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COMPARATIVE STUDY ON THE RESPONSES OF TWO LINES OF SUGAR BEET (Beta vulgaris L) TO SALINITY

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ABSTRACT

Sugar beet is a crop able to resist high levels of soil salinity after emergence and establishment. Considering the significant difference in the effect of nitrogen forms on sugar beet performance under normal conditions, the form of nitrogen may affect the performance of sugar beet plants under abiotic stress, particularly salinity. Additionally, exploring the most appropriate type of nitrogen for sugar beet could mean optimizing sucrose content. Therefore, here using two lines, sugar beet was grown in pots (filled with 4 kg soil), salt-resistance (line 7233- p.29 x Mst), and salt-sensitive (line 3929-21939), the effect of two different forms of nitrate in the form of calcium nitrate (1 g per pot) and ammonium in the form of ammonium sulfate (1 g per pot) under normal and salt stress condition (40 Millimoles per liter sodium chloride) were evaluated. The result revealed the positive influence of nitrate over ammonium by indicating higher dry weight in both sensitive line: 19.2 and 13.6 g, and tolerance line: 20.4 and 13.6 g, respectively alone and in combination with salinity stress. Similarly, root yield levels positively influenced by nitrate treatment either alone or under salinity stress (sensitive line: 194.5 and 243.2 g and tolerance line: 207 and 249.5 g). The outcomes additionally showed the accumulation of proline aerial parts in both lines, and however, the proline accumulation of sensitive line was higher (3.9 mg/g dry weight). Moreover, induction of proline aggregation was considerably higher in nitrate nitrogen-treated sensitive line (9.3 mg/g dry weight). The absence of significant difference was obseved between nitrogen treatments in terms of extractable sucrose and root molasses sugar. Also, the root impurities increased in those treated with nitrate-nitrogen and salinity. It can be concluded that nitrate-nitrogen has improved the performance of both sugar beet lines against salinity stress, and its practical application is adviseable.

CALCULATION FOR REDEMPTION OF COMPACT TESTING BY THE PROCTOR METHOD THROUGH NEWTON'S GRAVITATIONAL POTENTIAL ENERGY

TIMOSHIN, Anton; DOROFEEV, Aleksei; ERSHOV, Kirill; PUSTOKHINA, Inna; EMELINA, Elena

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ABSTRACT

An indicator of the health of the human body in the state of the oral mucosa. Mechanical and chemical factors constantly influence it. At the first stage of the study, a comparative analysis of the frequency of various forms of traumatic injuries of the oral mucosa was carried out. The distribution of patients into groups was also carried out, depending on the medicinal product used. Then clinical examinations were carried out. After that, the therapy of traumatic erosive and ulcerative lesions of the oral mucosa began. The developed method for treating traumatic lesions of the oral mucosa with medicine based on phytoecdysteroids provides for eliminating the traumatic factor, applying ointment based on phytoecdysteroids to the dried out focus twice a day. The use of phyto-ointment leads to complete repair of traumatic erosive and ulcerative lesions of the oral mucosa on average by the eighth day from the start of treatment; a similar effect with the use of "Solcoseryl dental adhesive paste" is achieved by the tenth day, and the gel "Cholisal Dental" - at a later date, which is confirmed in this study. The most significant positive effect on the level of quality of life associated with the effectiveness of treatment of traumatic erosive and ulcerative lesions of the oral mucosa in comparison with the dental "Solcoseryl dental adhesive paste" and the gel "Cholisal Dental" is exerted by phyto-ointment, where a decrease in the total points was recorded. When conducting routine examinations of patients, it is necessary to pay attention to the oral mucosa damage. Moreover, in treating traumatic injuries of the oral mucosa, it is recommended to use phytoointment, which contains phytoecdysteroids.

EVALUATION OF IMMUNE SYSTEM STIMULATION WITH VACCINE PREPARED AGAINST INDUCED BREAST CANCER IN ALBINO MICE

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ABSTRACT

This study was designed to prepare vaccines. Cancer vaccines promote the destruction of cancer cells, and the cancer cells contain special antigens on their surface when the vaccine is given, it acts as an antigen to activate the immune system. The IL-2 stimulates the response to a type of cells called T-cell, and outer membrane vesicle (OMV) targeting cancer cells by stimulating the immunity to respond with it two types including innate and adaptive immunity, that lead to stimulating the immune system to reduce mammary adenocarcinoma induction in lab mice using T4-1 cell line breast cancer by taking blood and serum to evaluate the immune system efficacy. The tumor induction success by monitoring mice body weight loss showed the lost weight began in the third week after tumor induction, so 23.2 g at first and second week to 14.35 g at the end of the fourth week, whereas the control animals were weighed 32.37 g. The immunity system efficacy results appear a difference in blood and serum parameters after cancer induction. The result shows an increase in total WBC and monocytes (5900, 0.2 cells/mm respectively) but non significantly decreased in neutrophils and lymphocytes count (2.6, 5.9 cells/mm, respectively). Therefore, the first and second doses of vaccines increased the antibody and complement of the immune system compared with control. While Eliaza data for cytokines profile referred to elevated IL2 (26.5 pg/ml) in the serum of vaccination mice but only significantly decreased in IL6 and IL-22 amount (20.4 and 19.6 pg/ml respectively) comparing with control.

