). PCR products were separated on an ABI 3500 capillary electrophoresis instrument (Applied Biosystems, Foster City, CA, USA) at the core laboratory of the Genome and Stem Cell Centre (GENKOK) in Erciyes University, Kayseri, Turkey. For the identification of the SC-haplotype, a 2-step approach was used. An allele-specific reverse primer, AprSC8R (Halasz et al., 2010), was designed to selectively amplify the Sc/ S_8 -RNase allele and used in combination with PaConsI F (Sonneveld et al., 2003). AprFBC8-F (5′- CAT GGA AAA AGC TGA CTT ATG G -3′) and AprFBC8-R (5′- GCC TCT AAT GTC ATC TAC TCT TAG -3′) were used for detecting $SFB_{C/8}$ allele (Halász et al., 2007). The amplification was carried out using a temperature profile according to Halász et al. (2010).

For the second intron, PCR was conducted according to Sutherland et al. (2004) using the degenerate primers EMPC2consFD and EM-PC3consRD. For PCR amplification in a 20-mL reaction volume, containing 1X PCR buffer (Thermo) with the final concentrations of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.0 mM MgCl2, 0.2 mM of dNTPs, 0.3 mM of each primer, and 1.0 U of Taq DNA polymerase (Thermo). The PCR products were electrophoresed in 1.5% (w/v) agarose gel, stained with ethidium bromide (0.5 lg/mL) using 1×TAE buffer, at 110 V for 2 h and visualized under UV light. Molecular size of the amplified fragments was estimated using a 100-bp ladder (Thermo). PCR's were repeated three times to determine the clear band size from apricot DNA.

Evaluation of data

To determine the exact size of the S-RNase first intron region fragments under 100 bp DNA ladder (Invitrogen), the fluorescently labelled products were run on an automated sequencer ABI Prism 3500 Genetic Analyzer. For the determination of size (genotyping), GENEMAPER software and the GS600 LIZ size standard (Applied Biosystems) were used.

The second intron PCR products were separated by electrophoresis in 1.2% TAE agarose gels for 2 h at 100 V, whereas DNA bands were visualised by ethidium bromide staining. Fragment lengths were estimated by comparison with the 1-kb DNA ladder (Promega, Madison, WI, USA). In the case of unknown alleles, PCR products were cloned and sequenced in an automated sequencer and analysed as described by Halász et al. (2010).

Results and Discussion

The determination of the S-genotypes of 61 Azerbaijan apricot genotypes was carried out using the SRc-F and SRcR consensus primers (Vilanova et al., 2005) for the first intron and EM-PC2consFD / EM-PC3consRD primers (Sutherland et al., 2004) for the second intron analysis of the S-RNase gene (Table 1). AprFBC8 F and R primers were used for discrimination of SFBC/8 allele (Halász et al., 2007). The size of the PCR products was compared with those previously published by other researchers (Vilanova et al., 2005; Halász et al., 2007, 2010). For S_8 and Sc alleles, although Vilanova et al. (2005) and Halasz et al. (2010) reported as 353 and also, Halasz et al. (2013) reported as 355 bp, the hereby result obtained was 354 bp band size. These differences might be explained by the genetic analyzers that affect the sensitivity of the method (Cachi and Wünsch, 2014). The sizes of the PCR products

obtained were compared with those previously published by Vilanova et al. (2005) and Hala´sz (2007).

A total of nine accessions failed to produce bands for

the second intron. The bands with the sizes of 310, 370, 500, 820, 900, 1200, 1250,

1300, 1700, 1980, and 2800 bp were produced by 11, 5, 15, 18, 10, 2, 12, 10, 5, 6,

and 12 accessions, respectively (Table 3).

In the present study a total of twenty accessions failed to produce bands for the second intron. The bands with the sizes of 280, 310, 370, 820, 900, 1250, 1300 and 1700 were produced by 10, 3, 3, 7, 4, 27, 4 and 8 accessions, respectively (Table 1). Previous studies have found that allele sizes of 310, 370, 500, 820, 900, 1250, 1300 and 1700 indicate S_3, S_{12}, S_9, S_7, S_2, S_{13}, S_6, S_{11}, respectively. In the present study, "Zeynabi", "Yeni forma" 1, "Gaysi", "Maychicheyi", "Yeni forma 2", "Shalakh 1", "Abu Talibi", “Teberze 2”, “Shalakh 3” and “Qırmiziyanag” produced by 280 bp sizes were not assigned to any known S locus. Four Azerbaijan cultivars (Jir Zeferani, Gaysi, Mayovka 1, Mayovka 2) a fragment of 900 bp was detected that indicated the presence of allele S_2. A fragment of 310 bp occurred in three Azerbaijan cultivars (Shemsi, Agja Nabad 2, Göyje Nabad confirming this allele as S_3. Four cultivars (Hampa, Yay Sherefi, Gecyetishen, Ordubad Sherefi) yielded a fragment of 1300 bp was detected that indicated the presence of allele S_6. The allele S_7 occurred in seven cultivars (Maychicheyi, Yeni forma 2, Teberze 1, Agja Nabad 2, Hagverdi 2, Alcha erik, Abu Talibi) as a fragment of 820 bp. Eight cultivars (Jir Zeferani, Gaysi, Mayovka 1, Mayovka 2) a fragment of 1700 bp was detected that indicated the presence of allele S_{11}. Fragment size characteristic for S_{12} allele was observed in three cultivars (Hampa, Jir Nakhchivan, Yay Sherefi). A fragment of 1250 bp occurred in twenty-seven cultivars (Ag erik Gülnar, Yeni forma 1, Balyarim, Shalakh 1, Teberze 1, Gecyetishen, Badami 1, Helena, Ag badami, Forma 1, Ag erik (early ripening), Ordubad eriyi, Ag erik, Shemsi, Göyje Nabad, Hagverdi 2, Heydari, Shalakh 2, May Goranboy, Badami 2, Limon erik 2) confirming this allele as S_{13}.

A total 9 S-RNase alleles (S_2, S_3, S_6, S_7, S_8, S_{11}, S_{12}, S_{13} and S_c) were determined in the 61 apricots genotypes (Table 1). Altogether, two cultivars (Forma 2, Mayovka 1) proved to be self-compatible. All apricot samples (except Forma 2, Mayovka 1) distributed in Azerbaijan used in the study showed self-incompatibility without SC-haplotype. Halasz et al. (2010) conducted a study to determine the S genotypes of a set of Turkish and Hungarian apricot cultivars by amplification of their S-RNase intron regions. A specific primer (AprSC8) for S_C and S_8 was designed to anneal

within the second intron region of the S_C- and S_8-RNase alleles. This primer pair amplified a fragment in the case of S_8/S_C-alleles. They reported that the presence of S_8/S_C-alleles was confirmed among the tested 18 cultivars. Some of them ('Canakkale', 'Ethembey', 'Kayisi Erigi', 'Mektep', 'Sam' and 'Yerli Izmir') were proved as self-compatible (ScSc). Two Turkish cultivars shared the S_CS_8-genotype ('Ethembey' and 'Mektep'). It was reported in their study that AprSC8 primer could distinguish between the SI and SC cultivars.

Nine previously described S-alleles were identified among the Azerbaijan cultivars. S_{13} was the most frequent S-allele in the tested Azerbaijan germplasm (occurring in 27 cultivars), followed by S_{11} (8), S_7 (7), S_2 (4), S_6 (4), S_{12} (3), S_3 (3), S_8 (3), S_c (2), while S_C-allele was only found in two cultivars.

In the present study, 'Ag erik Gülnar' ($S_{11}S_{13}$), 'Hampa' (S_6S_{12}), 'Yay Sherefi' (S_6S_{12}), 'Teberze 1' (S_7S_{13}), 'Gecyetishen' (S_6S_{13}), 'Ag erik' ($S_{11}S_{13}$), 'Shemsi' (S_3S_{13}), 'Agja Nabad 2' (S_3S_7), 'Göyje Nabad' (S_3S_{13}), 'Hagverdi 2' (S_7S_{13}), 'Ordubad Sherefi' (S_6S_{13})), 'Heydari' ($S_{11}S_{13}$), 'Shalakh 2' ($S_{11}S_{13}$), 'Alcha ərik' (S_7S_{13}), 'Badami 2' ($S_{11}S_{13}$), 'Limon erik 2' ($S_{11}S_{13}$), 'Mayovka 1' (S_CS_2), 'Forma 2' (S_CS_8) 18 S genotype combinations were determined.

Table 1. S-genotype profiles of apricot germplasms distributed in Nakhchivan, Tartar, Goranboy, Agdash regions of Azerbaijan

Cultivar	First intron (bp) of S-RNase gene	Second intron (bp) S-RNase gene	Paconsl-F/AprSC8R	AprFBC8-R/AprFBC8-F	S genotype
Zeynebi	268,268	280,280	-	150	
May Natig	-,-	-,-	-	150	
Ag erik Gülnar	378,304	1250,1700	-	150	$S_{11}S_{13}$
Yeni forma 1	268,378	280 ,1250	-	150	S_{13}
Jir Zeferani	332,332	900,900	550	150	S_2S_2
Jir erik	353,378	-,-	550	150	S_8
Gaysi	268,332	280,900	-	150, 500	S_2
Maychicheyi	268,402	280,820	-	150, 500	S_7
Balyarim	378,378	1250,1250	550	150	$S_{13}S_{13}$
Hampa	262,424	370,1300	-	150, 500	S_6S_{12}
Yeni forma 2	268,402	280,820	-	150, 500	S_7
Jir Nakhchivan	262,262	370,370	-	150, 500	$S_{12}S_{12}$

Yay Sherefi	262,424	370,1300	-	150, 500	S_6S_{12}
Shalakh 1	268,378	280,1250	-	150, 500	S_{13}
Teberze 1	402,378	820,1250	-	150,500	S_7S_{13}
Tokhum Shemsi	262,424	-,-	-	150,500	
Gejyetishen	378,424	1250,1300	-	150,500	S_6S_{13}
Badami 1	378,378	1250,1250	-	150	$S_{13}S_{13}$
Helena	378,402	1250,-	-	150	S_{13}
Mehmani	268,402	-,-	-	150	
Hagverdi 1	268,402	-,-	-	150	
Ag Nabati	378,378	-,-	-	150	
Kürdeshi	268,378	-,-	-	150	
Talibi	268,402	-,-	-	500	
Genotip 1	378,378	-,-	-	500	
Ag badami	378,378	1250,1250	-	-	$S_{13}S_{13}$
Agjanabad 1	378,402	-,-	-	150,500	
Limon erik 1	268,402	-,-	-	150,500	
Forma 1	378,378	1250,1250	-	-	$S_{13}S_{13}$
Ag erik (late ripening)	332,402	-,-	-	150,500	
Ağ erik (early ripening)	304,378	-,1250	-	150	S_{13}
Ordubad eriyi	304,378	-,1250	-	150,500	S_{13}
Ag erik	304,378	1700,1250	550	150	$S_{11}S_{13}$
Badam erik 1	304,304	1700,1700	,600	150	$S_{11}S_{11}$
Shemsi	268,378	310,1250	-	150	S_3S_{13}
Badam erik 2	304,402	-,-	-	150,500	
Agja Nabad 2	268,402	310,820	-	150,500	S_3S_7
Göyje Nabad	268,378	310,1250	-	150,500	S_3S_{13}
Hagverdi 2	402,378	820,1250	-	150,500	S_7S_{13}
Genotip 3	304,378	-,-	-	-	
Ordubad Sherefi	378,424	1250,1300	-	150	S_6S_{13}
Heydari	304,378	1700,1250	-	150,500	$S_{11}S_{13}$
Ordubad jiri	268,378	-,-	-	150	
Forma 2	304,353	-,-	-	150,500	S_CS_8

Genotip 2	304,378	-,1250	-	150,500	S_{13}
Ordubad Nabati	268,378	-;-	-	150	
Yeni forma 3	268,378	-,1250	-	150	S_{13}
Shalakh 2	304,378	1700,1250	-	150	$S_{11}S_{13}$
Alcha erik	402,378	820,1250	-	150	S_7S_{13}
Abu Talibi	268,402	280,820	-	-	S_7
Teberze 2	268,378	280,1250	-	-	S_{13}
Ag erik Elchin	304,424	-,-	-	150	
May Goranboy	304,353	1700,-	550	150	S_8S_{11}
Mayovka 1	332,353	900,-	550	500	S_CS_2
Badami 2	304,378	1700,1250	-	150,500	$S_{11}S_{13}$
Shalakh 3	268,378	280,1250	-	-	S_{13}
Girmiziyanag	-,-	280,1250	-	150,500	S_{13}
İrevan eriyi	-,-	-,-	-	-	
Mayovka 2	332,378	900,-	-	500	S_2
Limon erik 2	304,378	1700,1250	-	150	$S_{11}S_{13}$
Esgerabat	268,402	-,-	-	-	

Mehlenbacher et al. (1991) reported that the European group of apricot (Europe, North America, South Africa and Australia are included) may be described as self-compatible. It was reported by Halasz et al. (2013) to support the S-genotype determinations, as first intron lengths were also determined for all genotypes using fluorescently labelled primers and automated sizing on a capillary sequencer. Analysis of the first intron in 63 wild-grown apricot accessions from Turkey showed that 17 of 63 apricot accessions had 355 bp fragment. This fragment size was previously attributed to both the S_C- and S_8-RNase alleles (Halasz et al., 2007).

Vilanova et al. (2005) used SRc-R and SRc-F primer pair for 10 apricot cultivars to determine their S alleles. Six of 10 apricot genotypes were obtained via reciprocal crossing. They determined apricot genotypes that had Sc allele, which yielded at 353 bp. It was reported with previous studies that most of the European cultivars had Sc allele, whereas old Turkish cultivars were self-incompatible (Yilmaz, 2008; Halasz et al., 2010).

Since coding regions of the S_8- and S_C-RNase alleles are identical, discrimination between the 2 alleles was not possible. In apricot, self-compatibility is attributed to a pollen-part mutation: a 353 bp insertion in the SFB gene. To distinguish between

the self-incompatible (SI) and self-compatible (SC) accessions, a previously designed specific primer pair (AprFBC8) can be used (Halasz et al., 2010), which amplifies a fragment of approximately 500 bp in the case of SFB_C-allele, while genotypes carrying the SFB_8-allele show a fragment of approximately 150 bp (Halasz et al., 2013). Thus, Halasz et al. (2013) determined 17 apricot accessions carrying SFB8-allele among 63 apricots from Turkey using AprFBC8 primer pair and they were stated as self-incompatible.

Based on the structure of S-RNase, many pairs of primers have been developed for Prunus species, such as Pru-C2 and PCE-R (Tao et al., 1999a; Yamane et al., 2001), SRc-F and EMPC5consRD, SRc-F and PM-C5 (Vilanova et al., 2005; Sutherland et al., 2004; Habu et al., 2008), ASIII and AmyC5R (Tamura et al., 2000), EM-PC2consFD and ED-PC3cons-RD 70 (Sutherland et al., 2004), PaConsI-F and PaConsI-R, PaConsII-F and PaConsII-R (Sonneveld et al., 2003). Yaegaki et al. (2001) first determined S-RNase genotypes using the primer pair Pru-C2 and Pru-C5. Tao et al. (2002) cloned novel S8-RNase and Sc-RNase using Pru-C2 and PCE-R. Recently, the S-genotypes of 14 Japanese apricot cultivars native to Japan were determined using Pru-C2 and PCE-R, SRc-F and EM-PC5consRD, SRc-F and PM-C5 (Habu et al., 2008). The primer pair Pru-C2 and PCE-R was developed from C2 and C3 in Prunus by Tao et al. (1999) and Yamane et al. (2001) and is considered as the universal primer pair for determining the S-genotypes in Japanese apricot (Habu et al., 2008).

Halasz et al. (2013) carried out a study to determine S genotypes of wild-growing Turkish apricots by PCR amplification of the S-RNase intron regions and SFB gene, in order to characterize their sexual (in) compatibility phenotype. The authors determined the complete S-genotype of 63 wild-grown apricot accessions that originated in the Erzincan region. Ten previously described and 2 new S-alleles (provisionally labeled S_X and S_Y) were identified in the studied genotypes. S2 was the most frequent S allele in the tested germplasm (occurred in 19 accessions), followed by S_8 (17), S_{19} (16), S_3 (13), S_{12} (11), S_6 (10) and S_7 (10), while S_9-, S_{11}- and S_{13}-alleles were found in 8 accessions each. A total of 36 different S-genotypes were assigned to the tested accessions. The SC-allele responsible for self-compatibility in apricot was not present, indicating that all accessions were self-incompatible. The analysis of S-allele frequencies allowed to conclude the close relationship of wild-grown and cultivated apricots in Turkey and helped to raise hypotheses that may explain the high occurrences of S_2- and S_8-alleles.

One of the most important factors in apricot crop evolution was the emergence of self-compatibility, which has resulted in a serious loss of genetic diversity in Europe and the Mediterranean Basin (Pedryc et al., 2009; Bourguiba et al., 2012). In a

previous study, Halasz et al. (2010) detected an uneven distribution of the S_C-allele in Turkish apricot cultivars: no self-compatible cultivar was found among 11 tested genotypes in the Eastern Region, while 7 out of 14 tested cultivars from the Western part of the country were self-compatible. Although the 55 cultivars analyzed in their study did not reveal a sound conclusion regarding the place of the origin of self-compatibility in apricot, the increasing number of S_C cultivars from East to West was suggestive.

Conclusion

The development of apricot production in Azerbaijan is expanding day by day. There are also apricot growing areas with wide genetic diversity. Determining the S allele structure of apricot germplasm is very important for orchard management and breeding programs. Within the framework of this research, the S-allele structure of apricot germplasm in Azerbaijan was determined and the results showed that there are great differences between the studied apricot genetic materials according to the S allele formation.

References

Bourguiba, H., Audergon, J.M., Krichen, L., Trifi-Farah, N., Mamouni, A., Trabelsi, S., D'Onofrio, C., Asma, B.M., Santoni, S., Khadari, B. (2012). Loss of genetic diversity as a signature of apricot domestication and diffusion into the Mediterranean Basin. BMC Plant Biology, 12(1), 1.

Burgos, L., Pérez-Tornero, O., Ballester, J., Olmos, E. (1998). Detection and inheritance of stylar ribonucleases associated with incompatibility alleles in apricot. Sexual Plant Reproduction, (11), 153-158.

Cachi, A.M., Wünsch, A. (2014). S-genotyping of sweet cherry varieties from Spain and S-locus diversity in Europe. Euphytica ,197(2), 229-236.

de Nettancourt, D. (2001). Incompatibility and incongruity in wild and cultivated plants. 2nd ed. Springer-Verlag, New York.

Doyle, J.J., Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, (19), 11-15.

Egea, J., Burgos, L. (1996). Detecting cross-incompatibility of three North American apricot cultivars and establishing the first incompatibility group in apricot. J. Amer. Soc. Hort. Sci., (121), 1002– 1005.

Entani, T., Iwano, M., Shiba, H., Che, S.F., Isogai, A., Takayama, S. (2003). Comparative analysis of the self-incompatibility (S-) locus region of *Prunus mume*: identification of a pollen-expressed F-box gene with allelic-diversity. Genes Cells, (8), 203-213.

Faust, M., Sura´nyi, D., Nyujt´o, F. (1998). Origin and dissemination of apricot. Hort. Rev. (Amer. Soc. Hort. Sci.), (22), 225–266.

Habu, T., Matsumoto, D., Fukuta, K., Esumi, T., Tao, R., Yaegaki, H., Yamaguchi, M., Matsuda, M., Konishi, T., Kitajima, A., Yamada, T. (2008). Cloning and characterization of twelve S-RNase alleles in Japanese apricot (*Prunus mume* Sieb. et Zucc.). Journal Japanese Society Horticultural Science, (77), 374-381.

Halasz, J., Hegedus, A., Herman, R., Stefanovits-Banyai, E., Pedryc, A. (2005). New self-incompatibility alleles in apricot (*Prunus armeniaca* L.) revealed by stylar ribonuclease assay and S-PCR analysis. Euphytica, (145), 57-66.

Halasz, J., Hegedus, A., Szikriszt, B., Ercisli, S., Orhan, E., Unlu, H.M. (2013). The S-genotyping of wild-grown apricots reveals only self-incompatible accessions in the Erzincan region of Turkey. Turkish Journal of Biology, (l), 733-740.

Halasz, J., Pedryc, A., Ercisli, S., Yilmaz, K.U., Hegedus, A. (2010). S-genotyping support the genetic relationships between Turkish and Hungarian Apricot Germplasm. Journal of the American Society for Horticultural Science, (135), 410-417.

