Full Length Research Article
Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 15/02/2024;
Date Revised: 17/03/2024;
Available Online: 31/03/2024;

Author's Affiliation:
1. Department "Biological safety", Faculty "Veterinary medicine", Kazakh National Agrarian Research University, Almaty – Kazakhstan
2. School of Biology, Aristotle University of Thessaloniki, Thessaloniki – Greece
3. Veterinary Research Institute, ELGO DEMETER, Thermi, Thessaloniki – Greece
4. Department "Veterinary Medicine", Faculty "Agriculture", NJSC "Mukhtar Auezov South Kazakstan University", Shymkent - Kazakhstan

***Corresponding Author:**
Gulmira Tulepova
Email: erketai.91@mail.ru

How to Cite:
Tulepova G, Makhashov E, Ekateriniadou L, Nurkhojayev N, Sadiev S (2024). Developing a Nano Platform for Bovine Brucellosis Diagnostic Product. Adv. Life Sci. 11(2): 533-538.

Keywords:
Brucellosis; Brucella; Outer membrane proteins; Omp16

Open Access

Developing a Nano Platform for Bovine Brucellosis Diagnostic Product

Gulmira Tulepova[1]*, Edil Makhashov[1], Loukia Ekateriniadou[2,3], Nurzhan Nurkhojayev[4], Sagypash Sadiev[1]

Abstract

Background: Brucellosis is one of the most common zoonoses caused by Brucella species. Brucella is an intracellular pathogen that causes abortion in domestic animals and brucellosis in humans. The disease has no specific pathognomonic signs; hence a prompt and accurate diagnosis of the disease plays a very critical role in preventing brucella transmission and treating the disease.

Methods: Research was carried out at the Department of Veterinary Science of Kazakh National Agrarian Research University and in the Molecular Biology laboratory of the Institute for Plant Biology and Biotechnology under the Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan. Studies were carried out on a killed culture of the vaccine strain *Brucella abortus* RB19, genomic DNA of *B. abortus* RB19, Grapevine virus A, plasmid vectors for DNA cloning, a fluorescent nano marker, and Brucella membrane proteins.

Results: The research demonstrated that brucellosis remained one of the most common zoonotic diseases worldwide. This disease not only resulted in significant economic losses in agriculture but also posed a serious threat to public health.

Conclusions: The study successfully established prokaryotic expression vectors for efficient protein production, validated through sequencing and Western blotting, and proposed a promising nano-platform for plant-based synthesis of brucellosis proteins, with potential implications for diagnostic kit development and vaccine research in agriculture.

Introduction

Brucella abortus is a gram-negative facultative intracellular bacterium of the genus Brucella that is a common cause of brucellosis in cattle [1-3]. Brucellosis is characterized by several symptoms including fever, headache, joint pain, and fatigue [4-6]. The disease can persist for weeks to months and can lead to serious complications if left untreated [7-9]. Brucellosis poses a serious public health problem, especially in developing countries where control measures are limited and where the disease can have a significant impact on both human health and livestock productivity [10-13].

Despite advances in veterinary medicine, diagnosing brucellosis remains challenging due to its non-specific symptoms and the complexity of differentiating between vaccinated and infected animals. Recent advancements in nanotechnology and genetic engineering offer promising avenues for developing more accurate diagnostic tools.

Brucellosis presents a serious problem for the livestock industry [14-16]. It can give rise to reproductive problems in animals such as abortion, stillbirth, and infertility [17,18]. In dairy cattle, infected cows may show decreased milk production [19,20]. In addition to economic losses, infected animals end up being culled, which affects the genetic potential of the herd [21]. Animals with brucellosis produce antibodies against the field strain of the brucellosis pathogen [22,23]. Vaccinated animals also produce antibodies against brucellae of the vaccine strain [24,25]. However, diagnostic drugs on the global market are unable to differentiate antibodies produced to the vaccine strain from those produced to the brucellosis field strain [26]. As a result, vaccinated animals are diagnosed with the disease and slaughtered as per veterinary requirements [27-29].

This study aimed to create a platform for the development of brucellosis antigens in plants for a future diagnostic kit for bovine brucellosis.

Methods

Research was carried out at the Department of Veterinary Science of Kazakh National Agrarian Research University and in the Molecular Biology laboratory of the Institute for Plant Biology and Biotechnology under the Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan in 2018-2021.