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EXPERIMENTAL SUBSTANTIATION OF MANURE FRACTIONATION ON PIG FARMS USING A SPIRAL-SCREW MECHANISM

KLASNER, Georgy Georgievich; SPIRIDONOV, Alexandr Maximovich

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ABSTRACT

The main vector of mechanization and automation of livestock farming at the present stage of technological development of producers is the improvement of resource-saving technologies and technical devices that enables agricultural producers to produce relatively expensive and high-quality equipment to improve conditions for the animals. This article was considered a method for effective fractionation of manure on pig farms to further obtain humus for soil fertilization. The optimal conditions for the performance of the presented gadget were identified, namely: the time spent by the manure mass in the rotor is 0.1, with a separation factor of 170 to 180, the partition for the filter is made of metal sheet with holes whose diameter varies from 0.8 to 1.5 mm and a thickness of no more than 1 mm. The device presented in the manuscript has several advantages in the form of automation, low energy consumption and cost, novelty, and high efficiency.

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TO AUTHORS

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY

UTILIZATION OF PROTEOMICS APPROACH TO UNDERSTAND GENES ASSOCIATED WITH THE OCCURRENCE OF BIOTIC STRESS IN PLANTS

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ABSTRACT

Of the most fundamental fields of modern biology is transcriptomics, with a focal point on the expression pattern of plants under various conditions by assessing ribonucleic acid. So far, this approach has been a game-changer in revealing the gene structure, function, and most importantly, their cellular and biological role. Considering the criticality of pathogens for crop plants, understanding plant defense mechanisms against them is in high demand. This study aimed to review the principles of these approaches and their recent application in the plant. An Important method to address this gap is transcriptomics, which can effectively provide insight into plants against pathogens. This field has covered different aspects of plant biology besides the plant-pathogen relationship. Identifying pathogens in infected plants and the series of reactions they provoke at the gene level is crucial to finding the responsible gene (s). Finding the gene associated with resistance or vulnerability to a specific pathogen paves the way to differentiate the potential genotypes. Thus, the breeding attempts would be more successful. The advancement in biotechnology has revolutionized this field with some of the methods that have been commonly applied in studies on the plant-pathogen relationship, for instance, Northern blotting, microarray, real-time polymerase chain reaction.

Keywords: *plant-pathogen relationship, gene expression, polymerase chain reaction, microarray method, SAGE*

1. INTRODUCTION:

The precise and controlled pattern that causes different genes to be expressed in other cells and at different times is called the gene expression pattern. Different expression of genes in cells causes differences in leaf and root cells in plants or humans, causes differences in liver and muscle cells, and differentiates a healthy cell from a cancer cell. However, the question that arises is how researchers find out genes that are on and when these genes are turned on or off. Gene expression is an active phenomenon, and the same genes may function differently under various conditions. Simply put, it can be said that two organisms have identical genotypes but indicate different phenotypes, which is due to differences in the expression of various genes (Gibson, 2005; Anderson and Kedersha, 2009). Researchers often use laboratory methods such as Northern Blot or serial analysis of gene expression (SAGE) to answer various gene expression questions, especially defense genes in plants under stress. Most of these methods make it possible to identify genes that are turned on and off in the cell; consequently, this information can indicate the conditions that led to these genes expression (Ohtsu *et al.*, 2007; Weber *et al.*, 2008). The main groups of pests and diseases that damage plants include fungi, bacteria, nematodes, viruses, and insects. Plants protect themselves through various tools, including structural barriers that act as physical barriers and produce secondary metabolites with antimicrobial activity (Ambrose and Belanger, 2012). Plants can activate their immune system after understanding the pathogen. This speed of these processes is one of the most critical factors in the success of resistance in plants (Fregene *et al.*, 2004).

Activation of resistance genes leads to subsequent changes at the infection site and systematically throughout the plant, causing physical changes in the cells such as more lignin production and higher plant strength (Venu *et al.*, 2007). The essential part of effective plant defense against pathogens is the rapid induction of plant defense genes. A significant group of defense genes is genes encoding pathogenic proteins (PR). While the function of some PR protein genes is still unclear, some of them have chitinase and

glucanase activity, which are cell wall compounds. Other induced defense genes include diphenhydramines, enzymes involved in the biosynthesis of phytoalexins, plant protection enzymes, and messaging components such as specific transcription factors (Q. Zhu *et al.*, 1994; El Ghaouth *et al.*, 2003; Suryadi *et al.*, 2014). Induction of defense genes in plants responding to pathogens occurs primarily at the transcriptional level. Secondly, the regulation of temporal and spatial expression patterns of defense genes is an essential part of plant defense. Identifying message transmission components for the expression of plant defense genes, including crucial key transcription factors, has raised the hope that these factors are a reliable tool for increasing plant resistance to a wide range of pathogens. The studies have mainly been on the model plant, Arabidopsis, which will eventually lead to the transfer of these results to crops using recombinant DNA methods (Bolwell, 1999; K. B. Singh *et al.*, 2002; De Palma *et al.*, 2019).

The pathways involved in the expression of plant defense genes are regulated by several defense messenger molecules, including salicylic acid, ethylene, nitric oxide (NO), jasmonic acid, and reactive oxygen species (ROS) such as hydrogen peroxide. ROS and NO are critical signaling molecules during the hypersensitivity reaction, and their activity together seems to cause local death of plant cells (Ma *et al.*, 2018; De Palma *et al.*, 2019; N. Li *et al.*, 2019). A significant increase in salicylic acid levels at the site of infection and to a lesser extent in other parts of the plant and salicylic acid as a spray on the plant increases the plant resistance to many pathogens. Transgenic plants unable to accumulate salicylic acid due to the lack of a bacterial enzyme that converts salicylic acid to its inactive form cannot develop systemic acquired resistance (SAR) and induction of defense genes (A. Singh *et al.*, 2017; Kamle *et al.*, 2020). Rapid defense genes and their changes are obtained in different stress conditions with the pathogen (Yao *et al.*, 2020). In the early days of the study of gene expression, researchers mostly studied the expression of a single gene or a few of genes over a period of time. With the help of new methods, methods that make it possible to study many genes have recently been used. Fortunately, new techniques have made it possible to study gene expression on a large scale (H. Chen and Vierling, 2000; López-Maury *et al.*, 2008). It is essential to study the expression of plant defense genes during stress concerning pathogens. The results of this research increase information related to the mechanism of tolerance and resistance to pathogens and facilitate the production of tolerant cultivars through biotechnological methods (Nelson *et al.*, 2004; Gutierrez *et al.*, 2008).