Halász, J., Pedryc, A., Hegedűs, A. (2007). Origin and dissemination of the pollen-part mutated SC-haplotype which confers self-compatibility in apricot (*Prunus armeniaca* L.). New Phytologist, (176), 792-803.

Hormaza, J.I., Yamane, H., Rodrigo, J. (2007). Apricot genome mapping and molecular breeding in plants. In: Kole C (Ed). Fruits and Nuts,Springer-Verlag Berlin Heidelberg, (4).

Kostina, K.F. (1969). The use of varietal resources of apricots for breeding. Trudy Nikitskiy Botanicheskiy Sad, (40), 45–63.

Kostina, K.F. (1970). Self-fertility studies in apricot. Trudy Gosudarstvenny Nikitkiy Botanicheskiy Sad, (45), 7–17.

Mehlenbacher, S.A., Cociu, V., Hough, L.F. (1991). Apricots (*Prunus*), 65–107. In: J.N. Moore and J.R. Ballington (eds.). Genetic resources of temperate fruit and nut crops, Chap. 2. International Society for Horticultural Science, Wageningen, The Netherlands.

Pedryc, A., Ruthner, S., Herman, R., Krska, B., Hegedus, A., Halasz, J. (2009). Genetic diversity of apricot revealed by a set of SSR markers from linkage group G1. Scientia Horticulturae, (121), 19-26.

Romero, C., Vilanova, S., Burgos, L., Martinez-Calvo, J., Vicente, M., Llacer, G., Badanes, M.L. (2004). Analysis of the S-locus structure in *Prunus armeniaca* L. identification of S-haplotype S-RNase and F-box genes. Plant Molecular Biology, (56), 145-157.

Sonneveld, T., Tobutt, K.R., Robbins, T.P. (2003). Allele-specific PCR detection of sweet cherry self-incompatibility (S) alleles S1 to S16 using consensus and allele-specific primers. Theoretical and Applied Genetics, (107), 1059-1070.

Sutherland, B.G., Robbins, T.P., Tobutt, K.R. (2004). Primers amplifying a range of Prunus S-alleles. Plant Breeding, (123), 582-584.

Szab´o, Z., Ny´eki, J. (1991). Blossoming, fructification and combination of apricot varieties. Acta Hort, (293), 295–302.

Tamura, M., Ushijima, K., Sassa, H., Hirano, H., Tao, R., Gradziel, T.M., Dandekar, A.M. (2000). Identification of self-incompatibility genotypes of almond by allele-specific PCR analysis. Theoretical and Applied Genetics, (101), 344-349.

Tao, R., Habu, T., Namba, A., Yamane, H., Fuyuhiro, F., Iwamoto, K., Sugiura, A. (2002). Inheritance of Sf-RNase in Japanese apricot (*Prunus mume*) and its relation to self-compatibility. Theoretical and Applied Genetics (105), 222-228.

Tao, R., Yamane, H., Sugiura, A., Murayama, H., Sassa, H., Mori, H. (1999). Molecular typing of S-alleles through identification, characterization and cDNA cloning for S-RNases in sweet cherry. Journal of the American Society for Horticultural Science (124), 224-233.

Vilanova, S., Romero, C., Llacer, G., Badanes, M.L. (2005). Identification of self- (in) compatibility alleles in apricot by PCR and sequence analysis. Journal of the American Society for Horticultural Science, (130), 893-898.

Yaegaki, H., Shimada, T., Moriguchi, T., Hayama, H., Haji, T., Yamaguchi, M. (2001). Molecular characterization of S-RNase genes and S-genotypes in the Japanese apricot (*Prunus mume* Sieb. et Zucc.). Sexual Plant Reproduction, (13), 251-257.

Yamane, H., Tao, R., Sugiura, A., Hauck, N.R., Iezzoni, A.F. (2001). Identification and characterization of S-RNases in tetraploid sour cherry (*Prunus cerasus*). Journal of the American Society for Horticultural Science, (126), 661-667.

Yilmaz, K.U. (2008). Phenological, morphological and pomological characteristics of some local apricot genotypes and determination of their genetic relationships and self-incompatibility by molecular markers. Department of Horticulture Institute of Basic and Applied Sciences, University of Cukurova, PhD Thesis.

Yilmaz, K.U., Kafkas, S., Paydaş-Kargı, S. (2013). Determination of Self-(in)compatibility in Turkish Apricot Genotypes. Fruit Science, (1), 34-40.

Yilmaz, K.U., Basbug, B, Gürcan, K., Pinar, H., Halasz, J., Ercisli, S., Uzun, A., Cocen, E. (2016). S-Genotype Profiles of Turkish Apricot Germplasm. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 44(1), 67.

Zhang, S.L., Cao, S.M., Wu, H.Q. (2003). Self-incompatibility genotypes of fruit trees and their identification methods. Journal of Fruit Science, (20), 358- 363.

Khazar Journal of Science and Technology Volume 7 №1 2023, 32-43
Khazar University Press 2023 DOI: 10.5782/2520-613. 202.7.1.32

Role of Herbal Medicines in Glucose-6 Phosphate Dehydrogenase Deficiency

Ana Barati
Faculty of Graduate School of Science, Art, And Technology,
Khazar University, Baku, Azerbaijan
Email: Barati.ana2021@khazar.org

Abstract

Favism is an acute hemolytic reaction triggered in people with an inherited deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD). Glucose 6-phosphate dehydrogenase (G6PD) deficiency is one of the commonest human enzymopathies, caused by inherited mutations of the X-linked gene G6PD. It is estimated that about 400 million people are affected by this deficiency. The G6PD enzyme catalyzes the first step in the pentose phosphate pathway, leading to antioxidants that protect cells against oxidative damage. Most individuals with G6PD deficiency are asymptomatic, with no clinical manifestations of illness unless triggered by certain drugs, metabolic conditions, infections, and ingestion of fava beans. A G6PD-deficient patient, therefore, cannot protect red blood cells against oxidative stresses. Typically, hemolysis ensues in about 24-48 hr after a patient has ingested a substance with oxidant properties. The degree of hemolysis varies with the inciting agent, the amount ingested, and severity of the enzyme deficiency. On the other hand nowadays, more people are frequently turning to herbal medicines as treatments for various medical conditions, often without medical advice. It has been documented that as many patients who take herbal medicine are unaware of their potential adverse effects, so they continue to use the products. The objective of this study examines the herbal medicines that people with G6PD deficient use for other treatments, But their unaware consumption causes symptoms of this disease, so they shouldn't consume them, or about herbal medicines that studies have shown were able to protect against hemolytic damage in human with G6PD-deficient.

Keywords: glucose-6-phosphate dehydrogenase, Favism, herbal medicine

Introduction

Favism is an acute hemolytic reaction triggered by exposure either to fava beans (Vicia faba) or to certain drugs (e.g., sulfa-based antibiotics and the antimalarial primaquine) in people with an inherited deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD). G6PD enzyme generates nicotinamide adenine dinucleotide phosphate (NADPH), which represents the primary defense against oxidative stresses in red blood cells (RBC). Mutations in the G6PD gene can destabilize the enzyme and reduce its activity levels, leaving cells vulnerable to damage from exogenous triggers, including certain foods, infections, and a range of drugs, that may lead to RBC lysis and acute hemolytic anaemia (AHA) (Kattamis et al., 1969). In favism this, the patient can suffer from the destruction of red blood cells, severe anemia, and possibly death. There are two necessary conditions for the disease: (1) genetic inheritance of the "Mediterranean" variant of the abnormal gene trait for G6PD deficiency; and (2) ingestion of fava beans, usually fresh, or exposure to some drugs. The bean is a dietary staple in areas where favism is reported Only an estimated 20 percent of those with the genetic trait for G6PD are likely to experience episodes of favism (Diegues, et al., 2022). Under modern medical conditions the hemolytic anemia caused by favism is only rarely fatal. Strong evidence suggests that both the gene for G6PD deficiency and the cultural practice of fava bean consumption are evolutionarily adaptive traits that protect against death from all types of malaria (Brown., 1981). Favism, then, could be described as a negative outcome of the interaction of the positive adaptive qualities of both the gene and the bean. A consequence of this is that G6PD-deficiencies individuals are resistant the malaria causing parasite (Bienzle et al., 1972). Because G6PD deficiency is an X-linked recessive disorder, the main clinical manifestations are observed in hemizygous males, and most females are unaffected carriers (Schuurman, et al., 2009; Lim et al., 2005). The most frequent manifestations are neonatal jaundice and acute hemolytic anemia, which typically appear 2-4 days after exposure to a trigger, such as certain medications, toxins, or agents of infection (Schick, 2017). Favism is found primarily in the Mediterranean and Middle East regions where fava beans are a staple food and the Mediterranean variant of the G6PD deficiency gene is relatively common. Mark Belsey reports that it is frequently encountered in Greece, Sardinia, Italy, Cyprus, Egypt, Lebanon, Israel, Iran, Iraq, Algeria, and Bulgaria, and is particularly common among Sephardic Jews (Belsey, 1973). The WHO also defines herbal medicines as plant-derived materials or preparations intended for human therapeutic use or further health benefits in humans (WHO, 2005). Herbal products are usually ingested raw, as tea or as decoctions (concentrated extracts). Sometimes they are applied as a paste or powder on the skin. Some herbal traditions, such as traditional Chinese Medicine (TCM) and Ayurvedic

medicine, have medicinal products that are packaged in the form of pills or liquids for ease of consumption and retailing (Ko, 1999). These are sometimes called proprietary medicine, finished products, or patent medicine (Phua, 2009). It has been documented that as many patients who take herbal medicine are unaware of their potential adverse effects, so they continue to use the products. The objective of this study examine the herbal medicines that people by G6PD deficient use for other treatments, But their unaware consumption causes symptoms of this disease, so they shouldn't consume them, or about herbal medicines that studies have shown was able to protect against hemolytic damage in human with G6PD-deficient.

G6PD and G6PD deficiency: historical milestones

Warburg in Berlin, Germany, identified in yeast and in red cells an enzyme of carbohydrate metabolism that oxidized glucose-6-phosphate. Because the oxidation was not carried out by O_2 itself, but required as an intermediary the coenzyme NADP (then called TPN), they named the enzyme Zwischenferment: we now know it was G6PD (Warburg and Christian, 1932). Alving in Chicago, discovered that men who had developed AHA following administration of the antimalarial primaquine had severe deficiency of G6PD in their red cells (Alving et al., 1956). Sansone in Genoa, Italy, found G6PD deficiency in all patients who had a history of favism (Sansone and Segni., 1957). Szeinberg and colleagues in Tel Aviv, Israel, found that the inheritance of G6PD deficiency was consistent with the gene being on the X chromosome (Szeinberg et al., 1958). Panizon in Sassari, Italy (Panizon, 1960), and Doxiadis in Athens, Greece (Doxiadis et al., 1961), identified G6PD deficiency as a cause of severe neonatal jaundice. Shortly after the formulation of the "Lyon hypothesis" regarding inacttheivation of 1 of the 2 X chromosomes in somatic cells of female mice. 1995-2002 Embryonic stem cells in which G6PD had been knocked out by targeted homologous recombination have normal pentose synthesis but exquisite sensitivity to oxidative stress (Pandolfi, et al., 1995); and G6PD inactivation is lethal early in embryo development (Longo et al., 2002).

World Health Organization Classification

The World Health Organization has classified G6PD deficiency as class I-V according to the magnitude of the enzyme deficiency. Patients in class II have a severe enzyme deficiency, where the G6PD activity is <10% of the normal value. Class II patients experience intermittent hemolytic episodes, typically after exposure

to substances that are a source of oxidant stress, such as fava beans (as in this case) or oxidant medications. G6PD deficiency can also be classified according to mutations in the G6PD gene that exist within specific ethnic groups, such as Mediterranean-type G6PD deficiency, which is a class II deficiency (Cappellini and Fiorelli, 2008). (Table 1)

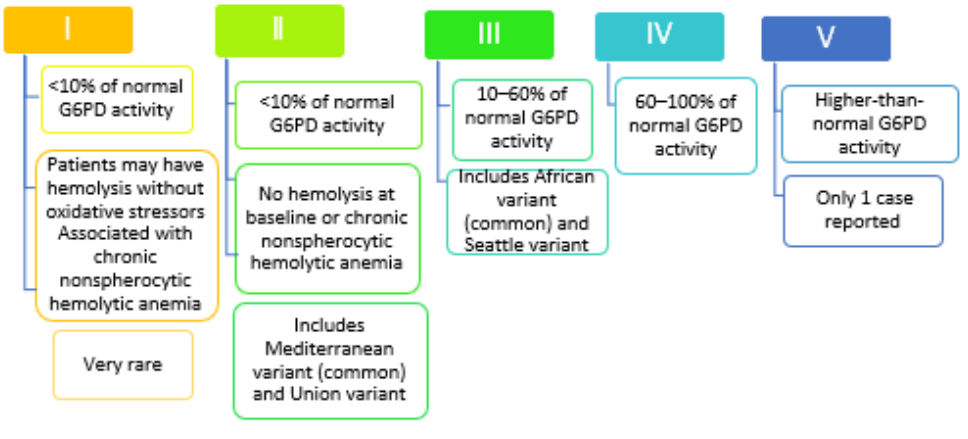


Table 1. World Health Organization Classification of Glucose-6-phosphate Dehydrogenase (G6PD) Deficiency Variants (WHO, 1989).

Herbal medicines cited in the literature

Centaurea ammocyanus

Centaurea species have received considerable interest in biological researches due to their many important biological activities. R. Joujeh et al study *Centaurea ammocyanus* aerial parts hydromethanolic extract for, investigate its hemolytic effect on human erythrocytes and anti-hemolytic activity in the protection of normal and G6PD enzyme-deficient human erythrocytes against oxidative damage. The results revealed that hemolysis caused by *C. ammocyanus* extract was very low with a maximum value of 4.06% at the concentration 3000 µg/ml. The results also showed that the hemolysis induced by H_2O_2 is reduced in a concentration-dependent manner in the presence of the *C. ammocyanus* extract. The highest protection values were about 88.14% and 92.59% for normal and G6PD deficient erythrocytes, respectively. Through this study, it can be concluded that *C. ammocyanus* aerial parts extract showed no obvious hemolytic effect, and could be considered safe for human erythrocytes. The extract had antioxidant activity and was able to protect normal and

G6PD-deficient human erythrocytes against hemolytic damage induced by hydrogen peroxide (Joujeh et al., 2020).

Salvia oficinalls

Salvia oficinalls is an old medicinal plant, which is rich source of different chemical constituents including terpenoids, polyphenol as well as essential oils. Because of its flavoring and seasoning properties, this plant has been widely used in preparation of many foods. In folk medicine of Asia and Latin America, it has been used for the treatment of different kinds of disorders including seizure, ulcers, gout, rheumatism, inflammation, dizziness, tremor, paralysis, diarrhea, and hyperglycemia (Garcia et al., 2016). In traditional medicine of Europe, *S. officinalis* has been used to treat mild dyspepsia (such as heartburn and bloating), excessive sweating, age-related cognitive disorders, and inflammations in the throat and skin (Perry et al., 1999; Adams et al., 2007). German Commission E has accepted the use of *S. officinalis* for a number of medical applications included inflammation and dyspepsia. *Salvia officinalis* extract is used to stabilize fat and fat-containing food against oxidation. The main antioxidant compounds in the sage are carnosic acid and carnosol rosmarinic acid. Al-Awaida showed *Salvia oficinalls* extracts were able to protect hemolysis in parallel to their activation extents on G6PD activities (Al-Awaida and Akash, 2014).

Diospyros lotus L

Diospyros lotus L. is indigenous to the temperate Asian forests, China and also seen in north of Iran from parts of the coast of Caspian Sea up to 1100 meter from sea level in Astara to Ramian, Gorgan in north of Iran (Mallvadhani et al., 1998). *Diospyros lotus L*., similar to other species of *Diospyros*, has a high amount of naphthoquinones especially 7- methyljuglone. Many studies have shown that it has numerous biological and pharmacological properties include: including its use as an antifebrile agent, secretions, as a sedative, and for controlling cough (Sabeti, 1997). Azadbakht et al evaluated the protective effect of *Diospyros lotus L*. fruit extract against the hemolytic damage induced by Vicia faba beans extract in both G6PD enzyme-deficient human and rat erythrocyte in vitro and in vivo.The results have shown that *Diospyros lotus L*. fruits extract has antioxidant activity that may protect against hemolytic damage induced by Vicia faba bean extract in both G6PD-deficient human and rat erythrocytes. The study gives a scientific basis for the efficacy of the fruit extract as used in Iran. The fact that this was shown in human erythrocytes in vitro is significant and provides a rationale for further testing in vivo in G6PD-deficient human populations (Azadbakht et al., 2011).

Tea extracts

Tea extracts are known to terminate inflammatory conditions, and its usage has been reported to prevent skin damage caused by UV irradiation (Vayalil et al., 2004). There are three types of tea: black, Oolong, and green tea. Green tea is widely consumed in Japan, China, and other Asian nations and is becoming more popular in Western nations. The difference between green tea and the others is that green tea is not fermented, thus preventing antioxidants from being lost during that process. Therefore, and in contrast to black tea, green tea contains high concentrations of polyphenols such as epigallocatechin-gallate (EGCG). Tea polyphenols have been shown to inhibit proteasome function, thereby terminating inflammation (Nam et al., 2001). Ko et al investigated the pro-oxidative effects of tea and some polyphenols (epigallocatechin-3-gallate and epigallocatechin) on G6PD-deficient erythrocytes in vitro. The tea extracts significantly decreased the level of reduced glutathione in G6PD-deficient erythrocytes in a dose-dependent manner but did not alter the level in normal erythrocytes. The authors believed it is highly unlikely the plasma concentration of these compounds would reach a harmful level in individuals with G6PD deficiency under conditions of normal consumption. Instead, they suggested that an additive effect might occur if individuals with G6PD deficiency take additional oxidative drugs. No case reports in the literature have described hemolysis when individuals with G6PD deficiency consumed tea and/or polyphenols, and, to date, involvement of tea and some polyphenols in hemolysis in individuals with G6PD deficiency has not been confirmed in vivo (Ko et al., 2006).

Fenugreek seeds

Fenugreek (Trigonella foenum graecum) is a plant that belongs to the Leguminosae family (Kaviarasan et al, 2006). It is established that *fenugreek* seed extract has anti-diabetic effects through several pathways, such as restoring pancreatic β cell function and inhibiting sucrase and alpha-amylase activities (Baquer et al., 2011). It is full of 4-hydroxyisoleucine, which directly induces insulin secretion from pancreatic β cells (Kaviarasan et al, 2006).. Furthermore, there are some evidences that its seed extract reduces serum triglycerides, total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) (Baquer et al., 2011). Beside these properties, some anti-inflammatory and anti-nociceptive actions were also attributed to its seed extract (Kawabata et al., 2011). In one case report, *fenugreek* seeds were suspected to have triggered hemolysis in a an individual with G6PD deficiency. Acute hepatitis and a1-antitrypsin deficiency were excluded as causes of hemolysis, but neither the diet nor possible exposure to drugs in this patient, who had an 8-month history of poorly controlled diabetes, was investigated, and therefore a causative relationship between

fenugreek and hemolysis could not be demonstrated conclusively (Sadler et al., 2009).

Henna (*Lawsonia inermis*)

Henna is a dye derived from the dried leaves of the flowering plant Lawsonia inermis (Raupp et al., 2001). The dye principally contains 2-hydroxy-1, 4-napthoquinone, but also flavonoids and steroids. Henna continues to be widely used in Africa, Asia and the Middle East, to colour or adorn the skin, nails and hair. Its use is often associated with religio-cultural events, including marriage. Reports of haemolysis with the topical use of henna have been reported (Kandil et al., 1996; Kok et al., 2004; Zinkham et al, 1996; Seyedzadeh et al., 2007; Katar et al., 2007). Raupp et al. presented four cases of G6PD deficient individuals who experienced haemolytic crisis following topical henna application. Acute renal failure occurred in one case and the patient died after 2 days of admission (Kandil et al., 1996). In another study by Kandil and colleagues, the authors reported (Kok et al., 2004), G6PD deficient individuals who were admitted due to acute haemolysis after 24–72 h post-henna application. Other authors have also reported similar features after application of henna. Taken together, existing data reviewed showed that henna could increase the risk of haemolysis in infants and children with G6PD deficiency (Kok et al., 2004).