The research protocol was discussed and approved at a meeting of the Ethical Commission of Kazakh National Agrarian Research University, Department of Obstetrics, Surgery, and Biotechnology of Reproduction on January 4, 2021.

The objects under study include a killed culture of the vaccine strain *B. abortus* RB19, genomic DNA of *B. abortus* RB19, Grapevine virus A, plasmid vectors for DNA cloning, a fluorescent nano marker, and Brucella membrane proteins to create a diagnostic test and a vaccine against brucellosis in cattle.

Detection of antibodies

The study employed a wide range of biochemistry, molecular biology, and bioinformatics methods. Importantly, the research methodology relies on VLps virus particle/antigenic peptide production technology and fluorescent polarization technology for the detection of antibodies against brucellosis in blood, serum, plasma, and milk. The diagnostic kit is based on peptide sequences mimicking *B. abortus* Rb19 vaccine strains, which are coupled to regions of viral VLP particles for subsequent synthesis in plants. A new Viron-Brucella diagnostic kit produced by a plant is purified and used as a viral protein diagnostic kit. The gene encoding the brucellosis surface antigen *Omp16* is introduced into the plant using Grapevine virus A (vector).

Detection of brucellosis

The outer membrane protein (Omp) is an important organelle of Gram-negative bacteria that performs many tasks and is crucial as a protective antigen. A universal method used for molecular weight separation and protein analysis is sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

To detect brucellosis based on recombinant *B. abortus* outer membrane protein 16kDa (rOmp16) as an antigen, the Omp16 gene of *B. abortus* was first synthesized and cloned in the pET-19b vector and then expressed in Escherichia coli cells. The expression was subjected to SDS-PAGE, immunoblotting, and western blotting.

Omp16 for diagnostic purposes proved to be a valuable marker for serological diagnosis of brucellosis. The immunogenicity, specificity, and susceptibility to various diagnostic methods contributed to the effectiveness of Omp16 in detecting brucellosis in both humans and animals.

The surface antigens Omp16 and Omp25 of the brucellosis vaccine strain RB19 were cloned in a bacterial system and their nucleotide sequences were sequenced. Their expression was also obtained.

DNA manipulation

DNA was extracted from a killed culture of *B. abortus* 19 vaccine strain using a DNA extraction kit (PureLink™ Genomic DNA Mini Kit, Invitrogen). *E. coli* strain TOP10F was used as a host for gene cloning, sequencing, and storage of the recombinant plasmid. The pET-19b cloning vector was employed for the

cloning and sequencing of the amplified gene. The full-length open reading frame of the Omp16 gene (530 bp) was amplified using Taq DNA polymerase (Thermo Fisher Scientific) and genomic DNA of B. abortus as a template. Specific primers were designed based on the DNA sequence of Omp16 retrieved from the NCBI GenBank database. Polymerase chain reaction (PCR) was conducted using a Real Time StepOnePlus thermocycler (Applied Biosystems), following the manufacturer's instructions for preparing the reaction mixture. DNA denaturation was performed at 94, 62, and 72°C, and the final DNA extension was performed at 72°C over 10 minutes.

Prokaryotic and Soluble Expressions

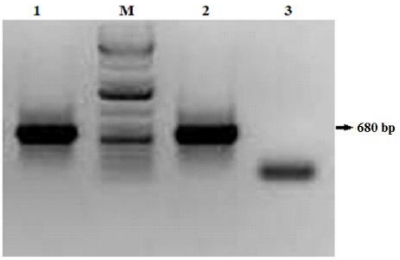
In the process of the research, the prokaryotic expression vectors *pET-19b-OMP25* and Omp16 were established and induced for expression in *E. coli*. Prokaryotic expression has simple requirements and high expression efficiency, but some proteins can integrate with host cell membrane cells, thereby reducing the expression level of foreign proteins in cells. Because of the importance of high levels of recombinant protein production in immunological studies, this hybrid was cloned into the pET-19b expression vector.

The expression of soluble proteins is influenced by several factors, such as enhanced aeration of the bacterial mass, its volume, and alterations in induction conditions and incubation temperature. Improved aeration of the bacterial culture contributes to elevated expression levels and solubility of the target proteins. The integrity of the cloning was validated through sequencing of the cloning product.