Numerous reviews exist on plant response to abiotic and biotic stress. Still, only a few systematic reviews have considered the application of proteomics techniques to unravel the underlying genes involved with these defense responses. Therefore, various popular proteomics methods for analyzing gene expression profiles in plants against pathogens and pests will be discussed in this review focusing on plant defense responses.

2. MATERIALS AND METHODS:

The focal point of this review was proteomics methodologies that had been utilized for elucidating the genes underlying the response mechanisms involved with biotic stress, excluding abiotic stress. The application of mitigating substances in plants against pests and pathogens is not covered in the review. However, the criticality of the topic is appreciate — a period of 1990 to 2020 covered in a systematic literature review. Given the richness of the literature in this area, specificity was taken to be more focused. This review is based on published works that have been conducted on plants, mainly crops or model species.

As it is a critical area of biology, several reviews have been published. However, the level of focus on plant defense systems is quite a different one. Scopus, ScienceDirect, ResearchGate and Google Scholar databases were used. The search was done in the English language. Specific keywords have opted to retrieve many results after identifying proteomics methodology to understand the abiotic stress-associated genes. The chosen keywords were: “abiotic stress”, “plant-pests relationship”, “plant-pathogen relationship”, “genetic mechanism in plants”, “influence of pests and pathogens on plants”, “microarray”, “real-time PCR”, “SAGE method”, “proteomics of plant”, “plant signaling against pathogens”, “inducing plant defense system”, “simultaneous defense systems in plants”, “gene families”, “northern blotting”, “southern blotting”, “northern blotting hybridization”, “rice”, “arabidopsis”, “wheat”, “maize”. The terms mentioned above were searched combinations with AND. The keywords were only searched in English.

Major peer-reviewed published papers were used in this paper; however, technical journals, books, and conference proceedings were

included in a couple of cases. After screening 550 abstracts and titles, the 75 references in this paper were selected. Some of the topics chosen were: (1) Northern blotting variants and their application detecting miRNAs expression (2) Seq—quantitative evaluation of gene expression (3) serial analysis of gene expression (SAGE) technology (4) characterization of bacterial isolates producing in plants (5) plant signaling against pathogens and pests (6) gene-level responses to plant-pathogen relationships and (7) plant defense system induction by pests and pathogens.

3. RESULTS AND DISCUSSION:

3.1. Methods of studying the expression of defense genes in plants

There are different methods for determining the quantity and quality of transcripts (Southern, 2006), divided into four general forms: the Northern blot and Northern ballet Reverse; Microarray; Real-time reverse transcription PCR; and the Serial Gene Expression Analysis Method (SAGE).

3.1.1. Methods based on hybridization of Northern blot and reverse Northern blot

The number of mRNA copies of each gene accurately indicates the expression of that gene. Tracing the expression of a gene ultimately suggests the intensity of transcription and expression of that gene. The Northern blotting method is one of the first methods to show differences in the number of mRNAs produced by each gene. This method was invented by James Alvin and Jog Stark at Stanford University in 1979. It was first used to track specific RNA sequences. The name is derived from Southern blotting (Brown *et al.*, 2004; Josefsen and Nielsen, 2011; Rio, 2015). In this method, mRNA is first extracted from biological samples, and then mRNA is isolated from cellular materials, including DNA, proteins, lipids, and other cellular organs. The different mRNA pieces are separated by gel electrophoresis (a method that separates molecules based on weight and electrical charge) and then transferred to a membrane called blotting (Figure 1).

To identify copies of mRNA produced by a specific gene, such as under pathogen stress, the samples would be incubated with a small piece called a probe from single-stranded RNA or DNA sequences. These molecules are labeled using radioactive molecules (Brown *et al.*, 2004; Wang and Yang, 2010). The probe or detector is designed based on the mRNA sequence of the target gene and then bound to the desired sequence. After the probe is bonded in the desired sequence, the probe is exposed to X-rays, and the radiation emitted from it causes a stain on the radiological film. The intensity of the signal on the photographic film shows the researchers how much mRNA was present in the prototype, and the intensity of the stain determines this; in this method of a gene that is expressed during stress, It always remains constant and is used as a fixed gene (Seki *et al.*, 2001; Rabbani *et al.*, 2003; Forment *et al.*, 2005). Another method used to study gene expression is reverse northern blotting, where the nucleic acid is permanently stained on a membrane.

In contrast, the stain is a collection of amplified cDNA fragments or the target gene (Figure 1). In this method, after DNA is stained on the membrane, a hybridization or two-vein reaction is performed using cDNA probes made from RNAs extracted from tissues. Therefore, this method is commonly used to study the expression profile of genes and study the expression of many genes in an organism (Fraser *et al.*, 1994; Barrett and Kawasaki, 2003; Brown *et al.*, 2004).