Gingko biloba

Gingko biloba is the fourth most frequently used source of herbal medicine in the United States, accounting for 4.3% of all single herb sales in 2001. Its extract is used for many clinical conditions, including dementia, mild cognitive impairment, cerebrovascular and arterial insufficiency, tinnitus, vertigo, asthma, and allergies (Bent et al., 2005). Increasing evidence suggests that *G. biloba* leaf extract can act as a prooxidant in vitro and in vivo. These extracts contain quercetin (Pawlikowska-Pawlega et al., 2003) and procyanidins (Robaszkiewicz et al., 2007), which induce oxidative stress, especially in high doses. *Gingko* is generally well tolerated, but can increase the risk of bleeding if used combination with warfarin, antiplatelet agents or in subjects with G6PD deficiency. One case report discussed a 55-year-old woman with a history of hypertension and dementia, who was given a 17.5 mg injection of *Gingko biloba* leaf extract to improve her memory and subsequently developed jaundice. Cessation of therapy improved her condition and she was discharged 5 days later. Taking into consideration the widespread use of this supplement and paucity of reports, it is highly improbable that *Gingko* can lead to haemolysis in G6PD deficiency (Lai et al., 2013).

Acalypha indica

Acalypha indica is a weed found in various parts of Asia, and widely used in Ayurveda for its claimed anti-inflammatory, antimicrobial and antitussive effects (Seebaluck et al., 2015). Sellahewa first described *Acalypha indica* induced haemolysis in four patients in Sri Lanka (Sellahewa, 1992). Since then, three other studies have similar reported incidences of acute haemolysis after ingestion of *Acalypha indica* (Narasimhan et al., 2014; Lamabadusuriya and Jayantha., 1994; Senanayake and Sanmuganathan., 1996). In all cases, the authors suggested that consumption of a broth containing *Acalypha indica* was the cause of haemolysis. Nevertheless, the actual dose and purity of these extracts were not reported. Indeed, toxicity studies from laboratory studies using low to very high doses of *Acalypha indica* extract in rats found it to be non-toxic to major organs. In view of these contradictory findings, we suggest caution in the consumption of *Acalypha indica* (Sathya et al., 2012; Lee et al., 2017).

Conclusion

In summary, herbal medicines play an important role in the general health-care system of many developing countries worldwide and are gaining popularity rapidly in many developed countries. Most individuals with G6PD deficiency are asymptomatic, with no clinical manifestations of illness and the unaware use of herbal medicines may have dangerous consequences.By examining herbal medicines in this review article, it can be seen that G6PD deficient patients should use herbal medicines cautiously because some of them, like henna, have been proven to cause hemolytic, but on the other hand, some plants can be used to Reducing the dangerous effects of this disease but more research is needed.

Reference

Adams, M., Gmünder, F., Hamburger, M. (2007). Plants traditionally used in age related brain disorders–a survey of ethnobotanical literature. J Ethnopharmacol. 113:363–381.

Al-Awaida, W.J., Akash, M. (2014). Protective role of aqueous medicinal herbal extracts against oxidative stress on Glucose-6-phosphate dehydrogenase activity and RBC fragility. Life Sci J, 11(2), 385-391.

Alving, A.S., Carson, P.E., Flanagan, C.L., Ickes, C.E. (1956). Enzymatic deficiency in primaquine-sensitive erythrocytes Science, 124 (3220), 484-485.

Azadbakht, M., Hosseinimehr, S.J., Shokrzadeh, M., Habibi, E., & Ahmadi, A. (2011). Diospyros lotus L. fruit extract protects G6PD-deficient erythrocytes from hemolytic injury in vitro and in vivo: prevention of favism disorder. European review for medical and pharmacological sciences, 15 (11), 1270-81.

Baquer, N.Z., Kumar, P., Taha, A., Kale, R.K, Cowsik, S.M, McLean, P. (2011). Metabolic and molecular action of Trigonella foenum-graecum (fenugreek) and trace metals in experimental diabetic tissues. J Biosci, 36:383–396.

Bent, S., Goldberg, H., Padula, A., Avins, A.L. (2005). pontaneous bleeding associated with Ginkgo biloba: a case report and systematic review of the literature. J Gen Intern Med, 20:657-661.

Belsey, M.A. (1973). The epidemiology of favism. Bulletin of the World Health Organization, 48 (1), 1 – 13.

Bienzle, U., Ayeni, O., Lucas, A.O., Luzzatto, L. (1972). Glucose-6-phosphate dehydrogenase and malaria. Greater resistance of females het-erozygous for enzyme deficiency and of males with non-deficient variant. Lancet, 1(7742):107-110.

Brown, P.J. (1981). New considerations on the distribution of malaria, thalassemia, and glucose-6-phosphate dehydrogenase deficiency in Sardinia. Hum Biol, 53(3):367-82.

Cappellini, M.D., Fiorelli, G. (2008). Glucose6-phosphate dehydrogenase deficiency. Lancet, 371:64-74.

Diegues, A., Simões, P., Ceriz, T., Lopes, A.R., Tomé, E. (2022). Favism: A Case Report. Cureus.14(3): e23269.

Doxiadis, S.A., Fessas, P., Valaes, T. (1961). Glucose-6-phosphate dehydrogenase deficiency. A new aetiological factor of severe neonatal jaundice Lancet, 1 (7172), 297-301.

Garcia, C.S.C., Menti C., Lambert, A.P.F. (2016). Pharmacological perspectives from Brazilian Salvia officinalis (Lamiaceae): antioxidant, and antitumor in mammalian cells. An Acad Bras Ciênc, 88:281–292.

Joujeh, R., Salim, Z., Sobhi, M. (2020). Reduction of oxidative stress of normal and G6PD enzyme-deficient human erythrocytes by Centaurea ammocyanus extract (in vitro study). Pharm Sci Asia, 7 (2), 113-120.

Kandil, H.H., Al-Ghanem, M.M., Sarwat, M.A., Al-Thallab, F.S. (1996). Henna (Lawsonia inermis Linn.) inducing haemolysis among G6PD-deficient newborns. A new clinical observation. Ann Trop Paediatr, 16: 287–91.

Katar, S., Devecioglu, C., Ozbek, M.N., Ecer, S. (2007). Henna causes life-threatening hyperbilirubinaemia in glucose-6-phosphate dehydrogenase deficiency. Clin Exp Dermatol, 32: 235–6.

Kattamis, C.A., Kyriazakou, M., Chaidas, S. (1969). Favism: clinical and biochemical data. J Med Genet, 6(1):34-41.

Kaviarasan, S., Ramamurty, N., Gunasekaran, P., Varalakshmi, E., Anuradha, C.V. (2006). Fenugreek (Trigonella foenum graecum) seed extract prevents ethanol-induced toxicity and apoptosis in Chang liver cells. Alcohol Alcohol, 41:267–273.

Kawabata, T., Cui, M.Y., Hasegawa, T., Takano, F., Ohta, T. (2011). Anti-inflammatory and anti-melanogenic steroidal saponin glycosides from Fenugreek (Trigonella foenum-graecum L.) seeds. Planta Med, 77:705–710.

Ko, C.H., Li, K., Ng, P.C. (2006). Pro-oxidative effects of tea and polyphenols, epigallocatechin-3-gallate and epigallocatechin, on G6PD-deficient erythrocytes in vitro. Int J Mol Med, 18:987–994.

Kok, A.N., Ertekin, M.V., Ertekin, V., Avci, B. (2004). Henna (Lawsonia inermis Linn.) induced haemolytic anaemia in siblings. Int J Clin Pract, 58: 530–2.

Ko, R.J. (1999). Causes, epidemiology, and clinical evaluation of suspected herbal poisoning. J Toxicol Clin Toxicol, 37:697–708. doi: 10.1081/CLT-100102447.

Lai, S.W., Chen, J.H., Kao, W.Y. (2013). Acute hemolytic anemia in glucose-6-phosphate dehydrogenase deficiency complicated by Ginkgo biloba. Acta Haematol, 130(4):288-90.

Lamabadusuriya, S.P., Jayantha, U.K. (1994). Acalypha indica Induced haemolysis in G6PD deficiency. Ceylon Med J, 39: 46–7.

Lee, S.W., Lai, N.M., Chaiyakunapruk, N., Chong, D.W. (2017). Adverse effects of herbal or dietary supplements in G6PD deficiency: a systematic review. Br J Clin Pharmacol. 83(1):172-179.

Lim, F., Vulliamy, T., Abdalla, S.H. (2005). An Ashkenazi Jewish woman presenting with favism. J Clin Pathol, 58:317–319.

Longo, L., Vanegas, O.C., Patel, M. (2002). Maternally transmitted severe glucose 6-phosphate dehydrogenase deficiency is an embryonic lethal EMBO J, 21 (16), 4229-4239.

Mallvadhani, U.V., Panda, A.K, Rao, Y.R. (1998). Pharmacology and chemotaxonomy of Diospyros. Phyto Med, 49: 901-951.

Nam, S., Smith, D.M, Dou, Q.P. (2001). Ester bond-containing tea polyphenols potently inhibit proteasome activity in vitro and in vivo. J Biol Chem, 276: 13322-13330.

Narasimhan, D., Kumar, S., Murali, A., Satish, M., Mambatta, A. (2014). Acute intravascular hemolysis triggered by herbal remedy. J Forensic Toxicol Pharmacol. 3: 1.

Panizon, F. (1960). Erythrocyte enzyme deficiency in unexplained kernicterus [letter] Lancet, 2 (7159), 1093.

Pandolfi, P.P., Sonati, F., Rivi, R., Mason, P., Grosveld, F., Luzzatto, L. (1995). Targeted disruption of the housekeeping gene encoding glucose 6-phosphate dehydrogenase (G6PD): G6PD is dispensable for pentose synthesis but essential for defense against oxidative stress EMBO J, 14 (21), 5209-5215.

Pawlikowska-Pawlega, B., Gruszecki, W.I., Misiak, L.E., Gawron A. (2003). The study of the quercetin action on human erythrocyte membranes. Biochem Pharmacol, 66:605-612.

Perry, E.K., Pickering, A.T., Wang, W.W., Houghton, P.J., Perry, N.S. (1999). Medicinal plants and Alzheimer's disease: from ethnobotany to phytotherapy. J Pharm Pharmacol, 51:527–534.

Phua, D.H., Zosel, A., Heard, K. (2009). Dietary supplements and herbal medicine toxicities-when to anticipate them and how to manage them. Int J Emerg Med, 2(2):69-76.

Raupp, P., Hassan, J.A., Varughese, M., Kristiansson, B. (2001). Henna causes life threatening haemolysis in glucose-6-phosphate dehydrogenase deficiency. Arch Dis Child, 85: 411–2.

Robaszkiewicz, A., Balcerczyk, A., Bartosz, G. (2007). Antioxidative and prooxidative effects of quercetin on A549 cells. Cell Biol Int, 31:1245-1250.

Sabeti, H. (1997). Forest, Trees and Shrubs of Iran. Yazd. University. Publishers, 806: 313-315 (Persian).

Sadler, J.M., Ash, S., & Brito-Babapulle, F. (2009). Possible fenugreek induced haemolysis in a patient with previously unknown G6PD deficiency. BMJ Case Reports, 2009(2009), no. pag.

Sansone, G., Segni, G. (1958). New aspects of the biochemical alterations in the erythrocytes of patients with favism; almost complete absence of glucose-6-phosphate dehydrogenase [in Italian] Boll Soc Ital Biol Sper, 34 (7), 327-329.

Sathya, M., Kokilavani, R., and Ananta Teepa, K. (2012). Acute and subacute toxicity studies of ethanolic extract of Acalypha indica Linn in male Wistar albino rats. Asian J Pharm Clin Res 5, 97-100.

Schuurman, M., van Waardenburg, D., Da Costa, J., Niemarkt, H., Leroy, P. (2009). Severe hemolysis and methemoglobinemia following fava beans ingestion in glucose-6-phosphatase dehydrogenase deficiency: case report and literature review. Eur J Pediatr. 168:779–782.

Schick, P. (2017). Glucose-6-phosphate dehydrogenase deficiency. Medscape, Schick P.

Seebaluck, R., Gurib-Fakim, A., Mahomoodally, F. (2015). Medicinal plants from the genus Acalypha (Euphorbiaceae)–a review of their ethnopharmacology and phytochemistry. J Ethnopharmacol, 159: 137–57.

Senanayake, N., Sanmuganathan, P.S. (1996). Acute intravascular haemolysis in glucose-6-phosphate dehydrogenase deficient patients following ingestion of herbal broth containing Acalypha indica. Trop Doct, 26: 32.

Sellahewa, K.H. (1992). Clinical study of intravascular haemolysis at Anurahapura. Sri Lanka Medial Association Annual Session, 20.

Seyedzadeh, A., Hemmati, M., GCheiny, S. (2007). Henna induced severe hemolysis in glucose-6-phosphate dehydrogenase deficiency. Pak J Med Sci, 23: 119–21.

Szeinberg, A., Sheba, C., Adam, A. (1958). Enzymatic abnormality in erythrocytes of a population sensitive to Vicia faba or haemolytic anemia induced by drugs Nature, 181 (4618), 1256.

Vayalil, P.K., Mittal, A., Hara, Y., Elmets, C.A, Katiyar, S.K. (2004). Green tea polyphenols prevent ultraviolet light-induced oxidative damage and matrix metalloproteinases expression in mouse skin. J Invest Dermatol, 122: 1480-1487.

Warburg, O and Christian, W. (1932). Über ein neues oxydationsferment und sein absorptionsspektrum. Biochem. Z. 254, 438-458.

WHO: National policy on traditional medicine and regulation of herbal medicines. In, 2005.

WHO Working Group. Glucose6-phosphate dehydrogenase deficiency. Bull World Health Organ. (1989). 67:601-11.

Zinkham, W.H., Oski, F.A. (1996). Henna: a potential cause of oxidative hemolysis and neonatal hyperbilirubinemia. Pediatrics, 97: 707–9.

Khazar Journal of Science and Technology
Khazar University Press 2022
Volume 7 №1 2023, 44-53
DOI: 10.5782/2520-613. 202.7.1.44

Dynamics of Changes in the Amount of Photosynthetic Pigments in Bean Seedlings Under NaCl and Na_2SO_4 Salinity Conditions

Jafarzade B.A
Zahid Khalilov st.23, Baku, Azerbaijan
Baku State University
cxanum@mail.ru

Abstract

One of the most important problems facing humanity currently is soil salinization. Excessive salt concentration in the soil is considered one of the main environmental factors limiting plant growth and productivity. The effect of different concentrations of NaCl and Na_2SO_4 in water (10-30mM) and soil (0.2-0.6%) cultures on the morphometric parameters of bean seedlings sensitive to salinity and the dynamics of changes in the amount of photosynthetic pigments (chl a, chl b, and carotenoids) in the initial stage of ontogenesis have been studied.

The amounts of photosynthetic pigments were found to decrease sharply in 7-and 14-day-old bean seedlings with enhancing NaCl and Na_2SO_4 concentrations (10-30mM). Compared to carotenoids, the decrease in the amount of chlorophylls (chl a, chl b) was sharper. It should be noted that under the influence of both salts, the decrease in the water culture was twice as much as in the soil culture.

Keywords: salinity, culture, photosynthetic pigments, chlorophyll, carotenoid, morphological traits, germination percentage

Introduction

Soil salinization is one of the most important problems facing humanity in modern times. Excess salt in the soil is considered one of the main environmental factors limiting plant growth and productivity (Flowers, 2008, 2004). According to various estimates, 15-23% of the total area of Earth, including agricultural land, is saline soils (Khitrov, 2009, Gamalero, 2009).

Saline soils have become a growing problem of irrigated agricultural lands (Kovda, 2008). According to available data, 50% of soils may be subject to salinization by 2050 (Ashraf, 1994).

From a chemical point of view, the type of salinity is determined mainly by the amount of anions in the soil, and therefore, it is divided into chloride, sulfate, chloride-sulfate, and carbonate salinization (Pilipenko, 2005). Chloride-sulfate salinization type is accounted for more than 50% of the area of saline soils (Khitrov, 2009). Since all the listed salts are well soluble in water, they are usually washed out of the soil by atmospheric precipitation in humid climates and remained in very small quantities. In a dry or hot climate, however, washing with rainwater does not occur, on the contrary, salt solutions accumulate in the upper layers of the soil with the rising flow of soil water. Water evaporates and salts remain in the upper layer of the soil (Ivanischev, 2020).

Salinity, which is one of the signs of soil degradation, significantly reduces its fertility (Isanova, 2017), as well as makes it difficult for plants to absorb macro and micronutrients and causes mineral starvation.As a result, the sensitivity of plants to pathogens and pests increases and it ultimately leads to a decrease in their ability to survive. This determines the relevance and importance of studying the problem of salinity and the effect of this factor on the growth and development of plants (Ivanischev, 2020).

Currently, there is a general opinion on the toxicity of the Na^+ ion and its negative effect on plants (Flowers, 2008).

To clarify the mechanism of action of Na salts on the plant organism, it is important to study the physiological processes in the first stages of the development of seedlings. The initial physical and chemical processes occurring in plant cells in the first stages of ontogenesis have a serious effect on the subsequent course of intracellular metabolism.

Thus, salinity has a negative effect on the formation of morphological traits of bean seedlings and reduces seed germination.

It is necessary to choose more tolerantto negative factors varieties at the initial stages of development, at the stage of sprout formation. According to some authors, it is possible to combine characteristics such as salt tolerance and productivity in one plant, that is, to grow high-yielding varieties (Jhuchenko, 1994; Kumakov, 1995).

Therefore, the effect of different concentrations of Na salts on the seed germination

percentage, growth indicators, and the dynamics of changes in the amount of photosynthetic pigments was studied.

The data presented on the importance and urgency of solving the global soil salinity problem and its impact on plant growth, development, and defense mechanisms are directly related to providing food products to the world population.

Materials and methods

The bean plant (local Piyada variety), which is widely used in agriculture in our Republic, waschosen as the research object. Bean is a protein-rich dicotyledonous plant belonging to the leguminous family (lat. Phaseolus). It occupies an important place among vegetable crops, both in terms of its nutritional value and itsuse extent. Technically ripe pods of beans contain 14.0% dry matter, including 6% protein, 4% nitrogen substances, 4-6% carbohydrates, 2.9% sugar, 1% cellulose, and 0.7% mineral substances. Dry seeds contain about 30% proteins.

Seed germination was carried out in the water (Knop's solution) and soil cultures under conditions of 20^0C and normal aeration in a special chamber with 420 lx of illumination. In experimental variants, 10, 20, and 30 mM NaCl and Na_2SO_4 were added to Knop's solution in the water culture.In the soil culture, 0.2% (1 g of salt per 500 g of soil), 0.4% (1 g of salt per 500 g of soil), and 0.6 % (1 g of salt per 500 g of soil) saline environments were created.

The germination percentage is determined based on the number of 5-day-old seedlings.

The amount of pigments was determined spectrophotometrically at wavelengths of 440.5, 644, and 662 nm after crushing leaves in the acetone solution.The amounts of chlorophyll a (Chl a), chlorophyll b (chl b), and carotenoids (Car) were estimated using the formula of HolmWettstein (Tretyakova, 1990):

$$\text{Chl a} = 9.784 \times D662 - 0.990 \times D644 \ (\text{ml}\backslash\text{l})$$
$$\text{Chl b} = 21.426 \times D644 - 4.650 \times D662 \ (\text{ml}\backslash\text{l})$$
$$\text{Ccar} = 4.695 \times D440.5 - 0.268(a+b) \ (\text{ml}\backslash\text{l})$$
and
$$C_0 = C \times V \times V_2 / (m \times V_1 \times 10) \ (\text{mg}\backslash 100\text{g}).$$

Where, C is the concentration of pigments, mg/l; V - volume of initial extract, ml; V_1 - volume of extract used for dilution, ml; V_2 – volume of the extract added to the

spectrophotometric cuvette, ml; m is the weight of the leaf used for the determination.

The studies were carried out with 3-4 replications. The obtained results were processed statistically (Lakin, 1990), and the error did not exceed 5%.

Results and Discussion

The negative effect of high salinity on plants is manifested starting from the first stages of development. Thus, the first stages of ontogenesis are more sensitive to extreme effects due to high metabolic activity.

To study the effect of NaCl and Na_2SO_4 on the germination energy of seeds, the germination percentage of the seeds used in the study was determined. For this purpose, the number of germinated seeds in 5 days was found. According to the obtained results,the isocation salts of Naaffect seed germination negatively. It is clear from the curves reflecting the effect of different concentrations of these saltson the germination percentage of bean seeds(Figures 1 and 2).