Results

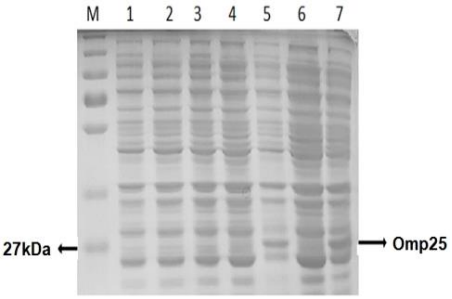
The validation of our Omp16 and Omp25 was confirmed through sequencing, SDS-PAGE, and western blotting analyses. The Omp25 gene was amplified, and its accuracy verified via agarose gel electrophoresis (Figure 1). Following successful amplification, the products were ligated into the cloning vector pET-19b and transformed into competent TOP10F E. coli cells. The integrity of the recombinant plasmids was confirmed through restriction enzyme digestion (NdeI and XhoI). Subsequently, sequencing of pET-19b+Omp25 was conducted using specific primers. The size of the Omp 25 gene is 642 bp, while the size of the noncomplementary primer nucleotides matrix containing restriction sites is 38 bp.

Western blotting using antibodies has shown specific reactivity with the purified rOmp25 produced in *E. coli* cells. The Omp16 family may not only play a part in protective humoral immunity but also induce specific cellular immunity. This brucella outer membrane protein can be recommended as a basis for the development of diagnostic and vaccine preparations, a brucellosis subunit vaccine, or a DNA vaccine.



M – marker 1Kb plus DNA ladder (Thermo Fisher Scientific);
1-2 – amplified fragment of *Omp25* gene DNA;
3 – negative control.
Figure 1: Amplification of the *Omp25* gene with specific primers.

The expression and purification of the recombinant protein were successful. Upon induction with IPTG, the expected size of the rOmp25 protein, 42 kDa (comprising 25 kDa for Omp25 and 17 kDa for pET-19b), was observed. Analysis of lysate from induced TOP10F E. coli and the purified protein through SDS-PAGE (10%) revealed the presence of the expected recombinant protein with a molecular mass of approximately 42 kDa (Figure 2).



M – marker PageRuler™ Plus Prestained Protein Ladder (Thermo Fisher Scientific);
1-3 – negative controls;
4 – expression control, clone 1;
5 – expression test, clone 1;
6 – expression control, clone 2;
7 – expression test, clone 2.
Figure 2: Electrophoresis of proteins isolated from *E. coli* cells in denaturing PAG (12.5%).

Discussion

In recent years, there have been many attempts to identify novel immunogens in the proteome of bacteria of the genus Brucella using immune proteomics approaches [30, 31]. The selection and production of new candidates for the development of diagnostics are the principal practical steps in the introduction of new diagnostic drugs and vaccines [32]. The major OMP proteins in Brucella spp. perform the role of immunogenic and protective antigens. Omp25 is one of the chief proteins in the OMP class in Brucella spp.

The research indicates that Omp25 plays a role in virulence, as evidenced by the weakening of *B. abortus* when infected with the B. abortus Omp25 mutant. Previous studies have reported that Omp25 inhibits TNF-α production in human macrophages infected with *B. suis* [33]. Previous reports have indicated that the DNA vaccine Omp25 from *B. melitensis* provides protection against infection with a wild-type virulent strain of *B. melitensis* in mice [34]. In our investigation, we selected the Omp25 gene, a dominant antigen of *B. abortus* RB19, for cloning, expression, and molecular analysis as a crucial component of the nano-platform alongside Grapevine virus A. To achieve high expression of the recombinant protein for immunologic studies, we used the positively charged expression vector pET32(a)+. The pET plasmid vector is widely recognized as the most efficient system for cloning and expressing recombinant proteins in *E. coli*. Specifically, the pET32 vector is optimized for high-level expression of peptide sequences when fused with Tha•Tag™ proteins, which consist of 109 amino acids.