Some advantages of Northern blotting include a standard and reproducible method for studying gene expression; identify mRNA size; ability to study RNA splicing; ability to study the half-life of RNA; preliminary probes can also be used; and membrane can be held after the reaction is completed and reviewed years later (Dallman *et al.*, 1991). On the Other hand, however, the disadvantages of Northern blotting are: it is difficult to identify multiple probes; if the RNAase enzyme sample is slightly damaged, the quality of the answers and the amount expressions have a false negative problem; and the Standard Northern blotting method is less sensitive than other methods such as Real-time PCR (Dallman *et al.*, 1991; Iskandar *et al.*, 2004).

Navabpour *et al.* (2011) studied the response of spinach and rapeseed to induced stress. In this study, three treatments of methyl viologen, silver nitrate, and 3-Amino-1,2,4-triazole with four different concentrations were used along with control and combination treatment (ascorbic acid + stress treatments). TBARM assay was performed to assess the level of cellular oxidation. Sampling was performed at 48, 24, 12, 6, and 72 hours after all treatments. Study of gene expression and Northern blot hybridization 48 h after the treatment took place. The results showed that all experimental treatments affected the

percentage of dead cells, the amount of TBARM, and the expression of genes through a relative increase in the level of reactive oxygen species (ROS). The Rubisco photosynthetic gene (RBCS) decreased activity with increasing concentration of stress treatments. Ascorbic acid pretreatment spray increased the relative expression of this gene by 51%. Although differences in expression patterns were observed for the other genes studied depending on the plant type and experimental treatments, they generally showed a positive response to stress treatments.

With increasing the concentration of treatments, a linear increase in gene expression was observed (Gholamnezhad *et al.*, 2016). In a study conducted by Ray *et al.* (2013) on the expression of genes involved in resistance to *Septoria tritici* blotch (STB) wheat, it was revealed that resistant cultivars express protein isomerase disulfide 3 hours after exposure to the disease while the increase in the expression of the same gene occurred 96 hours after infection in sensitive cultivars (Maskos and Southern, 1992).

3.2. Microarray method

This approach has been one of the first throughput-high tools to study transcripts over the past two decades (Kuhn, 2001). This method, by creating thousands of profiles of genes, simultaneously improves the study of transcript analysis (Gechev *et al.*, 2004; J. Y. Zhu *et al.*, 2011). Microarray technology can be divided into three general parts:

3.2.1 Step I. Chip design and preparation

At this stage, the necessary information must first be collected from the sequence of genes to be examined, and then, a fragment of a unique gene sequence with a length of 25 bp must be made. By placing these probes, about 1,000 spots can be placed on a chip and test the input, although the chips are readily available for the genes of several organisms on the market (Slonim and Yanai, 2009).

3.2.2 Step II: Reaction Preparation

This involves extracting mRNA and building a cDNA and simultaneously marking it with a fluorescent dye, the steps of hybridization, washing and drying the chip, and finally scanning the microbial chip. At this stage, after selecting the cell or tissue to be studied, mRNA is extracted from it, and then cDNA is made from the extracted mRNAs, then two populations of labeled cDNAs are mixed and hybridized with a DNA chip (Figure 2). Hybridization conditions and the regulation of different temperatures are critical (Nguyen *et al.*, 2002).

3.2.3 Step III: Data Analysis

Analysis of data is possible with the help of relevant software, hardware, and databases. By measuring the intensity difference between these two colors for each point, the results can be analyzed, and finally, the pattern of gene expression in each cell type can be drawn (Walter *et al.*, 2001). These three parts are entirely interdependent and should be tried to be done correctly because otherwise, the test result will not be good. Due to the variety of models and different types of microchips, the micro-data analysis section provides many algorithms and methods of analysis (Wilhelm and Landry, 2009). Micro-analysis allows biological researchers to perform their experiments in the shortest time and on a large scale. With this vast amount of information on gene expression, the relationship between DNA, RNA, and protein can be understood and compared with other organisms. There are pre-prepared kits for different organisms such as humans, mice, Arabidopsis, and many other organisms. Microbiology is used in various branches of science such as medicine, pharmaceutical, food industries, and agricultural sciences. This method is very useful in medical science in diagnosing cancers as well as microbial and viral pathogens. It also has applications in agriculture to diagnose plant pathogens and track changes in the expression of genes involved in the disease resistance process (K. Singh *et al.*, 1990). In 2018, a study was conducted to identify defense genes in rapeseed using microbial cDNA related to Arabidopsis. In this study, the changes in the frequency of 2000 expression labels or EST of Arabidopsis in rapeseed interactions with necrotrophic fungi *Alternaria brassicicola* were examined.

The results of studies on rapeseed were compared with previous results obtained on Arabidopsis. Search for homology using canola plant expression sequence tags from a database with about 6000 unique clones identified defense genes in canola. Genes identified in connection with rapeseed-pathogen interaction included genes involved in active oxygen metabolism, plant resistance genes, regulatory genes, and genes involved in secondary metabolism (Primrose *et al.*, 2001). Kumar *et al.* (2014) used the microarray method with 1,000 sequences of expression tags,

or ESTs, compared the genes involved in the SAR pathway in Arabidopsis. Besides, by knowing the genomic sequences of this plant, they were able to identify the promoters of genes that were expressed in the SAR reaction. Also, they used microarrays that had 1,000 sequences of expression tags, or ESTs.

Some of the limitations of the microarray method include the inability to detect new transcripts; the low dynamic range for transcript recognition; and problems with reproducibility and comparisons between experiments (Bumgarner, 2013).