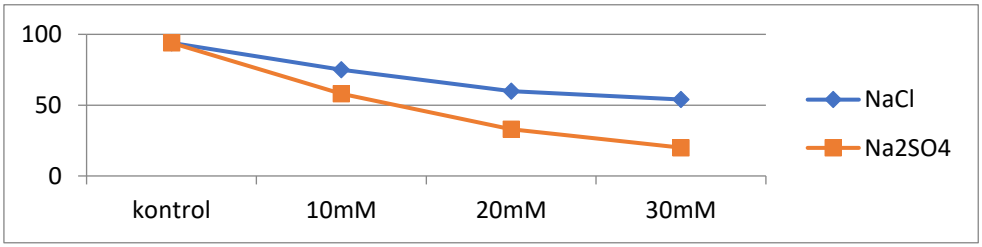


Figure1. Germination percentage of bean seeds at different concentrations of NaCl and Na_2SO_4 in water culture

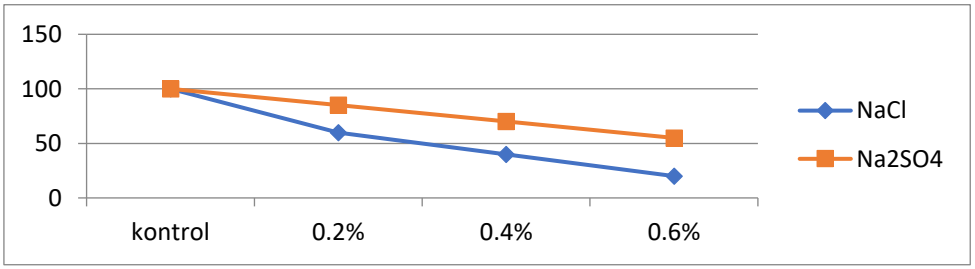


Figure 2. Germination percentage of bean seeds at different concentrations of NaCl and Na_2SO_4 in soil culture

As can be seen, the germination percentage of seeds decreased in both water (10-30mM) and soil cultures (0.2-0.6%) with the enhancing concentrations of sodium salts.

Although Na_2SO_4 has a stronger effect on the germination percentage of bean seeds than NaCl in water culture, the opposite picture is observed in the soil culture. Thus, the germination percentage decreased more with the increasing NaCl concentrations.

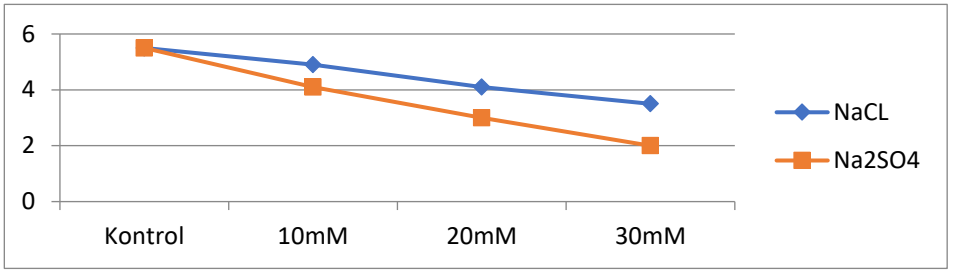


Figure3. The height (cm)of 5-day-old bean seedlings germinated in water culture at different concentrations of NaCl and Na_2SO_4

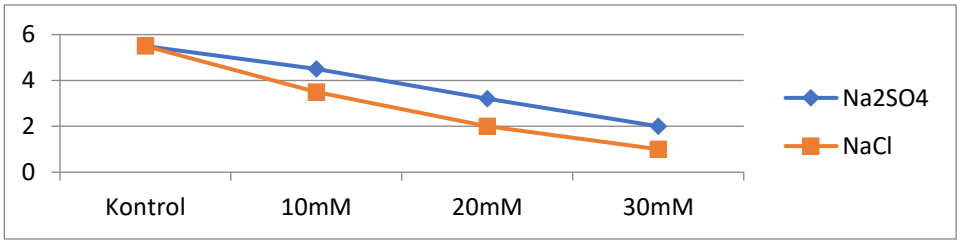


Figure 4. The height (cm)of 5-day-old bean seedlings germinated in soil culture at different concentrations of NaCl and Na_2SO_4

Salt stress also affects the growth parameters of 5-day-old seedlings of bean plants cultivated in water and soil cultures. Thus, under the effect of both salts, a significant reduction in the studied morphological parameters occurred in both water and soil cultures. According to literature data, salinity has a negative effect on the formation of morphological indicators of the plant and reduces seed germination (Besaliyev, 2021).

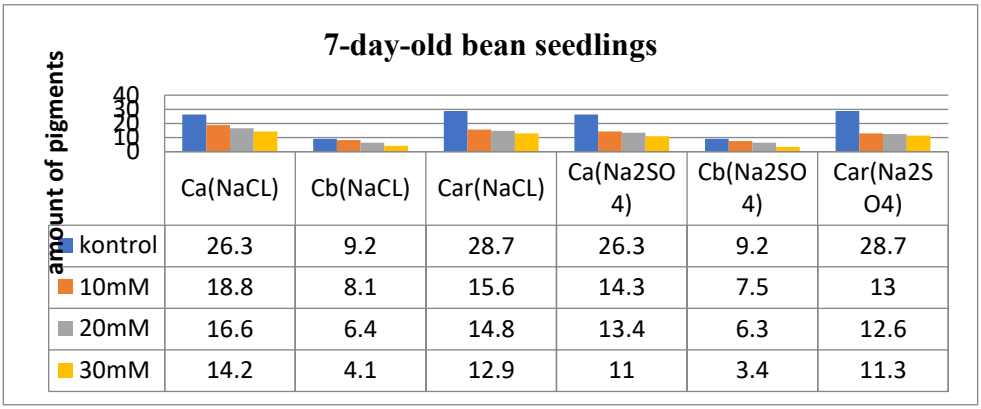
It should be noted that Na_2SO_4 salinity in water culture especially reduces the growth indicators. As seen in Figure 3, in water culture, SO^{4-} ion affects the growth indicators of the bean plant more than Cl^-, while in soil culture, the opposite picture is observed (Figure 4).

It is known from the literature that stress factors, including salt stress, reduce the intensity of photosynthesis in plants (Li, 2010). The excess amount of Na^+,SO_4^-, and Cl^-ions in the cell affects the photosynthesis pigments, causing the disintegration of

the granules, the disruption of carbon metabolism, and the photophosphorylation processes.

Since the ability of plants to tolerate salt depends on the intensity of photosynthesis, the determination of photosynthesis pigments in plants affected by salt stress is considered one of the important criteria. It should be noted that there are conflicting opinions in the literature about the salt-induced changes in the amount of photosynthetic pigments in plants (Li, 2010). Thus, according to some scientists, the amount of photosynthetic pigments in plants decreases under salinity conditions (Garifzyanov, 2006; Strogonov, 1970), while others stated that it increases or is not changed (Kusakina, 2003; Udovenko, 1977).

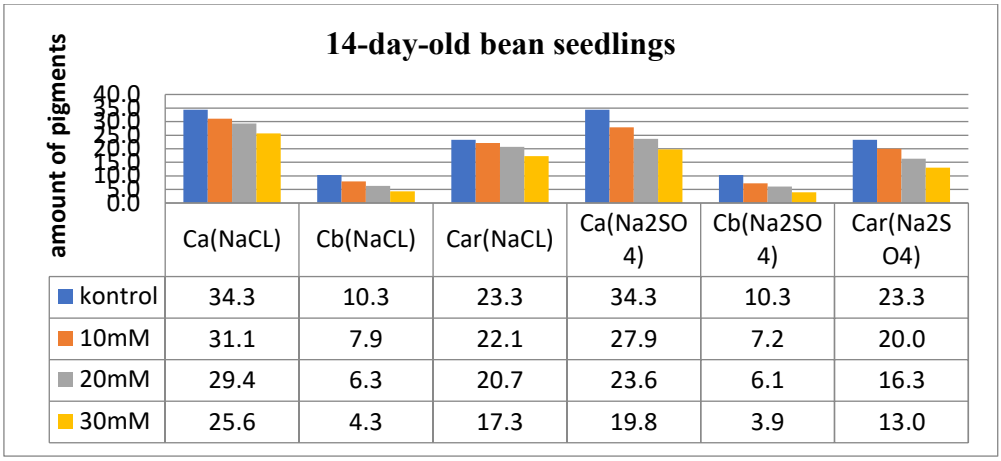
Our research revealed that the amount of pigments decreased sharply in 7-day-old bean seedlings grown in water culture with the enhancing(10-30 mM) concentrations of both salts (NaCl, Na_2SO_4). Thus, under the influence of 30 mM NaCl, the amount of chla decreased approximately 1.8-fold, the amounts of chl-b and carotenoids decreased 2.2 fold,while 30 mM Na_2SO_4 caused 2.3, 2.7, and 2.5-fold decreases in the amountsof chl a, chl b, and carotenoids, respectively (Figure 5).



	Ca(NaCL)	Cb(NaCL)	Car(NaCL)	Ca(Na2SO4)	Cb(Na2SO4)	Car(Na2SO4)
kontrol	26.3	9.2	28.7	26.3	9.2	28.7
10mM	18.8	8.1	15.6	14.3	7.5	13
20mM	16.6	6.4	14.8	13.4	6.3	12.6
30mM	14.2	4.1	12.9	11	3.4	11.3

Figure 5. Amounts of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids (Car) in 7-day-old bean seedlings grown in water culture

In 14-day-old bean seedlings, the amount of pigments decreased withincreasing salt concentrations (10-30 mM) (Figure 6). Thus, 30 mM NaCl caused approximately 1.3, 2.3, and 1.3-fold decreases in Chl a, Chl b, and carotenoids, respectively. Whereas, at 30 mM Na_2SO_4, the same parameters decreased 1.7, 2.6, and 1.7-fold, respectively.

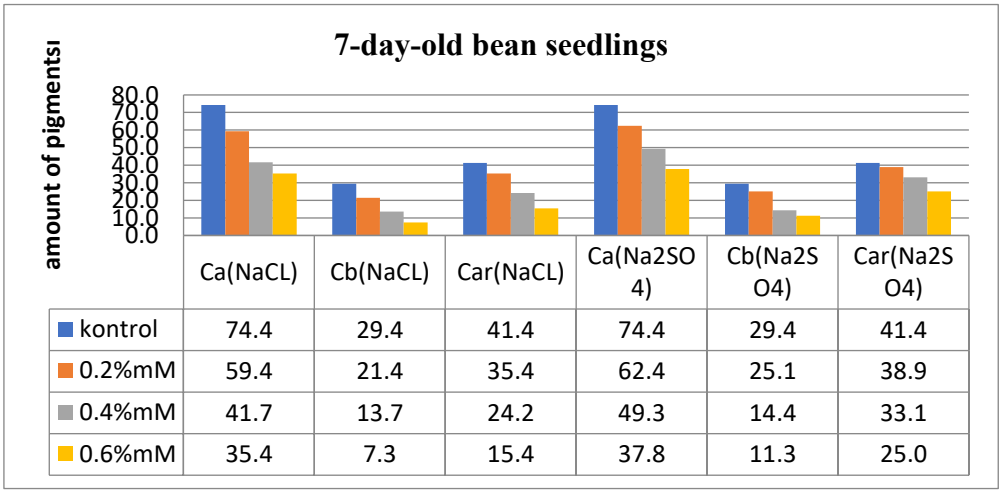


	Ca(NaCL)	Cb(NaCL)	Car(NaCL)	Ca(Na2SO4)	Cb(Na2SO4)	Car(Na2SO4)
kontrol	34.3	10.3	23.3	34.3	10.3	23.3
10mM	31.1	7.9	22.1	27.9	7.2	20.0
20mM	29.4	6.3	20.7	23.6	6.1	16.3
30mM	25.6	4.3	17.3	19.8	3.9	13.0

Figure 6. Amounts of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids (Car) in 14-day-old bean seedlings grown in water culture

It should be noted that although both salts reduced the amount of pigments, the negative effect of Na_2SO_4 was higher: $SO_4^- > Cl^-$

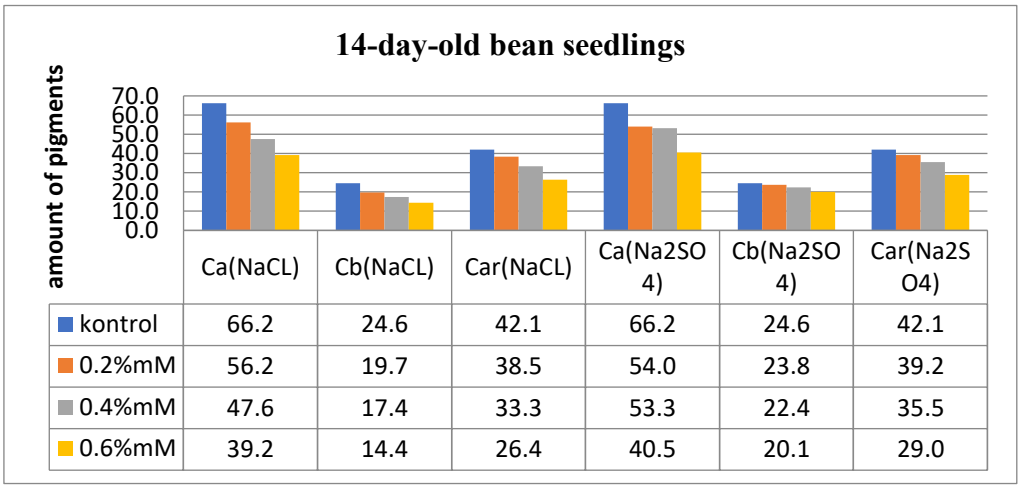
A similar situation is manifested in soil culture. The amount of pigments decreased sharply with increasing concentrations of NaCl and Na_2SO_4(10-30mM). At 30 mM NaCl, Chl a, Chl b, and carotenoids decreased approximately 2.1, 4.0, and 2.6-fold, respectively, in 7-day-old bean seedlings.While at 30 mM Na_2SO_4, the respective parameters decreased 1.9, 2.6, and 1.6-fold (Figure 7).



	Ca(NaCL)	Cb(NaCL)	Car(NaCL)	Ca(Na2SO4)	Cb(Na2SO4)	Car(Na2SO4)
kontrol	74.4	29.4	41.4	74.4	29.4	41.4
0.2%mM	59.4	21.4	35.4	62.4	25.1	38.9
0.4%mM	41.7	13.7	24.2	49.3	14.4	33.1
0.6%mM	35.4	7.3	15.4	37.8	11.3	25.0

Figure7. Amounts of chlorophyll a (Cl a), b (Cl b), and carotenoids (Car) in 7-day-old bean seedlings grown in soil culture.

In 14-day-old seedlings, 30 mM NaCl caused approximately 1.6, 1.7. and 1.5-fold decrease in the amounts of Chl a, Chl b, and carotenoids, respectively. While at 30mM Na_2SO_4, these parameters decreased 1.6, 1.2, and 1.4-fold, respectively (Figure 8).



	Ca(NaCL)	Cb(NaCL)	Car(NaCL)	Ca(Na2SO4)	Cb(Na2SO4)	Car(Na2SO4)
kontrol	66.2	24.6	42.1	66.2	24.6	42.1
0.2%mM	56.2	19.7	38.5	54.0	23.8	39.2
0.4%mM	47.6	17.4	33.3	53.3	22.4	35.5
0.6%mM	39.2	14.4	26.4	40.5	20.1	29.0

Figure8. Amounts of chlorophyll a (Cl a), b (Cl b), and carotenoids (Car) in 14-day-old bean seedlings grown in soil culture.

According to the obtained results, the inhibitory effect of different concentrations of the isocation salts(NaCl and Na_2SO_4) on the amount of photosynthetic pigments in 7- and 14-day-old bean seedlings was high in sulfate-type salinity in soil culture and chlorine-type salinity in water culture.

The amount of Chl a and Chl b decreased more in both water and soil cultures, under the effect of both salts.

High concentrations of Na^+ and Cl^- ions in soil culture cause osmotic stress in plants due to a sharp decrease in the water potential of the root environment, and their excessive entry into cells changes the ion balance and leads to the disruption of many physiological and biochemical processes (Munns, 2008**).**

It should be noted that a shortage of water does not occur in plants grown in water culture.

In general, the water potential in saline soil solutions is lower than the water potential of cells in plant roots. Water absorption by plants decreases sharply in saline soils (Kuznetsov, 2017). It should be noted that NaCl combines with soil particles and

accelerates water absorption in the soil. Thus, the potential of water in the soil solution is sharply reduced and a water deficit occurs in plants, as a result of which plant growth, development, photosynthesis, and other physiological processes weaken sharply.

References

Ashraf, M. (1994). Breeding for salinity tolerance in plants. Crit.Rev. Plant Sci., V.13,pp. 17-42

Besaliev, I.N., Panfilov, A.L., & Reger, N.S. (2021). Peculiarities of the formation of morphological traits of seedlings and the activity of spring common wheat enzymes against the background of induced salt stress. Animal Husbandry and Fodder Production. 104(3).

Flowers T. 2004. Improving crop salt tolerance. Journal of Experimental Botany Vol. 55. pp. 307-319.

Flowers, T.J., & Colmer T.D. (2008). Salinity tolerance in halophytes. New Phytol. 179. pp. 945–963.

Gamalero, E., Berta, G., & Bernard, R.G. (2009). The use of Microorganisms to Facilitate the Growth of Plants in Saline Soils. Microbial Strategies for Crop Improvement. Berlin Heidelberg. Springer. 1-22.

Garifzyanov, A.R., & Gorelova, S.V. (2006). Oxidative stress and plant tolerance. Tula Ecological Bulletin. Issue 2, 364–368.

Isanova, G.T. et. al. (2017). Saline soils and determination of the province of salt accumulation on the territory of Kazakhstan. Arid ecosystems. Vol. 23. No. 4(73). 35-43.

Ivanischev, V.V., Evgrashkina, T.N., Boykova, O.I., & Zhukov, N.N. (2020). Soil salinization and its effect on plants. No. 3, 28-42.

Khitrov, N.B., Rukhovich, D.I., Kalinina, N.V., Novikova, A.F., Pankova, E.I., & Chernousenko, G.I. (2009). Estimation of areas of saline soils on the territory of the European part of Russia (according to the electronic version of the map of soil salinity at a scale of 1:2.5 million) . Soil science. 6, p. 627.

Kovda, V.A. (2008). Problems of desertification and salinization of soils in arid areas of the world. M.: Nauka. p.415.

Kumakov, V.A. (1995). Physiology of Spring Wheat Yield Formation and Breeding Problems. Agricultural biology. 30(5), p. 56.

Kusakina, M.G., Yeremchenko, O.Z., & Chetina, O.A. (2013). Influence of technogenic salts on the pigment system of plants growing in the zone of influence of salt tailings piles. Protection of the natural environment and ecological and biological education: materials of the III All-Russian scientific-practical conference with international participation,Yelabuga, pp. 60–63.

Kuznetsov, V. V., & Dmitriyeva, G.A. (2017). Physiology of plants. Yurayt Publishing House. p. 437.

Lakin, G. F. (1990). Biometrics, M.: Visshaya shkola, p. 293.

Li, Z., Baldwin, C.M., Hu, Q., Liu, H., & Luo, H. (2010). Heterologous expression of Arabidopsis H+-pyrophosphatase enhances salt tolerance in transgenic creeping bentgrass (*Agrestisstolonifera* L) plant. Cell Environ. 33, 272-289

Munns, R., & Tester M. (2008). Mechanisms of salinity tolerance. Ann. Rev. Plant Biol. 59, 651–681.

Pilipenko, V.N., Yakovleva, L.V., & Fedotova, A.V. (2005). Current state of saline soils in the Volga delta . Fundamental research. No. 8, 145-149.

Strogonov, B.P. et al. (1970). Structure and functions of plant cells under salinity. M.: Nauka Publishing House, p.318.

Tretyakova, N.N. (1990). "Workshop on plant physiology" Moscow, 90 p.

Udovenko, G.V. (1977). Salt tolerance of cultivated plants. L.: Kolos. p.215.

Zhuchenko, A.A. (1994). Strategy for adaptive intensification of agriculture (concept). Pushchino: ONTI PNTs RAN, 148 p.