Cloning sites embedded in crosslinked proteins for expression also contain sequences of His•Tag® and S•Tag™ for cutting, which is essential for marker identification and subsequent purification. The target genes are inserted into pET plasmids, which contain robust bacteriophage T7 transcriptional and translational signals, allowing for controlled expression. Expression is triggered by the presence of T7 RNA polymerase in the host cells. This polymerase is highly active, such that upon full induction, nearly all cellular resources are devoted to expressing the target gene. The results show that brucellosis remains one of the most prevalent zoonotic diseases on the planet that not only causes large economic losses to the livestock industry but also poses a significant public health problem [35]. In our study, the prokaryotic expression vectors *pET-19b-OMP25* and Omp16 were established and induced for expression in *E. coli*. Prokaryotic expression has simple requirements and high expression efficiency, but some proteins can integrate with host cell membrane cells, thereby reducing the expression level of foreign proteins in cells. Because of the importance of high levels of recombinant protein production in immunological studies, this hybrid was cloned into the pET-19b expression vector [36].

Soluble protein expression is associated with many factors, including increased aeration of the bacterial mass, its volume, and changes in induction conditions and incubation temperature. Increased aeration of the bacterial culture enhances both the expression level and solubility of target proteins. The integrity of the cloning was confirmed through sequencing of the cloning product. Additionally, sequencing results, along with SDS-PAGE and western blotting analyses, validated the expression of Omp16 and Omp25.

The creation of a nano-platform where genes encoding surface antigens can be expressed in plants, specifically a nano platform to synthesize brucellosis proteins in plants, will enable the development of a diagnostic kit that will differentiate infected animals from those vaccinated against brucellosis. The proposed platform relies on VLps virus particle/antigenic peptide production technology and fluorescent polarization technology for the detection of antibodies against brucellosis in blood, serum, plasma, and milk.

The platform combining the gene of Grape virus A and the gene of brucellosis antigen is a unique development, as it allows the synthesizing of Brucella membrane proteins in plants. This, in turn, provides a basis for developing a diagnostic kit against brucellosis in agricultural animals. The described platform may also provide a foundation for developing other chimeric proteins important for agriculture, including vaccines against particularly dangerous animal diseases.

Acknowledgments

Work was carried out as part of a research project under the budget program 217 "Development of Science" on the topic "Development of ViroN-Brucella diagnostic kit to combat bovine brucellosis in the Republic of Kazakhstan"

Author Contributions

Gulmira Tulepova: Gulmira Tulepova contributed to the conceptualization and design of the study, the acquisition and analysis of data, and the drafting and revision of the manuscript. She played a key role in the development and optimization of the nano-platform technology for synthesizing brucellosis proteins in plants.

Edil Makhashov: Edil Makhashov participated in the experimental design, data collection, and analysis. He contributed to the optimization of prokaryotic expression vectors for the efficient expression of target proteins in E. coli. Additionally, Edil was involved in drafting and revising the manuscript.

Loukia Ekateriniadou: Loukia Ekateriniadou provided expertise in nanotechnology and assisted in the development of the nano-platform technology. She contributed to the interpretation of results and provided critical input during manuscript preparation and revision.

Nurzhan Nurkhojayev: Nurzhan Nurkhojayev contributed to the experimental design and execution, particularly in the validation of the synthesized brucellosis proteins using sequencing, SDS-PAGE, and

Western blotting techniques. He also participated in data analysis and manuscript revision.

Sagypash Sadiev: Sagypash Sadiev played a role in the conceptualization of the study and provided guidance throughout the research process. He contributed to the interpretation of results and critically reviewed the manuscript for intellectual content and accuracy.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