3.3. Real-time Reverse transcription PCR (Real-time qPCR)

Real-time qPCR means moment-by-moment observation of a process. Along with the need to accurately quantify gene expression, the many problems in semi-quantitative PCR paved the way for a new arena in PCR. The initial design of Time-Real PCR was first carried out by Higuchi et al. (1993). In this diagnostic system, a fluorescent substance is released during the reaction in proportion to the number of products per cycle. The amount of fluorescent is identified and recorded by an indicator (Hadi et al., 2012). The real-time RT-PCR is a suitable and accurate method for studying gene expression. The basis of this method is based on the quantitative measurement of copies amplified in the exponential stage of PCR reaction by measuring the amount of fluorescence light (Huggett et al., 2005).

This method has undergone many changes since its introduction, so that it is now one of the most accurate and fastest methods. Diagnostics are used in many fields of science. The product of this reaction is marked using labeled materials. The sensitivity of this method is higher than the electrophoresis (Klein, 2002), and the dynamic range of detection is increased. There is no need to quickly and hastily perform the PCR process because, in conventional PCR, samples should be stained with ethidium bromide immediately. This method has a high-resolution power to detect changes less than twice as much, while agarose gel resolution is inferior. This method is used to evaluate the exact amounts of DNA and RNA, while it does not have the difficulties of the conventional method (Dallas et al., 2005).

3.3.1 Real-Time PCR Assay

In general, there are several methods for performing quantitative PCR using the Real-time PCR method as follows:

3.3.1.1 The non-specific form

This method is performed using SYBR green binding agents such as DNA-binding agents (Figure 3). This dye is attached by being placed in a small DNA gap. The advantages of this method include cheap, convenience, and sensitivity. One of its major disadvantages is the connection of green SYBR to two strands, such as dimer primer and other non-specific bands that estimate the concentration higher than the original amount. Therefore the optimization of the reaction conditions should be such that the primer Dimmer and non-specific product should be created to a minimum. A melting curve is used to confirm the results of this experiment (Primrose *et al.*, 2001; Pantchev *et al.*, 2010). One of Time-Real PCR advantages is drawing the melting curve, carried out after the PCR process. It is specific for this molecule and depends on DNA structure and factors such as length and number of nucleotides, probe concentration, ambient salt content, and percentage of GC since SYBR Green can differentiate different products using curve melt. After the PCR is completed, the device can draw a melting diagram of each sample by measuring the fluorescence changes at different temperatures (Capote *et al.*, 2012).

3.3.1.2 The specific form

This model uses the FRET mechanism where the probes are designed so that at the beginning of the probe, there is a fluorescent dye called the reporter, and at the end, there is another fluorescence called quencher. When the reporter and the quencher are at a molecular distance close to each other (when connected to a probe), the light coming into the reporter creates an emission whose wavelength is in the quencher excitation region, and it absorbs this light as radiation. Emits at longer wavelengths that the device cannot measure. After the separation of quencher and reporter, the light emitted by the reporter is not absorbed by the quencher, and in this case, the device measures the emitted light fluorescently. In this method, several different probes can be used (Figure 3), including the TaqMan probe, Beacons probe, Scorpion probe, and hybridization probe. Of the essential Real-time PCR applications are Absolute Quantification and Relative Quantification (Mackay *et al.*, 2002; Löfström *et al.*, 2015; Kralik and Ricchi, 2017; X.

Chen *et al.*, 2020).

3.3.2. Applications of Real-time PCR

By using this method, differentiation of plant defense gene expression patterns under various conditions can be made. Differences between different growth stages stressed plants, or between infected plant samples vs. healthy plants have been studied (Gholamnezhad *et al.*, 2016). in a study using the real-time RT-PCR method based on scorpion probe and specific primers, leaf blight virus disease in grapes or nematode carrying them were detected (Hussain and Singh, 2016). In most studies using real-time PCR, the method is based on two methods, the first based on the SYBR Green fluorescence and the other based on the TaqMan detector. These two methods have helped identify plant viruses in different hosts (Santala and Valkonen, 2018). Owing to the absence of protein in the structure of viroids, one of the plant pathogens, real-time PCR and RT-PCR are considered two very reliable approaches for identifying these pathogens (Oliveira *et al.*, 2011).

Real-time PCR based on the fluorescent substance Green-SYBR was used to identify citrus exocortis viroid as well as citrus viroid IIb (Almeida *et al.*, 2018). Control of plant diseases caused by bacterial agents requires very accurate identification methods; using various real-time PCR has accelerated the specificity and sensitivity of identifying plant bacterial agents (Q. Li *et al.*, 2011).

A specific PCR was developed using Green SYBR dye to detect the bacterium Xanthomonas axonopodis citrus canker in 2014 specifically. Another study by Adhikari *et al.* (2020) on the expression of 14 candidate genes in resistance to wheat leaf blight on susceptible and resistant wheat cultivars by real-time PCR showed that the expression of these 14 genes in resistant and sensitive cultivars at different time points. Four genes of chitinase, phenylalanine ammonia-lyase, 1-PR, and peroxidase were expressed in the first 24 hours after infection. These results in seedlings wheat resistance to *M. graminicola* indicated the response is completed 24 days after infection and continues after that, especially in resistant cultivars. So, analysis of the expression pattern of these genes can be a reliable and rapid way to distinguish resistant and susceptible cultivars (Tomlinson *et al.*, 2010). Bilodeau et al. (2 017) evaluated the effects of three different chemical methods, including Green SYBR, TaqMan, and beacons molecules, using beta-tubulin, ITS, and elicitine gene sequences to identify *Phytophthora ramorum*, the cause of sudden death syndrome in elm trees. This study showed that all three methods could separate the isolate 65 of the pathogen from other pathogen species in all infected samples (Feau *et al.*, 2019).