Khazar Journal of Science and Technology
Khazar University Press 2022
Volume 7 №1 2023, 54-60
DOI: 10.5782/2520-613.202.7.1.54

Associative Infection of Cattle with Eimeriosis and Paramphistomatosis

Mahir Nasibov
Azerbaijan Veterinary Scientific Research Institute
E-mail: mahirnasibov.64@gmail.com

Abstract

The article talks about the research works on associative paramphistomatosis and eimeriosis carried out in individual livestock farms of Khachmaz, Siyazan, Shabran districts of Guba-Khachmaz economic district. The extensiveness and intensity of infection with paramphistomatosis and eimeriosis were determined during the scatological examinations conducted in farms. During the examinations in the farms of Khachmaz district, infections with paramphistomatosis were detected in 1-3-month-olds 36.4%, in 6-9-month-olds 45.0%, in 10-12-month-olds 33.3, and in adult animals 21.7%, infection with eimeriosis in 1-3-month-olds 54.5%, in 6-9-month-olds 50.0%, in 10-12-month-olds 28.6%, and in adult animals 13.0, in farms of Siyazan district infection with paramphistomatosis in 1-3-month-olds 30.4%, in 6-9-month-olds 38.1%, in 10-12-month-olds 30.0%, and in adult animals 17.4%, infection with eimeriosis in 1-3-month-olds 43.5%, in 6-9-month-olds 33.3%, in 10-12-month-olds 25.0%, and in adult animals4,3%; In the farms of the Shabran district, infection with paramphistomatosis was detected in 1-3-month-olds 15.0%, in 6-9-month-olds 18.2%, in 10-12-month-olds 14.3%, in adult animals 9.1%, infection with eimeriosis in 1-3-month-olds 10.0%, in 6-9-month-olds 13.6%, in 10-12-month-olds 9.5%, and in adult animals 4.5%.

Thus, summarizing the results of the scatological examinations conducted, we come to the conclusion that throughout the Khachmaz district, associative infection (extensiveness) with paramphistomatosis was established as 33.7%, with eimeriosis 36.0%, throughout Siyazan district with paramphistomatosis as 28.7%, with eimeriosis 26.4%, throughout Shabran district with paramphistomatosis as 14.1%, with eimeriosis 9.4%. During autopsy examinations, the intensity of infection with paramphistomatosis of cattle in the farms of Khachmaz district was 7-23 specimens,

in animals throughout the Siyazan district 4-11 specimens, and in animals in Shabran district was 2-7 specimens.

Keywords: cattle, eimeriosis, paramphistomatosis, farm, associative infection, scatological examination, autopsy examination

Introduction

To the development of animal husbandry, including cattle breeding, is negatively affected by many parasitic diseases (eimeriosis, paramphistomatosis). In this regard, the study of diseases that cause economic damage to cattle breeding, both primary intestinal parasites and helminthiasis, stands out for its topicality. Spreading widely, parasitic diseases negatively affect the development of animals (most often calves and heifers). From this point of view, it is necessary to identify parasites (Artemenko, 1971; Beyer, 1989; Yerbolatov, 1982; Musaev, Surkova, Gaibova, 1986). Against parasitic diseases, scientists from both foreign countries and our republic have carried out large-scale research work, which continues to this day (Berdnikov, 1978; Veselova, Doroshina, Arkhipov, 1987; Yerbolatov, 1977; Musaev, Manafova, 1983; Svanbaev, 1972).

The parasitic fauna of cattle is influenced by many factors, which positively affects their development and leads to the spreading of mixed (associative) invasions that cause economic damage to farms (Denev, co-authors, 1982; Deusov, Podberezsky, Gevedze, 1960; Orlovsky, 1984; Rekhviashvili, 2001). In this regard, the foundation is being laid for the detection of parasitic diseases in animals and testing of new generation preparations against parasitosis (Velichko, 1971; Lyusin, 2019; Oparin, 1985).

Therefore, we set a goal to study both protozoan intestinal parasites (eimeriosis) and helminthiasis (paramphistomatosis) in the cattle-breeding farms.

Materials and methods

Research work was carried out in 2022 in the laboratory of the Department of Parasitology of the Veterinary Scientific Research Institute on the basis of pathological materials (fecal samples) collected in order to study the dynamics of infection with parasitic diseases of animals of different ages in individual cattle-breeding farms of Khachmaz, Siyazan, Shabran districts throughout the Guba-Khachmaz economic district. 86 pathological materials were examined in the

Khachmaz district, 87 in the Siyazan district, 85 in the Shabran district, then the results were analyzed and the extensiveness was identified. In order to determine the intensity of infection with helminthiasis after slaughtering animals at the points for the slaughter of livestock according to each district, a study was conducted during the autopsy in the internal organs (intestines, rumen) of 6 head of animals.

Results and discussion

For the purpose of determintion the dynamics of associative infection of cattle with paramphistomatosis and eimeriosis in the Guba-Khachmaz economic district in the spring, scatological examinations were conducted on pathological materials (fecal samples) brought from farms.

During the examinations in the farms of Khachmaz district, infections with paramphistomatosis were detected in 1-3-month-olds 36.4%, in 6-9-month-olds 45.0%, in 10-12-month-olds 33.3, and in adult animals 21.7%, infection with eimeriosis in 1-3-month-olds 54.5%, in 6-9-month-olds 50.0%, in 10-12-month-olds 28.6%, and in adult animals 13.0, in farms of Siyazan district infection with paramphistomatosis in 1-3-month-olds 30.4%, in 6-9-month-olds 38.1%, in 10-12-month-olds 30.0%, and in adult animals 17.4%, infection with eimeriosis in 1-3-month-olds 43.5%, in 6-9-month-olds 33.3%, in 10-12-month-olds 25.0%, and in adult animals4,3%; In the farms of the Shabran district, infection with paramphistomatosis was detected in 1-3-month-olds 15.0%, in 6-9-month-olds 18.2%, in 10-12-month-olds 14.3%, in adult animals 9.1%, infection with eimeriosis in 1-3-month-olds 10.0%, in 6-9-month-olds 13.6%, in 10-12-month-olds 9.5%, and in adult animals 4.5% (Table 1).

Thus, summarizing the results of the scatological examinations conducted, we come to the conclusion that throughout the Khachmaz district, associative infection (extensiveness) with paramphistomatosis was established as 33.7%, with eimeriosis 36.0%, throughout Siyazan district with paramphistomatosis as 28.7%, with eimeriosis 26.4%, throughout Shabran district with paramphistomatosis as 14.1%, with eimeriosis 9.4%.

Table 1. Infection rate of cattle with paramphistomatosis and eimeriosis (in%)

Age of animals	Examined	With paramphistomatosis		With eimeriosis	
		Infected	Invasion extensiveness	Infected	Invasion extensiveness

Khacmaz district					
1-3-month-olds	22	8	36,4	12	54,5
6-9-month-olds	20	9	45,0	10	50,0
10-12-month-olds	21	7	33,3	6	28,6
The adults	23	5	21,7	3	13,0
In total	86	29	33,7	31	36,0
Siyazan district					
1-3-month-olds	23	7	30,4	10	43,5
6-9-months-olds	21	8	38,1	7	33,3
10-12-months-olds	20	6	30,0	5	25,0
The adults	23	4	17,4	1	4,3
In total	87	25	28,7	23	26,4
Shabran district					
1-3-month-olds	20	3	15,0	2	10,0
6-9-month-olds	22	4	18,2	3	13,6
10-12-month-olds	21	3	14,3	2	9,5
The adults	22	2	9,1	1	4,5
In total	85	12	14,1	8	9,4

On the territory of Khachmaz, Siyazan, Shabran districts of Guba-Khachmaz economic district, an incomplete helminthological examination was carried out during autopsy on animals slaughtered at the slaughterhouses, located on the territories of the above-mentioned districts. When examined during autopsy, the intensity of infection with paramphistomatosis of cattle in the farms of Khachmaz district was 7-23 specimens, of animals in Siyazan district 4-11 specimens, and animals in Shabran district 2-7 specimens.

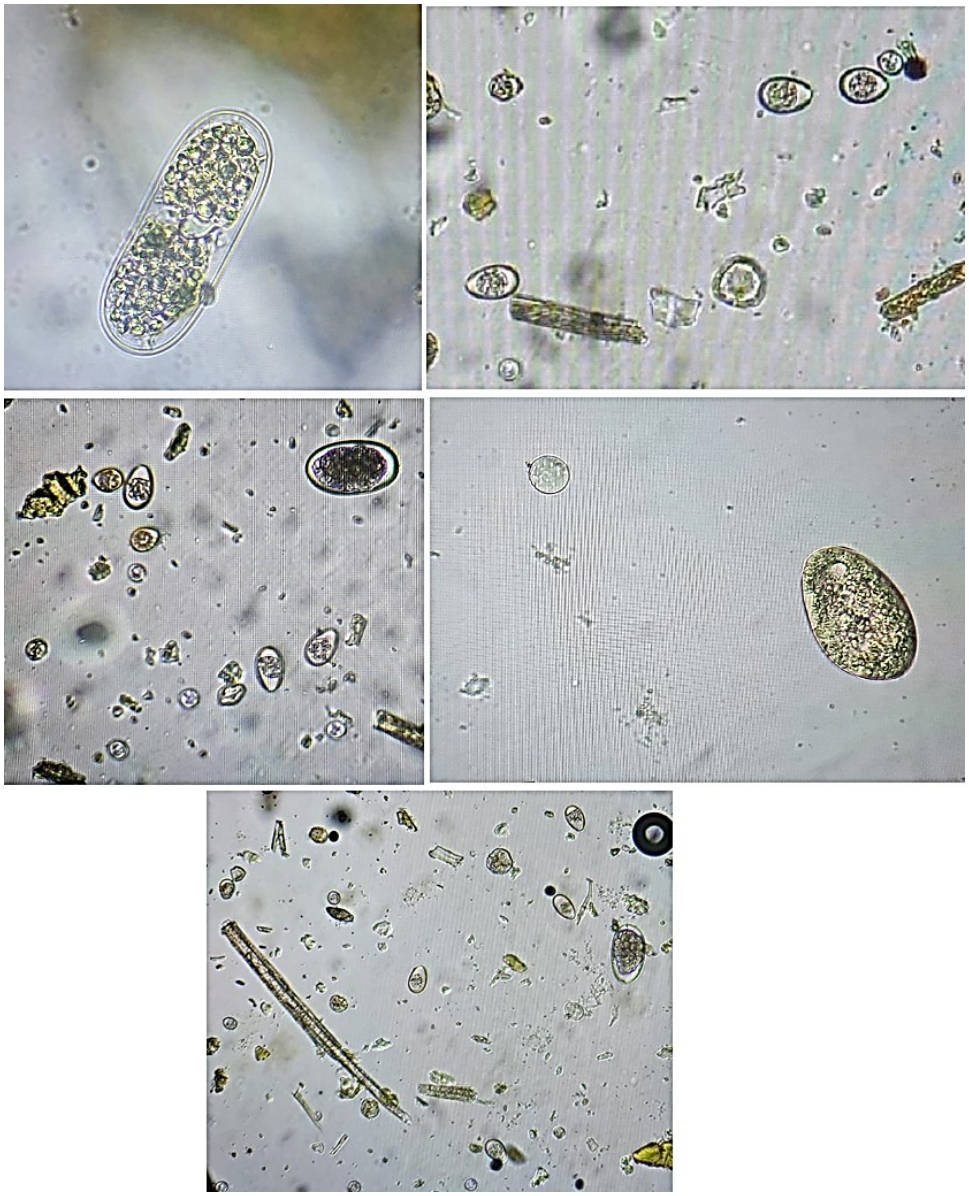


Figure 1. Causative agents of eimeriosis and paramphistomatosis in cattle

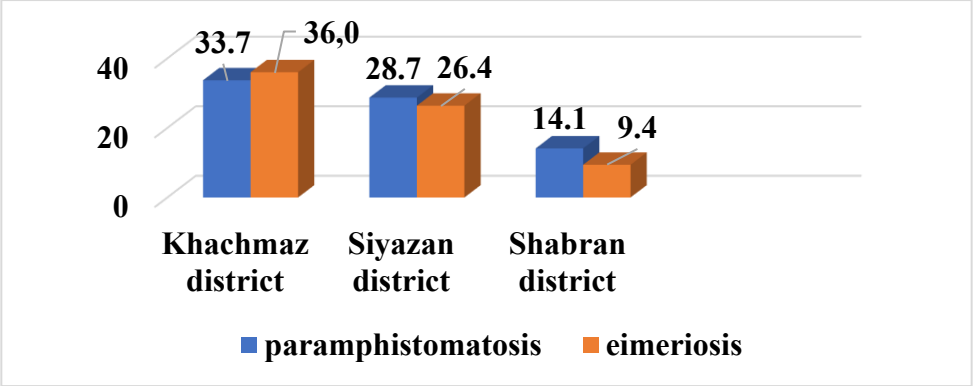


Figure 2. Infection rate of cattle with paramphistomatosis and eimeriosis

So, in the presence of favorable conditions and as a result of the development and achievement of the invasive stage of intestinal parasites, including helminth eggs and oocysts of eimeriosis, animals become infected. It should be noted that both the constant presence of intermediate hosts in favorable conditions leads to infection of cattle with paramphistomatosis, and the oocysts of eimeria, forming spores, acquire the ability to infect.

Conclusion

The extensiveness of associative infection throughout the Khachmaz district, with paramphistomatosis 33.7%, eimeriosis 36.0%, throughout the Siyazan district with paramphistomatosis 28.7%, with eimeriosis 26.4%, and throughout the Shabran district with paramphistomatosis 14.1%, with emeriosis 9.4% was identified. During the autopsy examinations, the intensity of infection with paramphistomatosis of cattle in the farms of Khachmaz district was 7-23 specimens, in animals throughout the Siyazan district 4-11 specimens, and in animals in Shabran district was 2-7 specimens.

References

Artemenko, Y.G. (1971). Paramphistomatosis of cattle in Ukraine. .Collection of works on helminthology. Publishing house "Kolos" M. 217-222.

Berdnikov P. (1978). Evaluation of pastures during paramphistomatosis. Veterinary medicine. No. 10, p. 13-14.

Beyer, T.V. (1989). Cell biology of sporozoa – pathogens of protozoal diseases of animals and humans. Leningrad: Nauka, p. 184.

Denev, I. et al. (1982). Economic damage in case of paramphistomatosis of animals..Veterinary medicine. No. 5, 12-13.

Deusov, N.J. L., Podberezsky, K.N., & Gevedze, K.I. (1960). Double invasion of young cattle with paramphistomatosis and fascioles. Veterinary medicine. No. 4, 24-26.

Lyusin, E. A. (2019) Prevention and treatment of bovine eimeriosis. Agricultural science. 7(8): 22-23.

Musaev, M. A., & Manafova, Sh.G. (1983). Age and seasonal dynamics of coccidiosis invasion and fauna of coccidia of cattle on Absheron .Information of AS of Azerbaijan SSR, series of biological sciences, No. 1, p. 56-58.

Musaev, M. A., Surkova, A. M., & Gaibova, G. D. (1986). Infection with eimeria of thoroughbred cattle imported to East Azerbaijan . Information of AS of Azerbaijan SSR, series of biological sciences, No. 5, p.35-40.

Oparin, P.G. (1985). Paramphistomatosis of cattle and sheep. In psalterium: Gastrointestinal trematodes of ruminants. M. "Agropromizdat" p.82-96.

Orlovsky, V. I. (1984) On the prediction of fascioliasis and parmphistomatosis in Belarus. Materials of scientific conference "Veterinary science to production". Minsk, 22, p.80-86.

Rekhviashvili, E. I. (2001). Distribution of individual trematode species in mixed invasion of cattle. .Materials of reports of scientifici conference on the "Theory and practice of combating parasitic diseases". VIGIS. 213-215.

Svanbaev, S. K. (1972). Clinical picture and pathological changes in cattle with coccidiosis. Bulletin of Agricultural Science. No. 2, 68-73.

Velichko, I.V. (1971). About paramphistomatids of ruminants in the USSR. Collection of works on helminthology. M. Kolos. 67-73.

Veselova, T.P., Doroshina, M.V., & Arkhipov, I.A. (1987). The effectiveness of hexychol C in fascioliasis and paramphistomatosis of cattle. .Veterinary Medicine. No.3, 57-58.

Yerbolatov, K. M. (1977). Paramphistomatosis of cattle and sheep in individual farms of Talda-Kurgan region. Abstract of reports of scientific conference of the All – Union society of helminthologists. No 1, p.65.

Yerbolatov, K. M. (1982) Paramphistomatoses.-Alma-Ata:Kainar. 183-238.

Khazar Journal of Science and Technology
Khazar University Press 2022
Volume 7 №1 2023, 61-67
DOI: 10.5782/2520-613.2023.7.1.61

Investigation of SARS-CoV-2 Infection in Domestic Animals

Qiyasaddin Jalladov, Aytan Hajiyeva, Sabina Mammadova, Chichak Aliyeva, Shabnam Mammadova, Shumshad Rustamli, Kamala Aliyeva, Rana Kangarli, Fakhranda Alizade, Narmin Akhundova

Scientific Research and Risk Assessment Division, Scientific Research Center Department
Azerbaijan Food Safety Institute, Azerbaijan

Abstract

Concerned with the COVID-19 pandemic, the study of this disease in animals has got a great scientific importance in clarifying the information about the source and circulation of the infection. The study aimed to investigate the source of infection of domestic animals (dog, cat, cattle, sheep, goat and poultry) with SARS-CoV-2, as well as to identify susceptible animal species and ways of transmission of the virus. Observations were made on the animals selected for the study, from which nasopharyngeal and oropharyngeal smears were taken for PCR, and blood samples were taken for enzyme-linked immunosorbent assay (ELISA).

The experimental part of the study was carried out in veterinary clinics, animal shelters and farms. Dogs and cats are kept in animal shelters and examined in veterinary clinics, as well as cattle, sheep, goats and poultry grown on various farms, were involved in the study.

Antibodies to SARS-CoV-2 were detected in 11 of 645 samples taken from animals whose clinical signs of COVID-19 disease were initially observed or whose owners were exposed to the disease.

Based on the results of the study, monitoring the dynamics of the spread of SARS-CoV-2 among animals is of great scientific and practical importance in preventing this process.

Keywords: Coronavirus disease 2019 (COVID-19), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), investigation, antibody, domestic animals.

Introduction

SARS-CoV-2 infection, caused the COVID-19 pandemic in the world and atypical pneumonia in humans, was first detected in late 2019 in the Chinese city of Wuhan and was named 2019-nCoV (Shchelkanov et al., 2020). The genome structure of the causative agent is homologous to 50% MERS-CoV, 79% SARS-CoV and 88% BtRsCoV, so it belongs to the second type of acute respiratory syndrome viruses (Pal et al., 2020). According to recent data, SARS-CoV-2 is classified as a highly mutagenic virus belonging to the Beta- and Deltacoronavirus groups of the *Orthocoronavirinae* subfamily of the *Coronaviridae* family (Zhou et al., 2020).

Although the exact source of the causative agent of infection is not known, it is believed that SARS-CoV-2 was originated in wild animals and, later, transmitted to humans (Ahmad et al., 2020). According to preliminary data, the spread of the disease was caused by bats sold at animal markets in the Chinese city of Wuhan (Mackenzie & Smith, 2020). In the later stages of the pandemic, rodents were also reported to be infected with Sars-CoV-2 in various parts of the world. Rodents are thought to have played an intermediate role in transmitting the virus from bats to humans (Yuan et al., 2020).

Although there are no solid scientific findings on the mechanism of transmission of SARS-CoV-2 from animals to humans or vice versa, in some countries, owners of dogs and cats infected with SARS-CoV-2 have been found to have the disease (Sit et al., 2020; Calvet et al., 2021). In the United States, there have also been reports of the disease being transmitted to zoo animals through contact with an employee infected with SARS-CoV-2 (USDA, 2020). In late April 2020, Netherlands reported the first case of SARS-CoV-2 infection in commercially farmed mink. Additional reports of SARS-CoV-2 infected farmed mink came later in 2020 from Denmark (June), Spain (July), the U.S. (August), Italy (August), Sweden (October), France (November), Greece (November), Lithuania (November), and Canada (December) (AVMA, 2021).

Material and methods

Dogs and cats are kept in animal shelters and examined in veterinary clinics, as well as cattle, sheep, goats and poultry grown on various farms, were involved in the study. At the initial stage of the study, blood samples were taken from animals (nasopharyngeal and oropharyngeal smears) for molecular genetic (PCR) examinations, and blood from peripheral veins for enzyme-linked immunosorbent assay (ELISA) examinations.