1. Abdullayev R, Karibaev T, Amantaev S, Zhumabayeva B. Brucellosis in Kazakhstan: current situation, challenges, and prospects. Revue Scientifique et Technique, (2020); 39(2): 545-555.
2. Arntzen CJ. Plant-made pharmaceuticals: from 'Edible Vaccines' to Ebola therapeutics. Plant Biotechnology Journal, (2015); 13(8): 1013-1016.
3. Corbel MJ. Brucellosis: an overview. Emerging Infectious Diseases, (1997); 3(2): 213-221.
4. Xu J, Dolan MC, Medrano G, Cramer CL, Weathers PJ. Green factory: plants as bioproduction platforms for recombinant proteins. Biotechnology Advances, (2012); 30(5): 1171-1184.
5. Lico C, Chen Q, Santi L. Viral vectors for production of recombinant proteins in plants. Journal of Cellular Physiology, (2008); 216(2): 366-377.
6. Hull R. Matthew's Plant Virology. 2002; 895. Academic Press, San Diego.
7. Vlasov PK, Komissarova NV, Kirikova MA, Atabekov DA. Sozdanie virusnogo vektora na osnove virusa A vinograda dlia ekspressii geterologichnykh genov [Creation of a viral vector based on Grape virus A for expression of heterologous genes]. Molecular Genetics, Microbiology and Virology, (2006); 1: 31-37.
8. Chen Q, Lai H, Hurtado J, Stahnke J, Leuzinger K. et al. Ot rasteniia k preparatu: novaia tekhnologiia dlia proizvodstva biologicheski aktivnykh substantsii [From plant to drug: a new technology for the production of biologically active substances]. Technologies of Living Systems, (2013); 10(2): 55-67.
9. Loh TY, Moon KB, Kim YS. Recent advances in plant-based VLP technology. Plant Biotechnology Reports, (2017); 11(3): 155-167.
10. Fischer R, Emans N. Molecular farming of pharmaceutical proteins. Transgenic Research, (2000); 9(4-5): 279-299
11. Hefferon KL. Plant virus expression vectors set the stage as production platforms for biopharmaceutical proteins. Virology, (2012); 433(1): 1-6.
12. Maclean J, Koekemoer M, Olivier AJ, Stewart D, Hitzeroth II, Rademacher T, Fischer R, Williamson AL, Rybicki EP. Optimization of human papillomavirus type 16 (HPV-16) L1 expression in plants: comparison of the suitability of different HPV-16 L1 gene variants and different cell-compartment localization. Journal of General Virology, (2007); 88(5): 1460-1469.
13. Peyret H, Lomonossoff GP. When plant virology met Agrobacterium: the rise of the deconstructed clones. Plant Biotechnology Journal, (2015); 13(8): 1121-1135.
14. Sahithi K, Reddy KV, Reddy KVR, Ananda Kumar P, Sudheer Kumar K, Reddy BK, Sudhakar K, Krishna MR, Reddy KS. Production of rotavirus-like particles (RLPs) in plants: a promising strategy for the development of oral vaccines against rotavirus. Molecular Biotechnology, (2019); 61(9): 686-695.
15. Cassataro J, Pasquevich KA, Estein SM, et al. Vaccination with the recombinant Brucella outer membrane protein 31 or the rough lipopolysaccharide RB51 protects mice against Brucella melitensis challenge. Vaccine, (2005); 23(31): 3947-3952.
16. Cloeckaert A, Vizcaíno N, Paquet JY, Bowden RA, Elzer PH. Major outer membrane proteins of Brucella spp.: past, present and future. Veterinary Microbiology, (2002); 90(1-4): 229-247.
17. Reshetnikova T, Krylova T. Serological and hematological studies of the blood of calves in the experimental use of the medication triazavirin. Advances in Life Sciences, (2023); 10(2): 265-269.
18. Ali A, Derar DR, Alhassun TM, Zeitoun MM. A comparison of the oxidant-antioxidant status of serum and seminal plasma from infertile male camels after zinc, selenium, and vitamin E treatment. International Journal of Veterinary Sciences, (2024); 13(2): 172-175.
19. Raketsky VA, Nametov AM, Sozinov VA, Baisakalov AA. Increasing the efficiency of the herd reproduction system by introducing innovative technologies into dairy farming in Northern Kazakhstan. Veterinary World, (2021); 14(11): 3028-3037.
20. Gnezdilova L, Fedotov S, Muradyan Z, Rozinsky S, Reddy Gade R. Effect of mycotoxins on reproduction and milk quality of dairy cattle and its amelioration by natural adaptogen "Betulin". Qubahan Academic Journal, (2023); 3(4): 400-408.
21. Jain-Gupta N, Contreras MA, Pacheco S, et al. Expression and immunogenicity of the Brucella Omp16 antigen delivered as a fusion to an alfalfa mosaic virus coat protein. Clinical and Vaccine Immunology, (2011); 18(3): 476-482.
22. Beishova I, Nurgaliyev B, Belaya A, Chuzhebayeva G, Ulyanov V, Ulyanova T, Kovalchuk A, Dushayeva L, Murzabayev K, Taipova A, Zholdasbekova A, Isabaev A. Marking of genetic resistance to chlamydia, brucellosis and mastitis in Holstein cows by using polymorphic variants of LTF, MBL1 and TLR9 genes. American Journal of Animal and Veterinary Sciences, (2023); 18(2): 89-97.
23. Shevchenko P, Rychshanova R, Suleimanova K, Bermukhametov Z, Miciński J, Brel-Kisseleva I. Breeding qualities of Aberdeen-Angus cows of different genotypes in Northern Kazakhstan. American Journal of Animal and Veterinary Sciences, (2023); 18(3): 217-222.
24. Reshetnikova TI, Zenkin AS, Krylova TG. Experimental use of the triazavirin antiviral medication in conditions of group administration at the pig-breeding unit. Advancements in Life Sciences, (2021); 8(4): 381-386.
25. Rabah IM, Nossair MA, Elkamshishi MM, Khalifa E. Serological and molecular epidemiological study on ruminant brucellosis in Matrouh Province, Egypt. International Journal of Veterinary Sciences, (2022); 11(1): 82-90.
26. Karabassova AS, Sultanov AA, Saduakassova MA, King DP, Ludi AB, Browning CFJ, Wilsden G. Toward the calibration of serological assays using sera collected from cattle and sheep following a single dose of foot-and-mouth disease vaccine. Veterinary World, (2022); 15(2): 524-530.
27. Saaidi M, Khorshidi A, Gargari SLM, et al. Expression, purification, and in vitro evaluation of the Brucella abortus outer membrane protein 16 (Omp16) in Escherichia coli. Molecular Biology Reports, (2019); 46(4): 4155-4162.
28. Alizadeh H, Dezfulian M, Rahnema M, Fallah J, Esmaeili D. Protection of BALB/c mice against pathogenic Brucella abortus and Brucella melitensis by vaccination with recombinant Omp16. Iranian Journal of Basic Medical Sciences, (2019); 22(11): 1302-1307.
29. Golchin M, Mollayi S, Mohammadi E, Eskandarzade N. Development of a diagnostic indirect Elisa test for detection of Brucella antibody using recomenbinant outer membrane