3.4. Serial analysis of gene expression (SAGE)

This method was first used by Velculescu *et al.* (1995) at Johns Hopkins University in the United States. This method is used to generate gene expression profiles for a particular cell or tissue and to identify specific genes expressed under specific cellular conditions. SAGE is also widely used in the study of microorganisms, cancer, and evolution. SAGE method is based on three essential principles (Marioni *et al.*, 2008).

The first principle of using short oligonucleotide sequences (tags) (Figure 4), is about 11 to 27 bp, derived from a specific part of the cDNA and sufficient to identify an mRNA transcript uniquely. The second principle is the sequential connection of tag sequences, which allows serial analysis of transcripts. Tags about 25 to 51 are connected and placed in a vector structure and then sequenced automatically. As a result, the information will be obtained for more than 31-35 different genes by performing a sequencing reaction. The third principle states that the number of times a particular tag is viewed accurately reflects the level of expression of the associated copies. Instead of studying the complete cDNA, a short sequence of 12 bp is provided, each of which is representing an mRNA in the transcript. The basis of this method is the same 12-bp sequences, despite their small size, are sufficient to identify mRNA-encoding genes.

In the next step, the mRNA is converted to a double-stranded cDNA and then treated with a restriction enzyme with a quadruple recognition site such as AluI to cleave the cDNA at many sites. The end-restriction fragments remain attached to the cellulose granules, and the other pieces can be washed to remove them from the column (Matsumura *et al.*, 1999; Yamamoto *et al.*, 2001; Gowda *et al.*, 2004; Hu and Polyak, 2006). A short linker is then attached to the free end of each cDNA. This linker has a BsmEI enzyme recognition site. This enzyme is a unique restriction enzyme that, in addition to cutting its recognition site, also cuts below its identification site at a distance of 11 to 14 nucleotides. Therefore, it treats BsmEI and separates fragments with an average length of 12 bp from each cDNA end. These pieces are collected and connected head-to-tail in a chain and sequenced,

and separate sequences can be identified in the chain; Because BsmEI sites separate them. At the end of the SAGE reaction, the polymer is made up of sequentially linked nucleotide tags and identifiers by locating the enzyme to which they are cleaved. The resulting sequence analysis by the software leads to a list of gene identities expressed in the cell or tissue under study, the frequency of which will be an estimate of their expression. There is now a wealth of SAGE project information in databases (Noureddine *et al.*, 2005; Anisimov, 2008).

Some of the advantages of the SAGE method are: the SAGE allows extensive analysis of mRNA transcripts without prior knowledge of the organism transcriptome; and the sequence of each tag is sufficient to search for it in the database, and the frequency of each tag directly indicates the frequency of the relevant copy (Anisimov, 2008).

3.4.1 Applications of SAGE in plant studies

Biological and non-biological, the study of toxin metabolism and the analysis of tissue or organ expression profiles. However, these studies mainly have been carried out on plant models such as rice and Arabidopsis. The application of SAGE method in studying other plants is expanding (Westermann *et al.*, 2012). a study using the SuperSAGE method attempted to elucidate the interaction of plant host and pathogen. The gene expression profile in both rice and pathogenic fungi was investigated simultaneously. Genes were identified that increased and decreased in expression in response to the pathogen elicitor. Reports have indicated that a large number of genes whose expression is reduced are related to proteins involved in photosynthesis (Matsumura *et al.*, 2005). This review showed that is an advantageous method to check cell transcriptome is the interaction of host and pathogen, especially in organisms whose genome yet to be known (Venu *et al.*, 2007).

4. CONCLUSIONS:

It is imperative to study the expression of defense genes associated with pathogens. The results of the current research available can enable scholars to elucidate the mechanism of tolerance and resistance to pathogens and facilitate the production of tolerant cultivars through biotechnological methods. Pathogenic proteins are produced by molecules such as ethylene, salicylic acid, and phytoalexins, which activate plant defense responses and increase cell wall strength and increase lignin formation. Together, these responses lead to the development or growth in resistance to pathogenic fungi. Specific transcription factors regulate the expression of these proteins. Transcription factors are essential and critical components in controlling gene expression in all living tissues and cause phenotypic diversity and adaptation of organisms during evolution. Thus, obtaining a reliable understanding from the controlling genes involved in defense response to biotic stress requires robust mythologies in transcriptomics that tools such as northern blot, microarray, real-time qPCR, and SAGE can provide. Fortunately, the advancement and introduction of sequencing techniques have been accelerated during the last decade. Therefore significant progress has been predicted, particularly in developing cultivars capable of resisting deadly pathogens.

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6. OPEN ACCESS:

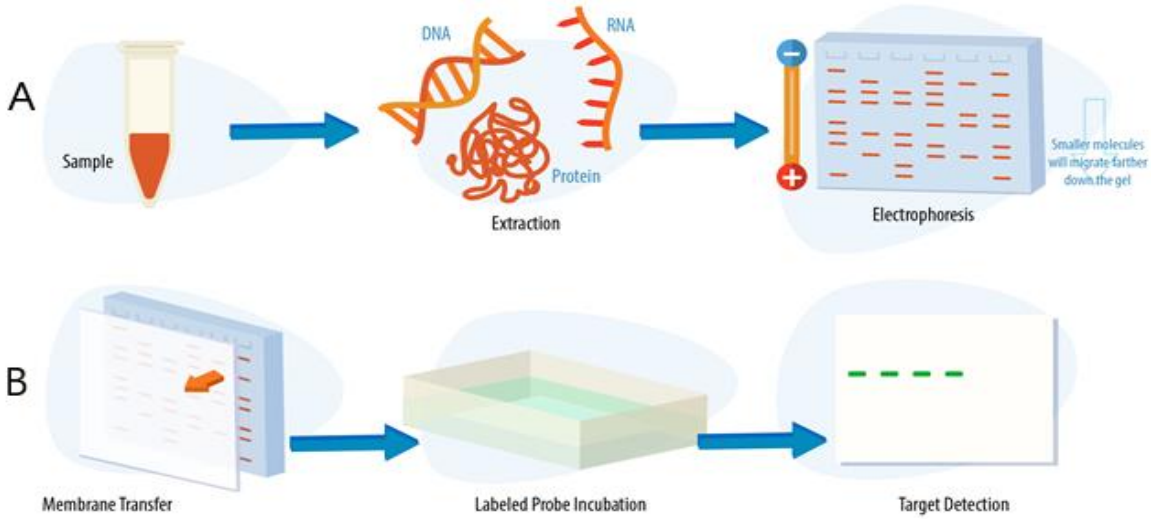

Figure 1. *A schematic of the processes of two types of Northern blot. A) Northern blotting and B) Reverse northern blot. (Retrieved from https://www.labmanager.com/insights/southern-vs-northern-vs-western-blotting-techniques-854).*

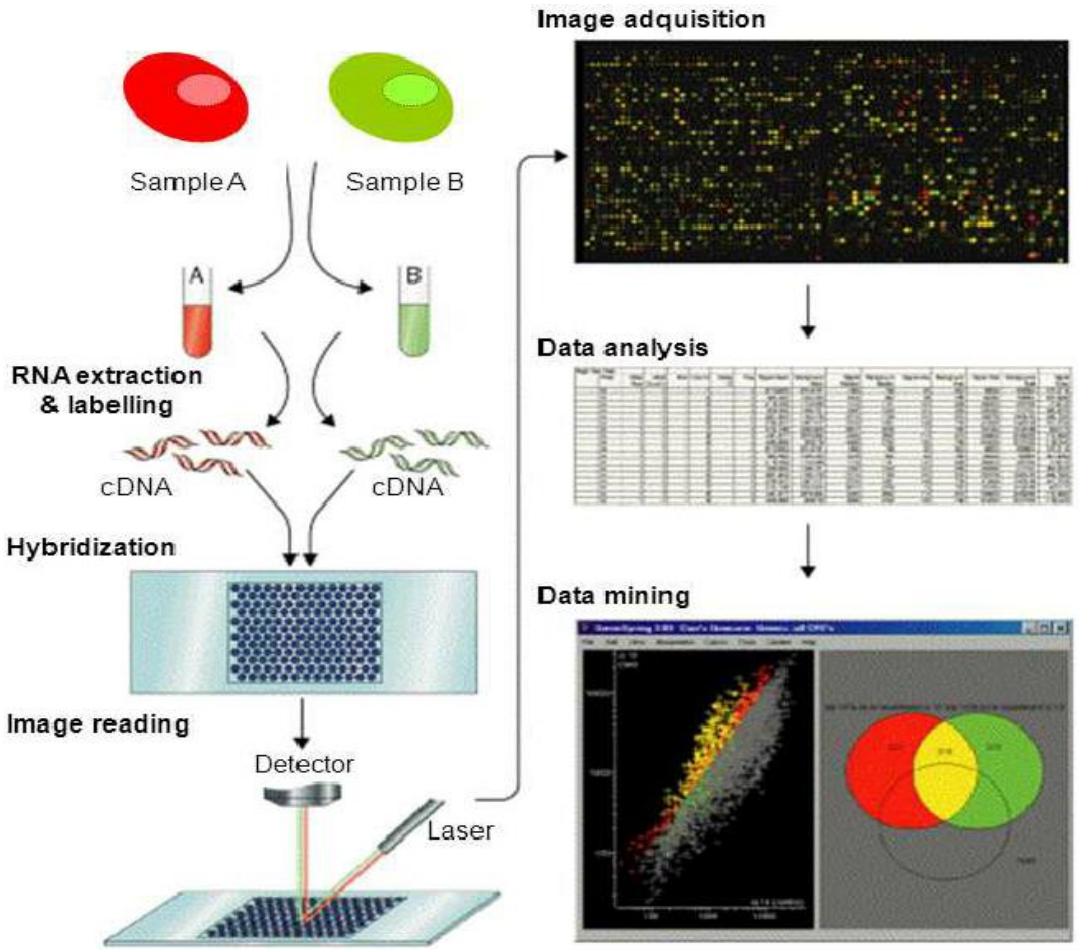

Figure 2. *Schematic displaying the DNA microarray methodology. This method often utilizes for identifying messenger RNAs (mRNA), so-called expression profiling. The approach composed of the fluorescently labelling of RNA while the RNA is transformed into complementary DNA (cDNA) (Lamas et al., 2012).*