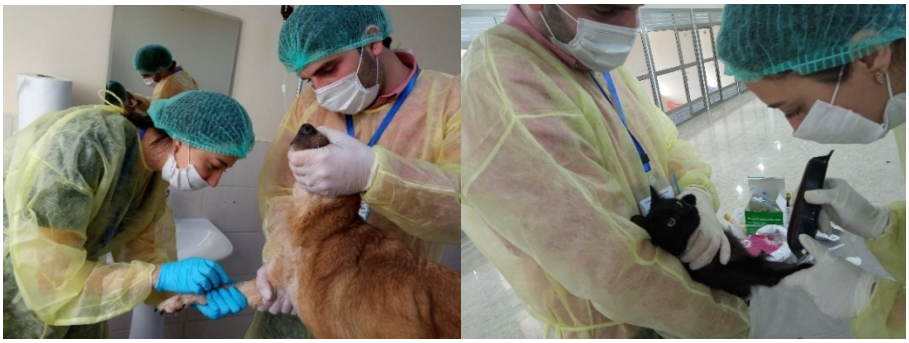


Figure 1. Blood sampling procedure

Samples were taken from 211 dogs, 136 cats, 19 cattle, 268 ruminants (sheep, goats) and 11 chickens. PCR and ELISA tests (virus in swab samples, antibodies in blood samples against the virus) were performed on samples taken from dogs and cats, and ELISA tests (antibodies in blood samples) were performed on samples taken from other animals. Blood samples were collected via leg venipuncture and sera were separated and stored at −20°C until further processing. All samples were collected under full personal-protective equipment.

Molecular genetic analyzes were performed using the BIO-RAD CFX96 Real-Time device. Extraction process done by the QIAamp® Viral RNA Mini kit. During the Extraction process, 140µl of the sample was taken at the initial stage and 160µl of RNA was isolated. Purification carried through Oasig™ lyophilized OneStep 2X RT-qPCR Master Mix kit, 5µl of extracted RNA was added to 20µl of Master mix. In the last stage, 25µl of the mixture was placed on the device for reading and recording.

Enzyme-linked immunosorbent assay (ELISA) analyzes were performed using the Thermo Multiskan FC device using the ID Screen SARS-CoV-2 Double Antigen Multi-species kit. Double antigen ELISA for the detection of antibodies directed against the nucleocapsid of SARS-CoV-2 in animal serum or plasma. Before the start

of analysis, all kit reagents were kept at room temperature (21°C± 5°C) and homogenized by vortexing. Firstly, 25µl Dilution buffer was added to each well. Secondly, an equal amount of Negative and Positive control was added properly to wells A1 and B1, C1 and D1. Then, 25µl sample was added to each remaining wells. The plate was covered and placed in a thermostat for 45 minutes of incubation. The plate was emptied and washed 3 times with 300µl wash solution. Then 300µl conjugate was added to each well. The plate was covered and kept at room temperature for 30 minutes. Again, the plate was emptied and washed 3 times with 300µl wash solution. In the next step, 100µl of substrate solution was added. The plate was covered and kept in the dark condition at room temperature for 20 minutes. In the end, 100µl of stop solution was added. The results were read and recorded at 450 nm.

Result and discussion

It should be noted that no SARS-CoV-2 agent was detected in the smear samples as a result of the research. No antibodies to SARS-CoV-2 were detected in immunosorbent assays analysis in blood samples from cattle, sheep, goats, and poultry. However, blood samples taken from 7 dogs and 4 cats with symptoms of the disease (runny nose, cough, diarrhea, etc.) revealed the presence of antibodies against the virus. The results demonstrate that the disease is limited among dogs and cats. Information on the type, age, residential area (owner/stray), and the amount (titer) of specific antibodies in the blood of dogs and cats, for which antibodies to SARS-CoV-2 have been detected, are presented in Tables 1 and 2.

Table 1. The table provides information on dogs with ≥60% antibodies in their blood.

Dog N^{o}	Gender of animal	Age	Sampling place	Titer (%)
1	Male	10 months	Animal shelter	143,58
2	Female	1 year	Animal shelter	157,18
3	Female	2 years	Animal shelter	64,24
4	Female	4 years	Vet. clinic	61,00
5	Female	3,5 months	Vet. clinic	237,86
6	Male	2 years	Vet. clinic	60,23
7	Male	1 years	Vet. clinic	98,97

As can be seen, the number of dogs with positive results is 7(Dog numbers are conditional). Of these, 62.24% of titers were found in the 3rd dog, 61.00% in the 4th dog, and 60.23% in the 6th dog. The antibody titer is 98.97 in the 7th dog. The 1st, 2nd and 5th dogs were found to have the highest titers, which are 143.58%, 157.18% and 237.86%, respectively. (≥ 60% positive) *

Table 2. The table provides information on cats with ≥60% antibodies in their blood.

Cat Nº	Gender of animal	Age	Sampling place	Titer (%)
1	Female	2 years	Vet. clinic	104,39
2	Female	3 years	Vet. clinic	162,19
3	Male	1,5 months	Vet. clinic	226,58
4	Female	8 months	Vet. clinic	153,63

As can be seen, the number of dogs with positive results is 4(Cat numbers are conditional). Of these, 104.39% of titers were found in the 1st cat, 162.19% in the 2nd cat, 226.58% in the 3rd and 153.63% in the 4th dog. (≥ 60% positive) *

* According to the instructions of the ID Screen SARS-CoV-2 Double Antigen Multi-species set, the result is considered positive if the antibody titer is ≥ 60%.

347 samples were examined by PCR and the result was negative. This means that no active patients were recorded in the sampled dogs and cats.

The results of the study show that dogs and cats are more susceptible to the disease among experimental animals, and the incidence of the disease among dogs is higher than among cats. Thus, the incidence was 3.32% among dogs and 2.94% among cats.

At the same time, the incidence of SARS-CoV-2 infection in females, both dogs and cats, is high, and the incidence of disease in domesticated animals is predominant (73%). Given the direct contact of dogs with antibodies in the blood of animals living in shelters, it can be assumed that there is a risk of transmission of the coronavirus from animal to animal.

The level of antibody titer, which is important in the formation of immunodeficiency, is inversely proportional to the age of the animals in the dog population, and the

amount of titer produced decreases with age. In cats, however, it can be concluded that the antibody titer in the blood is not related to the age of the animal.

During the collection of survey data on infected animals, it was determined that there were people infected with COVID-19 or with clinical symptoms of the disease among the owners of the animals or the staff serving them in the shelters. This, in turn, indicates that the human factor plays a role in the circulation of the pathogen in the transmission of the pathogen to animals.

Our results including 7 dogs and 4 cats with symptoms of the disease (runny nose, cough, diarrhea, etc.) revealed the presence of antibodies against the virus.

Since no antibodies to SARS-CoV-2 or the pathogen have been found in cattle and ruminants (cattle, sheep and goats), as well as poultry, it can be concluded that these animals are insensitive to the disease. Cases of SARS-CoV-2 infection have been reported in domestic dogs and cats. Thus, the formation of specific antibodies in the blood of domestic animals was observed by immunosorbent assays. Exposure of animals to SARS-CoV-2 infection increases the likelihood of contact with their infected owners or infected caregivers. Therefore, while contacting with animals during the COVID-19 pandemic, people should strictly follow the sanitary and hygienic rules and take into account the possibility of cross-transmission of zoonotic SARS-CoV-2. Research should also be continued to investigate the possibility of the virus circulating among animals and transmitting it from animals to humans.

More studies are needed to investigate the transmission route of SARS-CoV-2 from humans to cats and dogs. Finally, it is imperative that further studies be quickly carried out in order to better establish the risk of contamination of pets from humans, as well as the risk that infected pets would have as a source of infection for humans. Importantly, immediate action should be implemented to keep a suitable distance between humans and pet animals such as cats and dogs, and strict hygiene and quarantine measures should also be carried out for these animals.

References

Ahmad, T., Khan, M., Taha H., Musa, H., Nasir, S., Hui, J., Bonilla-Aldana, D.K., & Rodriguez-Morales, A. J. (2020). COVID-19: Zoonotic aspects, 1-4.

AVMA. (2021). SARS-CoV-2 in animals, 1-6.

Calvet, G. A., Pereira, S. A., Ogrzewalska, M., Pauvolid-Corrêa, A., Resende, P. S., Tassinari, W. S., Costa, A. P., Keidel, L. O., da Rocha, A. S. B., da Silva, M. F. B., dos Santos, S. A., Machado Lima, A. B., de Moraes, I. C. V., & Menezes, R. C.

(2021). Investigation of SARS-CoV-2 infection in dogs and cats of humans diagnosed with COVID-19 in Rio de Janeiro, Brazil, 4-8.

Mackenzie, J. S., & Smith, D. W. (2020). COVID-19: a novel zoonotic disease caused by a coronavirus from China, 2-6.

Pal, M., Berhanu, G., Desalegn, C. & Kandi, V. (2020). Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), 1-14.

Shchelkanov, M.Yu., Popov, A.Yu., Dedkov, V.G., Akimkin, V.G., & Maleev, V.V. (2020). The history of the study and modern classification of coronaviruses (nidovirales: coronaviridae), 221–246.

Sit, T. H. C., Brackman, C. J., Ming Ip, S., Tam, K. W. S., Law, P. Y. T., To, E. M. W., Yu, V. Y. T., Sims, L. D., Tsang, D. N. C., Chu, D. K. W., Perera, R. A. P. M., Poon, L. L. M. & Peiri, M. (2020). Infection of dogs with SARS-CoV-2, 586: 776–778.

USDA. (2020). List of SARS-CoV-2 Cases in the United States, 1-9.

Yuan, S., Jiang, S. C., & Li, Z. L. (2020). Analysis of Possible Intermediate Hosts of the New Coronavirus SARS-CoV-2, 2-5

Zhou, Z., Qiu, Y., & Ge, X. (2020). The taxonomy, host range and pathogenicity of coronaviruses and other viruses, 3-90

Khazar Journal of Science and Technology Volume 7 №1 2023, 68-71
Khazar University Press 2022 DOI: 10.5782/2520-613.202.7.1.68

Modern Agrarian Policy of the State

Rahim Bilalov
Lankaran State University
rahim19511951@mail.ru

Abstracc

Although the culture of agriculture and animal husbandry in Azerbaijan began in the ancient Mesolithic period before Christ, these opportunities given to us by nature began to be used more widely and efficiently in this field during/under/in the light of the leadership of Heydar Aliyev in the 70s of the last century. At present, his worthy successor, Ilham Aliyev, is successfully continuing the sustainable development of the agricultural sector. One of the important directions of agrarian reforms implemented in our country is the activity of agrarian science and educational institutions.

Keywords: State, agrarian, politics, education

Introcuction

The sustainable development of the agricultural sector in our country is one of the priorities of the economic development strategy implemented under the leadership of President Ilham Aliyev. Special attention is paid to the continuous improvement of competitiveness in agriculture and, for this purpose, improvement of the business environment, improvement of the legislative base, application of modern technologies and scientific and technical achievements to the attraction of local and foreign investments. Thanks to the complex works carried out in recent years, Azerbaijan has entered into the stage of modern development. This development is based on a political course that determines the provision of our independence, security, progress, modernization, social welfare and economic development. Like other fields, the agricultural field has entered into a qualitatively new stage by responding to these challenges with its dynamic development.

Azerbaijan is one of the settlements where the first people settled in the world. People living in this land with favorable climatic conditions, rich nature, high

mountains, wide plains, and many sources of fresh water have been living a nomadic and sedentary lifestyle since ancient times, engaged in agriculture and animal husbandry.

In the ancient Mesolithic period BC, the primary agricultural culture was formed in Azerbaijan, grain growing and animal husbandry became the main occupation of people. However, these opportunities and potentials given to Azerbaijan by nature began to be used more widely, properly and efficiently only in the 70s of the last century, that is, in the years when Heydar Aliyev began to lead the republic. It was H. Aliyev who proved the possibility of developing agriculture in Azerbaijan and literally created a revolution in this field. H. Aliyev's "Veterinarianism is a difficult profession, specialists in this field must always be vigilant." famous physiologist Pavlov said, "If medical doctors guard the health of people, veterinarians guard the health of all mankind." Opinion is completely coincident with today. The appreciation given to veterinary medicine by these prominent people is the Bird Flu, the Coronavirus, etc., which are currently making the planet more dangerous than even the most terrible wars. Today once again, proves the fact that diseases that can cause pandemics are passed from animals and birds to humans and that their health depends on animals. Today once again, this fact is proved that diseases causing pandemics are transmitting from animals and birds to human and hence their health deapends on animals. Recently, as a result of the impact of anthropogenic activity on our planet, the ecological consequences of some works have been done without taking into account the forecast have reached their climax, leaving humanity between two paths ahead- death and life. The lithosphere, hydrosphere, atmosphere, flora and fauna have been severely and irreparably damaged. Nature has been oppressed, resulting in global climate changes, unstoppable ecological cataclysms and natural disasters. As a result, torrential rains, strong floods, hurricanes, tsunamis, landslides, earthquakes and volcanic eruptions bring the genetic remains of human and animal corpses underground, along with infectious agents to the surface of the soil, causing dangerous epidemics, epizootics and pandemics. On the other hand, climate anomalies, solar explosions, and other environmental problems have changed the existing flora and fauna of the world, reducing the number and productivity of agricultural animals and birds, weakening their resistance to diseases, and increasing their susceptibility. On the contrary, they have increased the pathogenicity and virulence of microorganisms, viruses, and parasites caused it to appear in a new form. Therefore, some classic infectious diseases, not observed in the last centuries, have caused the reoccurrence of people and animals in an unusual form. Another very serious concern is that these diseases have acquired new properties and cannot be treated and prevented with existing drugs. Therefore, all international organizations, country leaders, scientists and every citizen of the planet, especially

the future generation, young people and students, must fulfill their human and humanitarian duty to protect nature and know that the end of genocidal and terrorist attitudes towards nature is the end of the entire living world, including it means a decrease in the number and productivity of animals, birds, fish and bees, which are the main source of human food, and lack of food.

According to the forecast of related experts, the growth level of the population on earth cannot fully meet their daily food needs. At the same time, it is expected that the rate of production of agricultural products will be low in the future. However, science has proven that food shortage can be eliminated in an intensive way, i.e. by increasing productivity, along with extensive factors. So, as you can see, all resources for improving food production have not been exhausted yet. For this, the application of non-loss technology, use of non-traditional sources of raw materials, and other sources of food supply should be sought and found. At the same time, more profitable, efficient and effective scientific achievements should be found and applied. To do this, modern biotechnology, molecular biology, genetics, electronics, and. the cutting-edge scientific achievements should be adopted and widely applied in our country. It has been proven that 70-80% of the food shortage, which is a great threat to humanity, can be covered by these progressive methods. These progressive achievements of science and technology should be delivered, taught, taught and applied in detail through our lectures and practical experiences to students studying in various teaching and educational institutions, including our university.

One of the important directions of the agrarian reforms carried out in the country is related to the strengthening of the activities of the agrarian-oriented scientific and educational institutions. In this framework, the research carried out in all scientific-research institutes and educational institutions should be improved on the basis of new challenges. Modern format exchange programs should be organizedamong international research centers and educational institutions under joint action programs. It is advisable to establish relations with prestigious international educational institutions.

In order to solve the problems mentioned above and posed by the head of the country, it is the responsibility of the professor-teacher team of our university to teach the state-of-the-art scientific, and technological achievements and practice them to our students who are studying in the us today, and those who will work in the agricultural field in the future, as well as highly qualified, professional and the task of preparing skilled specialists falls. To put this fact into act, , a joint activity program was created with the prestigious Kafkas University of Turkey in the field of veterinary medicine, of which the teachers were invited and got involved in the teaching process of

various subjects and disciplines. At the same time, it was mutually agreed that our students will have their industrial experience at that university.

References

1. **Aliyev, I. N.** (2001). Heydar Aliyev and Azerbaijan's Agriculture. Baku. 2001

2. **Mustafayev, F., Huseynov, & E., Salmanov, M.** (2013). Veterinary Medicine Genetics. Baku. 2013

3. "Veterinary science in the XXI century - Innovations towards the future". International scientific-practical conference materials. Baku. 2021

Khazar Journal of Science and Technology Volume 7 №1 2023, 72-76
Khazar University Press 2022 DOI: 10.5782/2520-613.202.7.1.72

Studying the Azerbaijani barley accessions to drought Stress Induced by Polyethylene glycol (PEG)

Moozhan Serpoush
Center for Cell Pathology Research, Department of Life Sciences, Khazar University, Azerbaijan

Abstract

Population growth and climate change necessitate the expansion of agricultural products. Drought stress has a significant impact on crop growth as a result of global warming. Drought stress can occur at any stage of plants growth and reduce production. One of the most important crops with high resistance to environmental abiotic stresses is barley (Hordeum vulgare). In order to choose the best barley accessions resistance to drought stress, 8 barley genotypes with different levels of (PEG) 0, -3, -6 and -9 bar were tested in a randomized complete block design with three replications at Khazar University's Center for Cell Pathology Research laboratory in November 2022. Traits such as root length, coleoptile length, plumule length, weight dry matter, percentage and rate of germination were measured. Selected drought tolerance genotypes can be used in barley breeding programs.

Keywords: Barley, Drought stress, Polyethylene glycol (PEG), Germination

Introduction

Drought stress (Ds) is one of the most serious and widespread environmental stresses on the planet. Ds, the most prevalent abiotic cause, has impacted agricultural production and growth (Kour D, et al., , 2019). Drought stress can be caused by any human or natural environmental actions (Shahid S, *et al*., 2018). Due to climate change, global warming, and water shortages, drought stress is inevitable (Sallam, Alqudah, Dawood, Baenziger, & Börner, 2019). Azerbaijan is located in the south Caucasus and has a variety of climates, including particularly hot summers with temperatures raising up to 40 to 45 degrees Celsius. Drought-tolerant cultivar selection is vital for sustainable agricultural production. Barley (*Hordeum vulgare)* belongs to the cereal grass family, one of the primary crops that has been domesticated and used as human food and livestock feed as well as the beverage

industry (Stein and Muehlbauer 2018). Due to high resistance to environmental changes and its diploid genome, barley has been implemented in breeding programs, wheat studies as a model and genetic resource (Ullrich, 2010). Plant physiological condition and changes play a significant part in drought stress tolerance; for example, proline regulates plant osmosis and prevents leaf chlorophyll degradation under stress conditions (Miller G, *et al*., 2005). Ds can occur at any point of plant growth and has the highest effect on yield loss (Kadam, 2014). Ds influences germination and the early development stage of barley.

This study was conducted to investigate genetic diversity among barley accessions under drought stress, comparing genotypes' responses to different levels of drought, and selecting drought tolerance barley accessions for use in breeding programs.

Material and method

This research has been conducted at Center for Cell Pathology Research laboratory, Khazar University, in November 2022. Seeds were obtained from the National Gene bank of Azerbaijan, ANAS (Azerbaijan National Academy of Sciences' Genetic Resources Institute). To estimate drought stress on seedling growth of barley cultivars, a completely randomized design (CRD) with 3 replicates was used (N=24). In this research 8 barley genotypes (table 1) with different levels of PEG (0, -3, -6 and -9 bar) were tested. Seeds were sterilized in sodium hypochlorite solution 2.5% for 10 minutes and after 3 times washed with distilled water. Seeds of each genotype as per treatment are placed on filter paper in petri dish with a diameter of 9 cm. Ten milliliters of concentration in varying levels of drought were given to each treatment. Petri dishes were kept for 8 days at room temperature of 20–22 °C with 16 hours of light and 8 hours of darkness for seven days. Polyethylene glycol (PEG) at pressures of 0, -3, -6 and -9 bar was used for the initial irrigation, and distilled water was utilized for the subsequent irrigation. Lids were used to avoid evaporation.

Barley morphological treats such as root length, coleoptile length, plumule length, weight dry matter, percentage and rate of germination were measured. The mean squares for the traits under study were calculated using the SPSS software and the Tukey test and Excel's bar-drawing software.

Table 1. Barley genotypes and gene bank code

No.	Code	Cultivar
1	2540	*Hordeum vulgare* var. nigropallidum
2	8830	*Hordeum vulgare* var. pallidum
3	8834	*Hordeum vulgare* var. nutans
4	8837	*Hordeum vulgare* var. nutans
5	8842	*Hordeum vulgare* var. nutans
6	8845	*Hordeum vulgare* var. erectum
7	8846	*Hordeum vulgare* var. pallidum
8	8853	*Hordeum vulgare* var. pallidum

Results and discussion

Based on the result of ANOVA data analysis, drought stress had significant effect on all treats such as root length, coleoptile length, plumule length, weight dry matter and rate of germination. Also, analysis shows high genetic variation among barley accessions based on the drought stress traits (table 2).