protein 16 kDa (rOMP16). Veterinary Research Forum, (2022); 13(3): 387-391.
30. Beishova I, Nurgaliyev B, Belaya A, Chuzhebayeva G, Ulyanov V, Ulyanova T, Kovalchuk A, Dushayeva L, Murzabayev K, Taipova A, Zholdasbekova A, Isabaev A. Marking of Genetic Resistance to Chlamydia, Brucellosis and Mastitis in Holstein Cows by Using Polymorphic Variants of LTF, MBL1 and TLR9 Genes. American Journal of Animal and Veterinary Sciences, (2023); 18(2): 89-97.
31. Karabassova AS, Sultanov AA, Saduakassova MA, King DP, Ludi AB, Browning CFJ, Wilsden G. Toward the calibration of serological assays using sera collected from cattle and sheep following a single dose of foot-and-mouth disease vaccine, Veterinary World, (2023); 15(2): 524-530
32. Gavrilova NN, Sadanov AK, Seitbattalova AI, Bayakyshova K, Kaptagai RZ. Selecting Active Strains of Lactic Acid Bacteria Antagonistic to the Pathogens of Brucellosis. Annals of Agri-Bio Research, (2023); 24(2): 255-259.
33. Boko KC, Ibrahim AI, Akpo Y, Alladassi ME, Aguidissou NO, Zoclanclounon A, Farougou S. Sero-Prevalence of Rift Valley Fever and Brucellosis in Cattle of Northern Benin, West Africa. American Journal of Animal and Veterinary Sciences, (2022); 17(4): 267-273.
34. Gnezdilova L, Laga V, Marzanova S, Yarygina E, Pozyabin S, Selina M. Molecular Genetic Assessment of Nodular Dermatitis Virus in Cattle Herds. Advances in Life Sciences, (2024); 11(1): 125-129.
35. Glazunov Y, Kabitskaya Y, Glazunova L, Donnik I, Boyko E, Vinogradova Y. Participation of Dermacentor Reticulatus Imago in the Reservation of Bovine Leukemia Virus. OnLine Journal of Biological Sciences, (2022); 22(4): 456-462.
36. Omelchuk N. Effect of The Rs-10 Radioprotector on Protein-Steroid Interaction in Irradiated Animals with Biphasic Adrenocortical Response to Irradiation. Qubahan Academic Journal, (2023); 3(4): 70–76.