Barley genotype 5 and 8 have the highest coleoptile length by 5.1 cm and 4.12 cm among all seeds and 3.28 cm & 1.35 cm (in order) growth in -0.9 level of PEG, but first barley accession with 3.82 cm coleoptile growth as third longest length at control level had no growth at -0.9 level of PEG (table 3). The same highest numbers were studied for root and plumule length in both genotype no 5 and no 8. Root length increased in all genotypes at level -0.3 bar in comparison with control level, except genotype no 2, 6 & 7. First and second genotypes showed high germination rate as 1.7and germination rate decreased in almost all genotypes by increasing PEG levels (figure 1). Hellal reported that PEG% had a negative effect on the germinated seeds in the early stage (Hellal *et al*., 2018)

Table 2. main squares for studied traits of barley accessions evaluated under PEG treatment

TRAITS	REP.	LE.	GENO.	G×LE	ERROR
DF	1	3	7	21	31
COL.	$0.005^{n.s}$	7.48^{**}	5.66^{**}	0.24^{**}	0.06
ROOT	$1.23^{n.s}$	26.73^{**}	25.47^{**}	7.14^{**}	0.46
PLUM	$0.74^{n.s}$	208.73^{**}	14.16^{**}	$1.48^{n.s}$	3.32
WEIGH	$0.32^{n.s}$	1.9^{*}	1.39^{*}	$1.27^{n.s}$	0.54
PER	$6.89^{n.s}$	11264.3^{**}	462.85^{**}	207.62^{**}	15.66
RATE	$0.05^{N.S}$	2.19^{**}	1.2^{*}	$0.08^{N.S}$	0.088

Table 3. effect of different levels of PEG on barley growth.

	COLEOPTILE LENGTH				ROOT LENGTH				PLUMULE LENGTH			
	0	**-0.3**	**-0.6**	**-0.9**	**0**	**-0.3**	**-0.6**	**-0.9**	**0**	**-0.3**	**-0.6**	**-0.9**
1	3.82	3.42	2.92	0	5.77	7.15	5.64	0	14.51	16.02	7.325	0
2	3.15	2.35	2.52	1.31	6.78	5.6	4.39	0	9.8	7.51	7.19	0
3	1.95	1.51	1.33	0.99	4.81	9.5	6.16	4.12	11.6	10.35	8.51	2.6
4	2.26	1.95	1.67	1.38	2.7	4.01	1.97	1.81	10.25	11.82	7.65	6.55
5	5.1	3.98	3.61	3.28	6.12	9.79	5.55	11.24	14.44	12.54	6.21	4.5
6	2.32	1.85	1.79	0.65	7.87	3.73	2.99	0	10.27	7.37	6.78	0
7	3.51	3.04	1.73	0	5.14	3.99	2.7	0	10.24	9.53	6.02	0
8	4.12	3.12	2.74	1.35	10.63	7.49	4.44	3.92	13.24	10.16	5.14	3.15

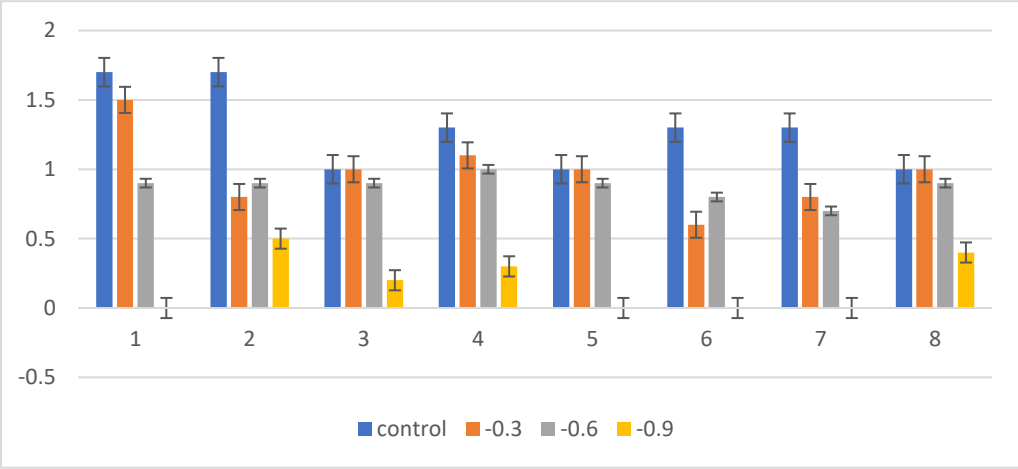


Figure 1. germination rate of barley cultivars affected by PEG levels

References

Hellal, F. A., El-Shabrawi, H. M., Abd El-Hady, M., Khatab, I. A., El-Sayed, S. A. A., & Abdelly, C. (2018). Influence of PEG induced drought stress on molecular and biochemical constituents and seedling growth of Egyptian barley cultivars. *J Genet Eng Biotechnol, 16*(1), 203-212. doi:10.1016/j.jgeb.2017.10.009

Kadam, N. N. (2014). Agronomic and physiological responses to high temperature, drought, and elevated CO2 interactions in cereals. . In Advances in agronomy , Vol. 127, pp. 111-156. Academic Press.

Kour D, et al., . (2019). Drought-Tolerant Phosphorus-Solubilizing Microbes: Biodiversity and Biotechnological Applications for Alleviation of Drought Stress in Plants. In: Sayyed R., Arora N., Reddy M. (eds) Plant Growth Promoting Rhizobacteria for Sustainab Microorganisms for Su. Springer, Singapore., vol 12. https://doi.org/10.1007/978-981-13-6536-2_13.

Miller, G., H. Stein, A. Honig, Y. Kapulnik and A. Zilberstein. (2005). Responsive modes of Medicago sativa proline dehydrogenase genes during salt stress and recovery dictate free proline accumulation. Planta 222: 70-79

Sallam, A., Alqudah, A., Dawood, M., Baenziger, P., & Börner, A. (2019). Drought Stress Tolerance in Wheat and Barley: Advances in Physiology. Breeding and Genetics Research. International Journal of Molecular Sciences., 20(13):3137. doi:https://doi.org/10.3390/ijms20133137

Ullrich, S. E. (2010). Barley: Production, improvement, and uses . (Vol. 12). John Wiley & Sons.

Khazar Journal of Science and Technology
Khazar University Press 2022
Volume 7 №1 2023, 77-90
DOI: 10.5782/2520-613.2023.7.1.77

Development of Ecological Effective Cleaning Method in Oil Industrial Wastewater (Iw) Mechanical Cleaning Plants

Narmin Rakida, Nazim Shamilov, Giyas Bayramov, Sevinj Gadjiyeva, Fakhraddin Yusubov
Baku State University, Baku, Azerbaijan,
E-mail: nara.rakida@mail.ru

Abstract

Due to the complex composition and dispersed systems of industrial wastewater generated in the oil refining industry, which have a colloidal solution, deep cleaning of these waters, up to 100% of oil product waste, is one of the main unresolved environmental problems. Due to the high content of MOPW in the IW obtained from ORI, the initial treatment of these waters is carried out by the method of mechanical precipitation due to the minimum of 1–2 g/l and the maximum concentration of 5–10 g/l.Depending on the amount of 1000–2000 mg/l of MOPW included in these devices, the purification of IW is often carried out in the actual range of 250–500 mg/l.In the next stages, physicochemical, chemical, biological, etc. the methods are carried out only in accordance with the environmental standards of the remaining MOPW to a certain extent. Although some of this water is transported to the periodic water system after the final biological treatment of the IW of the industry, the MOPW mixture, which remains in the ecological norms due to the discharge of the rest into the water basins, has a negative environmental impact on the flora and fauna of the basin. The ecological problem of the destruction of ecosystems still persists.

Keywords: Oil refining, industry, production, wastewater, mechanical, extractant, coagulant, flocculant

Introduction

Wastewater containing oil has its own specific characteristics in terms of composition and behavior of oil products in the aqueous environment, and ultimately determines the methods of treatment of this type of wastewater.The main features that determine the behavior of oil products in water are that they have a lower density than the density of water (gasoline 0.70–0.73, diesel fuel 0.8–0.9, jet fuel 0.8–0.85, fuel oil 0.94–1.0 g / cm^3) and low solubility. The latter does not exceed 20–30 mg / l in water for light oil fractions (gasoline), 70–90 mg / l for kerosene, and is practically zero for heavy fractions.When petroleum products fall into the water,

their bulk is in the form of coarse dispersions (droplets), and because of their low density, they easily rise to the surface of the water to form a floating layer or layer. The rest of the petroleum products are in a finely dispersed state and can form an "oil in water" emulsion. It is assumed that the true emulsion is formed in the colloidal dimensions of oil droplets (approximately 0.1 μm). However, persistent emulsions are also observed in larger droplets in wastewater containing oil products. The durability of the emulsion is determined by the surface tension, the kinetic resistance of the particles, their small density, the stabilizers of emulsions can be particles, depending on the wastewater. At present, various surfactants are finding a wider field of application, and their exposure to petroleum-containing effluents significantly disrupts the cleaning process by stabilizing the emulsion (Khaidarov et. al., 2005; Abrosimov et al., 2002; Komissarov et al., 2002; Nevsky et al., 2004; Glukhova et al., 2003; Bukhgalter et al., 2003; Kuchera et al., 2007; Glueckstern et al., 2000; Magid et al., 2006).

Coagulation is one of the most commonly used treatment methods for wastewater, refined and insoluble oils, and petrochemicals. Numerous studies in this area show that aluminum and iron oxides are used as coagulants in wastewater treatment (Kuchera et al., 2007; Glueckstern et al., 2000; Magid et al., 2006; Magid et al., 2002; Magid et al., 2007; Gentsler et al., 2004; Ksenofontov et al., 2004).

As noted in many technical literatures, the IW formed in the oil refining industry (ORI) is very different in terms of complex composition, flow rate and volume compared to the industrial wastewater generated in the oil extraction (extraction) industry. That is why ORI mixes wastewater of different compositions formed in very different technological processes and initially enters the mechanical treatment plant with a common sewage system (Kuchera et al., 2007).

Due to the high content of MOPW in the IW included in these units, these units are initially precipitated in the oil or oil separator sections and cleaned in large additional sediments belonging to the specified treatment area. Then, in the stages of physical-chemical, chemical and biological treatment, the cleaning technological processes are continued, part of the IW purified in accordance with the ecological norms is discharged into the recirculating water system and part into the water basins. Despite treatment in accordance with environmental standards, long-term environmental problems arise due to the presence of MOPW in the IW, even in the amount of less than 5–9 mg/l, which has a negative environmental impact on the flora, fauna and ultimately the ecosystem of the IW (Glueckstern et al., 2000).

We have been conducting long-term research and experimental work to clean up 100% of MOPW with various chemical components in several directions from ORI samples with very complex composition taken from the entrance of ORI mechanical treatment plants.

As noted in the literature of many technicians, we have conducted an ecological analysis of the reasons for the failure to separate and purify the IW of ORI, which is

a colloidal solution with a continuous dispersed system, up to 100% MOPW emulsion.

An emulsion of an oil, petroleum product waste mixture, which is used to purify some selective substances, such as solvent CCl_4, as well as other coagulants in different concentrations, precipitates at the bottom of the IW with the same solvent or coagulant. This leads to the loss of large amounts of MOPW in the subsequent treatment and the problem of separation from the water.

That is why, based on our environmental research - analysis and the results of experimental work, we have achieved a new ecological approach to the deep purification of IW samples from ORI, which is of great economic and environmental importance, up to 100% from MOPW, dependent substances an effective chemical method has been developed (Ilyin et al., 2006; Hadjieva et al., 2021; Bayakhmetova etal., 2011; Sapina et al., 2011; Shapkin et al., 2012).

During the purification of IW, which is formed in ORI and has a very complex composition, in several stages, ie during the process of transporting IW by centrifugal pump to each stage, the hydrophobic emulsions of these waters increase, and a continuous dispersed system colloidal solution is arises.

Therefore, the maximum amount of IW included in mechanical treatment plants (300–1000 mg/l), ie the maximum amount of MOPW containing 100–250 mg/l MOPW, depending on the amount of emulsion of MOPW in the IW it is possible to carry out at least 3–5 stages in the mentioned devices.

That is why, with the new chemical method we are working on, in the initial stage of the existing mechanical treatment plants of ORI, regardless of the highest concentration of MOPW in IW, these waters can be cleaned up to 100% from MOPW, dependent substances and completely discolored achieving transparency has been identified (Ilyin et al., 2006; Hadjieva et al., 2021; Bayakhmetova etal., **2011; Sapina et al., 2011; Shapkin et al., 2012).**

Materials and methods

According to the new method developed by us, the degree of purification of the IW sample was also determined by the device QX-KS 6890–5975 (Manufacturer Agilent Technologies, USA).Thus, the analysis of the device was carried out in a system including an Agilent 6890N gas chromatograph which has an interface with an Agilent 5975 high-performance mass selective detector manufactured by Agilent Technologies (USA).The chromatograph is equipped with an injector without flow splitting and a ZB-5 capillary column (Phenomenex, USA).The ZB-5 column has the following specifications - copolymer 5% diphenyl 95% dimethylpolysiloxane, length 60 m, inner diameter 0.25 mm, film thickness 0.25 μm. Helium (purity

99.999%) is used as the carrier gas at a flow rate of 1.5 ml/min.Ionizing source voltage 70 eV, source temperature 230°C, quadrupole temperature 150°C, injector temperature 270°C.

The amount of organic (PAH) compounds remaining in the treated water was 10 times less than the reference substances taken for spectral sampling, as can be seen from the spectrum wiped outby the device taken in Example VI. An overview of the spectrum device is shown in Figure 1.

Figure 1. **QX-KS 6890-5975 overwiev**

Chemical treatment of IW samples taken from the entrance of ORI mechanical treatment plants in different directions was carried out in the laboratory. During the experimental research works, petroleum ether as an extractant with high ecological effect, 5% solution of $Al_2(SO_4)_3$ salt as a coagulant, 5% H_2SO_4 solution as a flocculant and neutralizer and, if necessary, 5% - the optimal technological regime for the use of $NaHCO_3$ salt as a neutralizer to obtain pH=7 from 5 solutions was determined. That is why the IW sample was cleaned in the following direction by mixing in the following sequence and at each stage.

Experimental part

In the first stage: 1L IW sample is filled into the separating funnel, 0.5% of petroleum ether is added to it and mixed several times at 10–20.C for 0.5 minutes add 0.5% IW of 5% $Al_2(SO_4)_3$ solution coagulant to the extracted mixture and mix for 1 minute.

To speed up and complete the coagulation process, a flocculant (simultaneously neutralizing) 5% H_2SO_4 solution of 0.1% IW is added to the mixture and the mixture is stirred several times for 2 minutes. The coagulation process takes 15 minutes. The organic layer is separated from the water layer. The analysis of the amount of MOPW (total organic compounds) in the aquifer was performed by known methods and the spectrum was recorded with the device "QX-KS 6890-5975".

The spectrum captured by this device is shown in Figure 2 below.

Conclusions and discussions

Test T-VI

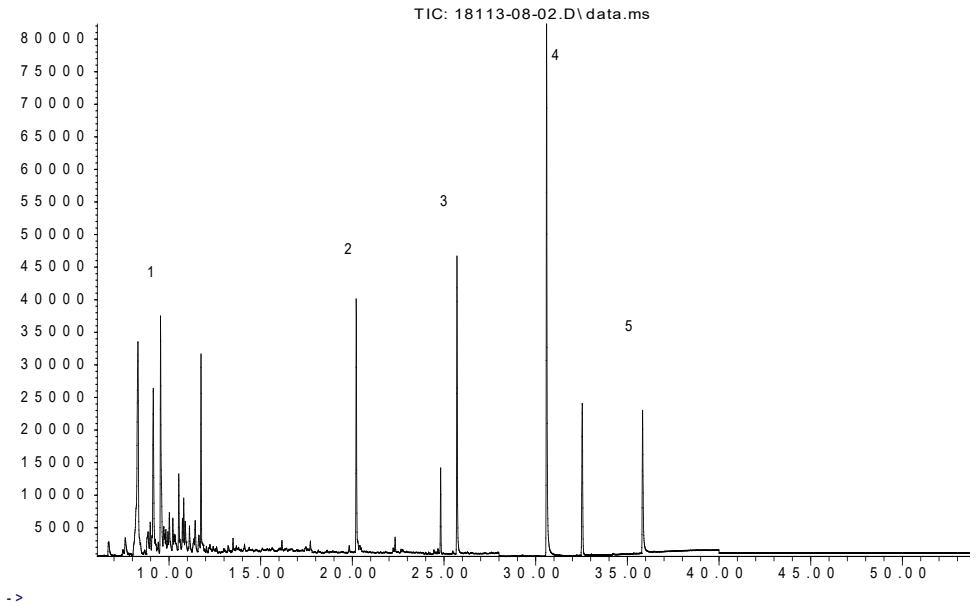


Figure 2. Spectrum was recorded with the device "QX-KS 6890-5975".

Peaks of the internal standard: 1.Naphthalene -d8; 2.Phenanthrene -d10;3.Pyrene -d10;4.Chrysen -d12';5.Washing machine -d12

The amount of organic matter remaining in the treated water sample was ten times lower than the not only environmental but also sanitary norms, as shown in Table 1. At the same time, the IW samples were spectrumed on a BRUKER device to determine the amount of residual organic compounds in the water sample purified by the new method developed above by us.

Table 1. QX-KS 6890-5975Concentration of individual PAHs automatically obtained on the device

Compound	naphthalene	acenaphthylene	acenaphthene	fluorene	phenanthrene	anthracene	fluoranthene	pyrene	Benzo(a)anthracene	chrysene	benzob(k)fluoranthene	benzo(a)pyrene	indeno(1,2,3-cd)pyrene	benzo(ghi)preylene	Dibenzo(ah)anthracene	Total EPA 16 PAH
T-VI (ug/l)	1.14	0.11	0.12	0.41	0.82	0.19	0.13	0.26	0.15	0.32	0.30	0.21	<0.01	0.07	<0.01	4.23

As can be seen from Figure 3, the hydrogen atoms in the water were obtained at high peaks. However, the peaks of the groups of hydrocarbon compounds remaining in the water are very small. This shows that the organic compounds in the IW sample purified by the new method are ten times lower than the environmental and sanitary norms. Thus, based on the above spectra and the results of the analysis of water treated by other gravimetric methods, it can be noted that up to one hundred percent treatment of oil industry wastewater can be carried out by this method. In addition to the above-mentioned experimental work, up to 5% of the IW purified to remove the extractant from the MOPW mixture obtained from the initial method of purification of IW samples of ORI is added to the IW in the separating funnel and mixed for 1 minute. It was then proved that up to 5% of the IW on the mixture could be used as a substitute for a coagulant solution from the purified IW obtained from the initial treatment and containing sulfate and chloride salts $Al(OH)_3$.

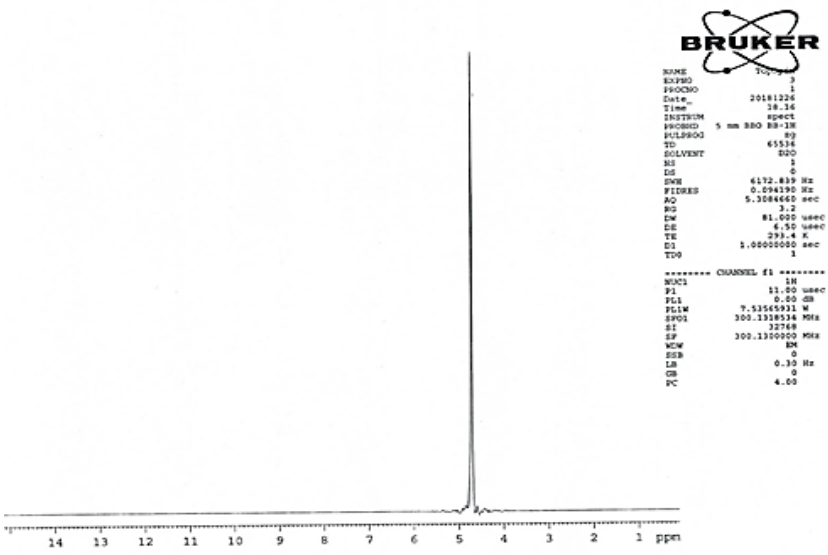


Figure 3. Spectrum of purified IW water sample taken with NMR BRUKER device.

In general$Al(OH)_3$ is formed by hydrolysis of the coagulant process after its addition to the IW sample after mixing, as noted in the literature (Ilyin et al., 2006; Hadjieva et al., 2021; Bayakhmetova et al., 2011; Sapina et al., 2011; Shapkin et al., 2012).

$$Al_2(SO_4)_3+6H_2O=Al(OH)_3+ 3H_2SO_4 \text{ is obtained.}$$

In this case, the metal compounds in the released H_2SO_4 IW are converted by their hydroxides (as pH=9–10) and their salts into sulfate salts. These salts are transferred to the water separated from the MOPW. $Al(OH)_3$, on the other hand, sinks to the bottom of the water phase of the IW by attracting positively charged mechanical compounds..

To increase the speed of the coagulation process after the addition of an extractant, coagulant to the IW sample, as a flocculant, and also to bring the IW medium to pH = 9–10 to pH =7.0–7.5, using H_2SO_4 acid. NaOH, $CaCO_3$, etc. H_2SO_4 reacts with compounds and enters the aqueous phase.

$$2NaOH+H_2SO_4=Na_2SO_4+2H_2O$$

$$CaCO_3+H_2SO_4=CaSO_4+H_2O+CO_2$$

Using the new method developed based on the results of the research, it is possible to achieve the complete in-depth purification of the IW formed in the ORI from the required MOPW-dependent substances, as well as the transparency of these waters.

The application of this method in production can be considered scientifically based. During the experiment, it was determined that the process of coagulation of the IW sample, the effectiveness of the purification depends on the sequence of application of the extractant, the concentration and amount of coagulant, as well as the consistency and amount of flocculant. The results of the coagulation process, depending on the sequence and concentration of the reagents used in the developed method, are given in Figure 4–7. The results of the method developed for the environmental treatment of the IW sample of ORI are given in Table 2–4.

IW sample during purification 5% $Al_2(SO_4)_3$ solution in the first stage, 5% H_2SO_4 acid solution in the second stage and petroleum ether as an extractant in the third stage a graph showing the coagulation process when used is shown in Figure 5 below.

At the same time, the technological scheme related to the method of environmentally effective cleaning of NES in mechanical cleaning units of ITS is shown in figure 8.

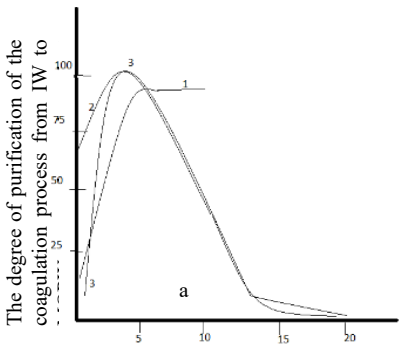


Figure 4. Dependence of the coagulation process (purification of MOPW and dependent substances from IW) on the amount of extagent (petroleum ether), coagulant 5% $Al_2(SO_4)_3$ solution) and flocculant (5% H_2SO_4 solution) 1) petroleum ether, 2) 5 % $Al_2(SO_4)_3$ solution, 3) 5% H_2SO_4.

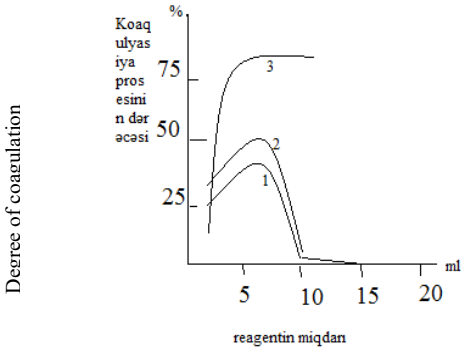


Figure 5. Dependence of the coagulation process (purification of MOPW and dependent substances from IW) on the amount of extagent (petroleum ether), coagulant 5% $Al_2(SO_4)_3$ solution) and flocculant (5% H_2SO_4 solution). 1–coagulant (5% $Al_2(SO_4)_3$ solution), 2– flocculant (5% H_2SO_4 acid), 3– extractant (petroleum ether).

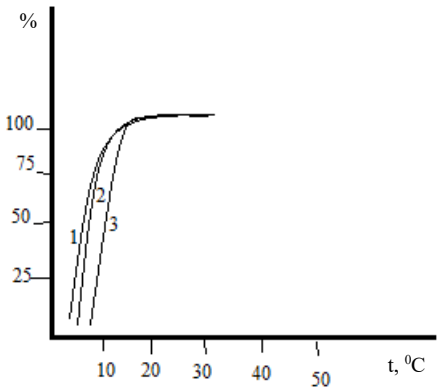


Figure 6. Graph of temperature dependence of the coagulation process. 1– extagent; 2– coagulant; 3– flocculant.

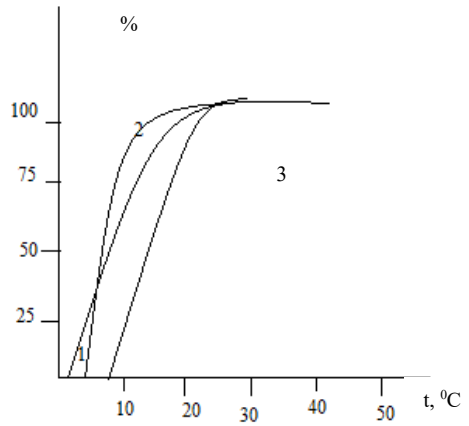


Figure 7. Time-dependent graph of the coagulation process. 1 – extagent; 2– coagulation; 3– flocculant

The table below shows the results of a new method developed for the environmental treatment of IW samples taken from ORI and those in which the amount of MOPW increased in those samples.

Table 2. The results of the method developed for the environmental treatment of the IW sample of ORI

IW-composition of samples															
Indicators before cleaning the sample					Reagents and mixtures used in cleaning						Indicators after cleaning				
Number and volume	MOPW amount and mg/l	The amount-of depen-dent substances is mg/l	Color	pH	Petro-ley ether ml	5% $Al_2(SO_4)_3$ ml	5% H_2SO_4 ml	K*-1	K*-2	Cleaning duration	XIMTQ amount	The amoun tof depen-dent substan-ces is mg/l	Color	pH	Note
I 1l	500–1000	100-150	Dark black	9-10	2.5	5	1.5	10.0	10.0	20	~0.001	~0.001	Transparent	7.03	
II 1l	1000-2000	100-150	Dark black	9-10	2.5	5	1.5	10.0	10.0	20	~0.001	~0.001	Transparent	7.02	
III 1l	2000-5000	150-200	Dark black	9-11	5.0	10	3	15.0	15.0	20	~0.001	~0.001	Transparent	7.04	An example taken in an emergency situation
IV 1l	5000-10000	200-250	Dark black	9-11	5.0	10	3	20.0	20.0	20	~0.001	~0.001	Transparent	7.05	An example taken in an emergency situation

Note: K*-1 MOPW, which was captured during cleaning and contained as an extractant, was used as an extractant substitute.

K*-2purified IW was used as a coagulant substitute.

Table 3. Results of the developed method for the deep, new environmentally effective treatment of industrial waste water obtained in the oil refining industry

Examples of industrial waste water for ecological scientific research																		
Composition before treatment: pH, color and components used during treatment											after the components are given				pH, color, composition after treatment			
Conditional number №, volume	The amount of amount of mixture of waste oil products in the composition, mq/*l*	pH	color	EK-1, ml	EK-2, ml	EK-3, ml	EKN compo-nent, ml	5% K-1 solution, ml	5% K-2 solution, ml	5% K-3* solution, ml	m.p., min	o.p.o.l., min	p.c.c., min	t.c.p., min	pH	color	The amount of mixture of waste oil products in the compo-sition, mq/*l*	Organic compounds mixture amount, mg/l
N-1, 1 *l*	500-1000	9-10	Deep black	0.3	0.7	–	–	5.0	2.0	0.3	2	3	5	15	7.3-7.4	clear	~ 0	<0.001
N-1, 1 *l*	500-1000	9-10	Deep black	–	–	1.0	–	5.0	1.0	0.3	2	3	5	15	7.3-7.4	clear	~ 0	<0.001
N-1, 1 *l*	500-1000	9-10	Deep black	–	–	–	5.0	5.0	1.0	–	2	3	5	15	7.3-7.4	clear	~ 0	<0.001
N-2, 1 *l*	1000<2000	9-10	Deep black	0.6	1.4	–	–	5.5-6.0	2.5	0.5	2	4-5	7	15	7.3-7.4	clear	~ 0	<0.001
N-2, 1 *l*	1000<2000	9-10	Deep black	–	–	2.0	–	5.5	2.5	0.5	2	5	7	15	7.3-7.4	clear	~ 0	<0.001
N-2, 1 *l*	1000<2000	9-10	Deep black	–	–	–	5.0	5.0	1.0	–	2	5	7	15	7.3-7.4	clear	~ 0	<0.001
N-3*, 1 *l*	2000<5000	9-10	Deep black	1.5	3.5	–	–	6.0	2.0	1.0	2	5	10	18	7.3-7.4	clear	~ 0	<0.001
N-3*, 1 *l*	2000<5000	9-10	Deep black	–	–	5.0	–	6.0	2.0	1.0	2	5	10	18	7.3-7.4	clear	~ 0	<0.001
N-3*, 1 *l*	2000<5000	9-10	Deep black	–	–	–	10-15	7.0	2.5	1.5	2	5	10	18	7.3-7.4	clear	~ 0	<0.001

Note: EK-1 – extraction component;
EK-2 – extraction component;
EK-3 – a component that replaces the mixture of EK-1 and EK-2 extraction components;
EKN – mixture of waste oil products containing extraction and coagulation components obtained without purification;
m.p. – mixing period;
o.p.o.l. -obtaining period of organic layer;
p.c.c. -the period of color change;
t.c.p. –treatment completion period;

Table 4. Mode of the technological process, the application of a new ecologically effective method of deep treatment of industrial wastewater in the oil refining industry in mechanical treatment facilities

Components to be used for industrial wastewater treatment												The composition of industrial wastewater after treatment		
Volume flow rate	The amount of amount of mixture of waste oil products in the composition, mq/l	pH	the volume											
			EK-1, ml	EK-2, ml	EK-3, ml	EKN component, ml	K-1 solution, ml	K-2 solution, ml	K-3* solution, ml	The period of the water color change	treatment completion period	The amount of amount of mixture of waste oil products in the composition, mq/l	Organic compounds mixture amount, mg/l	pH
1000 m^3/hour 24000 m^3/day	500–1000 500–1000	9–10	0.3 l 7.2 l	0.7 l 16.8 l			5.0 l 120.0 l	2.0 l 48.0 l	0.1 l 2.4 l	20 min	20 min	0	<0.001	7.3–7.4
1000 m^3/hour 24000 m^3/day	500–1000 500–1000	9–10			1.0 l 24 l		5.0 l 120.0 l	2.0 l 48.0 l	0.1 l 2.4 l	20 min	20 min	0	<0.001	7.3–7.4
1000 m^3/ hour 24000 m^3/ day	500–1000 500–1000	9–10				5.0 l 120.0 l	5.0 l 120.0 l	2.0 l 48.0 l	0.1 l 2.4 l	20 min	20 min	0	<0.001	7.3–7.4
2000 m^3/ hour 48000 m^3/ day	1000–2000	9–10	0.6 l 14.4 l	1.4 l 23.6 l			10.0 l 240.0 l	4.0 l 96.0 l	0.2 l 4.8 l	20 min	20 min	0	<0.001	7.3–7.4
2000 m^3/saat 48000 m^3/ day	1000–2000	9–10			2.0 l 48.0 l		10.0 l 240.0 l	4.0 l 96.0 l	0.2 l 4.8 l	20 min	20 min	0	<0.001	7.3–7.4
2000 m^3/ hour 48000 m^3/ day	1000–2000	9–10				10.0 l 240.0 l	10.0 l 240.0 l	4.0 l 96.0 l	0.2 l 4.8 l	20 min	20 min	0	<0.001	7.3–7.4
120000 m^3/ day	2000–5000	9–10			5.0 l 120 l		600.0 l	240.0 l	12.0 l	20 min	20 min	0	<0.001	7.3–7.4
120000 m^3/ day	2000–5000	9–10				600.0 l	600.0 l	240.0 l	12.0 l	20 min	20 min	0	<0.001	7.3–7.4

* – when required.

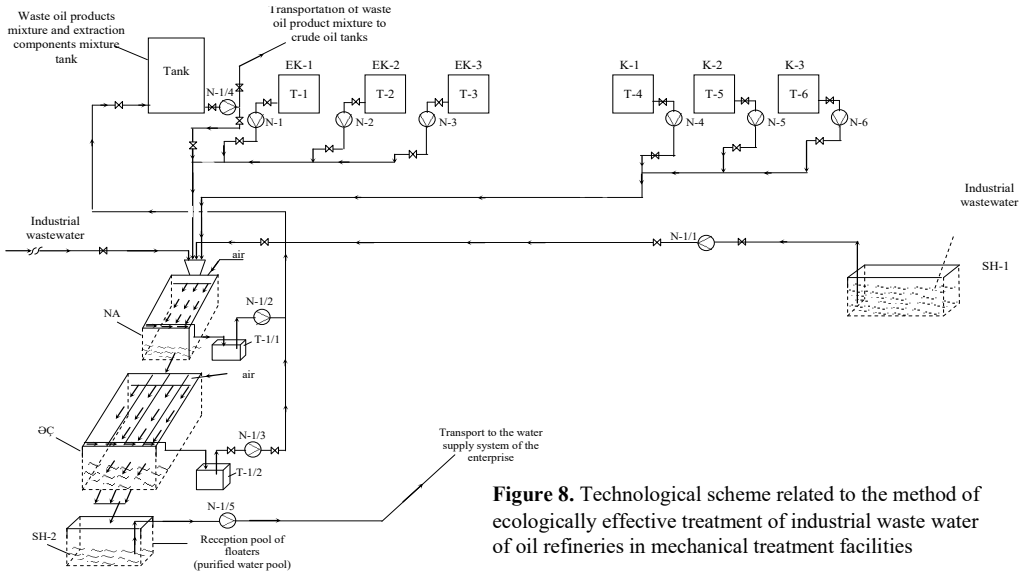


Figure 8. Technological scheme related to the method of ecologically effective treatment of industrial waste water of oil refineries in mechanical treatment facilities

Explanation:

SH-1 – receiving water pool of industrial waste water of oil refineries
SH-2 – receiving water pool of treated industrial waste water
Ç – Waste oil products mixture and extraction components mixture tank
T-1 – tank for the extraction component EK-1
T-2 – tank for the extraction component EK-2
T-3 – tank for the extraction component EK-3
T-4 – - tank for the component K-1
T-5 –- tank for the component K-2
T-6 –- tank for the component K-3
T-1/1 – underground capacity for accumulation of oil refinery waste mixture in oil separator neft ayırıcıda
T-1/2 – underground capacity for accumulation of oil refinery waste mixture in additional precipitator
N-1/5 – The pump for the transfer of industrial waste water purified by a new method from the SH-2 pool to the circulating water system

N-1 – transfer pump for the component EK-1
N-2 – transfer pump for the component EK-2
N-3 – transfer pump for the component EK-3
N-4 – transfer pump for the component K-1
N-5 – transfer pump for the component K-2
N-6 – transfer pump for the component K-3
N-1/1 – Transportation of industrial wastewater from SH-1 to treatment plants
N-1/4 – transfer pump for the Waste oil products mixture and extraction components mixture from tank 1
N-1/2 – transfer pump for the Waste oil products mixture and extraction components mixture from T-1/1
N-1/3 – transfer pump for the Waste oil products mixture and extraction components mixture from T-1/2

NA – oil separator

ap – additional precipitator

Conclusion

For the first time we have MOPW with the highest concentration of IW in mechanical treatment plants from a 5% solution of $Al_2(SO_4)_3$ as a coagulant from petroleum ether as a coagulant in a specially defined amount of extractant, flogulant (in some cases also pH_2 9–10 neutralizer)an environmentally friendly method has been developed to completely clean up to 100% of MOPW and dependent substances using 5% H_2SO_4 acid as a neutralizer and 5% $NaHCO_3$ as a neutralizer if required. Using this method, it is justified that there is no need to clean the IW formed in the ORI with flotates at other stages by physicochemical and biological purification methods. The new method is developed has of great ecological and economic importance.

References

Abrosimov, A.A. (2002). Ecology of hydrocarbon systems processing. Ed. dr. chem. sciences, prof. M.Yu. Dolomatov, Dr. tech. sciences, prof. E.G. Telyasheva. Moscow: Chemistry, 608. (in Russian).

Bukhgalter E.B., Golubeva I.A., & Lykov O.P. and others. (2003). Ecology of the oil and gas complex: Textbook in 2 volumes. Volume 1, Ed. ed. Vladimirova A.I. and Remizova V.V. M.: GUP Publishing House "Oil and Gas" Russian State University of Oil and Gas. THEM. Gubkina, 416.

Bayakhmetova, Z.K., Nurgaliyeva, G.O., & Dzhusipbekov, K. (2011). Purification of oil-containing waste waters using humacite-containing composite materials. Collection of materials of the All-Russian Youth Conference "Innovations in Chemistry: Achievements and Prospects. Kazan: KSTU, 6.

Gentsler, G.L., & Sharkov, A.M. (2004). Waste water treatment in the oil refining industry. Ecology and Industry of Russia, (10), 15 - 17.

Glueckstern, P. (2000). Desalination by reverse osmosis in Izrael. Euromembrane: Program and abstracts of conf., Jerusalem, Sept. (24 – 27), 38 - 39.

Glukhova, M.V., & Kudinov Yu.S. (2003). Fuel and energy complex of the Russian Federation and environmental safety. M.: CJSC "Publishing House Novy Vek", 172.

Hadjieva, S.R., Shamilov, N.T., Bayramov, G.I., & Rakida, N.M. (2021). In the country, the Azerbaijani National Academy of Sciences, the News Magazine. The Chemical Ecological Effect of Petroleum Production, 3(82), 80–83.

Hadjieva, S.R., Shamilov, N.T., Bayramov, G.I., Samadova, A.A., & Rakida, N.M. (2021). Effective cleaning of manufacturing wastewater formed in the petroleum industry by means of coagulation methods. Azerbaijani National Academy of

Sciences Ghana Department, News Magazine. No. 3(83) pages 56-60. Senior Bulletin. No.7. c-5

Ilyin V.I., & Kolesnikov V.A. (2006). Denisova M.A. Improving the technology of wastewater treatment from oil products and surfactants using the electroflotation method. Environmental Protection in the Oil and Gas Complex. No. 5. S. 3–4.

Khaidarov, F.R., Khisaev, R.N., & Shaidakov, V.V. etc. (2005). Ecological problems of the oil industry. Ufa: LLC "Publishing house of scientific and technical literature "Monograph", 190.

Komissarov, Yu.A., Gordeev, L.S., & Nguyen, X.N. (2002). Analysis and synthesis of water supply systems for chemical industries. Moscow: Chemistry, 496 p.

Ksenofontov, B.S. (2004). Purification of water and soil by flotation. M.: New technologies, 224.

Kuchera, J. (2007). Reverse osmosis is an important technology for water purification. Oil and gas technologies, (3), 101–102.

Magid, A.B., & Kuptsov, A.V. (2006). The main implemented developments of the State Unitary Enterprise "Institute of Petrochemical Processing of the Republic of Belarus" for the treatment of wastewater at refineries. World of Oil Products, (2), 13 - 14.

Magid, A.B., Kuptsov, A.V., & Rasvetalov, V.A. (2002). Modern facilities for physical and chemical treatment of oily wastewater. Corrosion protection and environmental protection, (3), 3–5.

Magid, A.B., Telyashev, E.G., & Kuptsov, A.V. (2007). Environmental technologies developed by the department of ecology of the State Unitary Enterprise INCP, introduced and proposed for implementation. Environmental Protection in the Oil and Gas Complex, (2), 37 - 39.

Nevsky, A.V., Meshalkin, V.P., & Sharnin, V.A. (2004). Analysis and synthesis of water resource-saving chemical-technological systems. Responsible ed. Labutin A.N. M. Nauka, 212.

Sapina, V.N., & Temnikova, E.V. (2011). Patent 2415815 Russia. MPC CO2F3, 02. Wastewater treatment method,. Bul.10.

Shapkin, N.P. and others. (2012). Patent 2440931 Russia. MPC CO2F1, 465. Wastewater treatment method, Bul. 3.