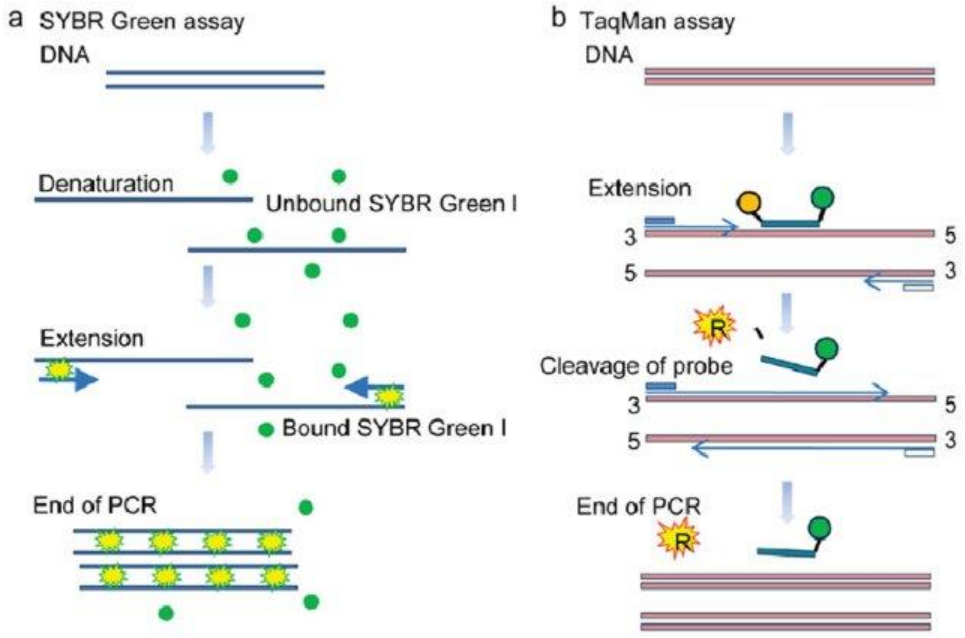

Figure 3. *Real-time polymerase chain reaction (PCR) chemistry: (a) SYBR Green assay, and (b) TagMan (Bae et al., 2013).*

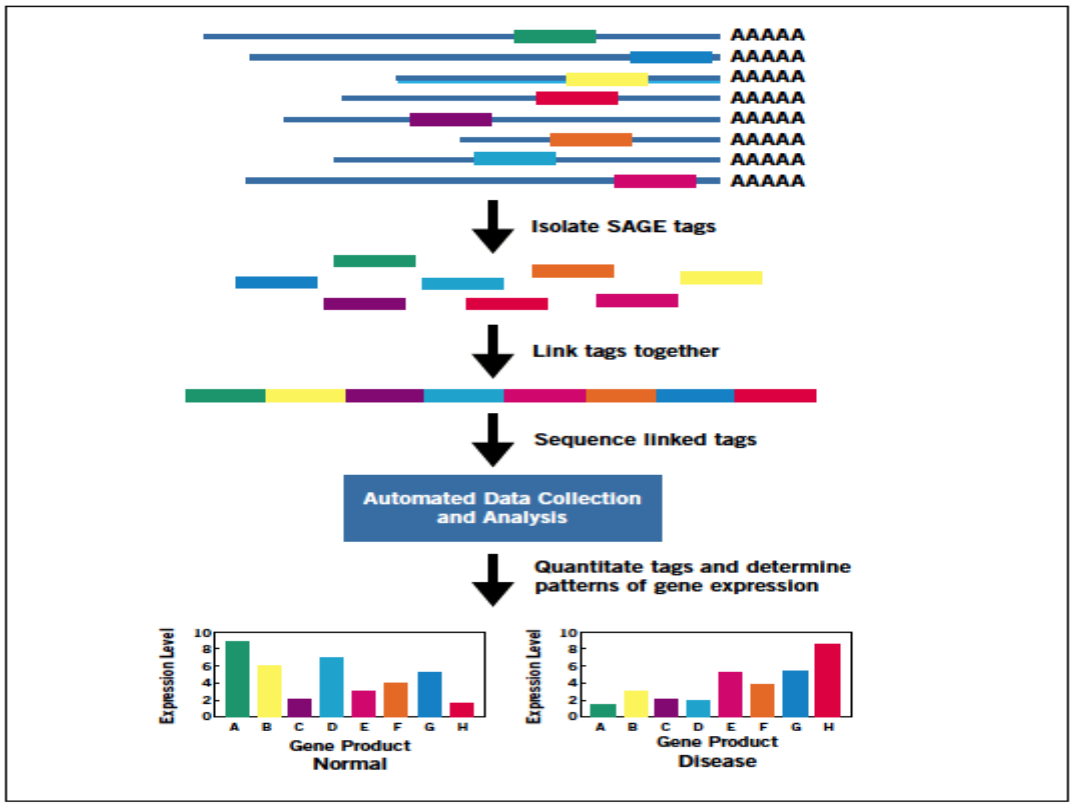

Figure 4. *The SAGE process starts with splicing and transcribing the genes to generate mature mRNA transcripts, then isolate the SAGE tags. The extracted mRNA turns into stable double-stranded–cDNA. Restriction enzymes digest the ds-cDNA to produce 11 linked 'tag' fragments. These tags are then sequenced using long-read Sanger sequencing (Different colors indicate different tags). The transcription of the genes of interest can be reported using their tag frequency and comparing the gene product of the infected sample with the control (Shafee and Lowe, 2017).*

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY

COMPARISON OF THE EFFECT OF SILICON AND SILICON NANO-CHELATE IN REDUCING THE IMPACT OF SALINITY STRESS ON WHEAT SEEDLINGS

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ABSTRACT

Today, salinity stress causes extensive damage to crops, and high soil salinity is one of the limiting factors for crop yields. A practical approach to lessen the negative effect of salinity stress is to use mineral nutrition methods such as spraying plants with silicone. To investigate and compare the effect of silicon and silicon nano-chelate on the wheat plant resistance (Shiroodi cultivar) to salinity stress, a factorial experiment was designed and conducted in a completely randomized design with five replications under hydroponic conditions. Experimental treatments included concentrations of 0 and 2 mmol/L silicon, 0 and 0.424 g/L silicon nano-chelate, 0 and 150 mmol/L sodium chloride, and their interaction. The growth and physiological indices showed that salinity stress decreasing effect on shoot dry weight, root fresh weight, catalase activity, and ascorbate peroxidase. These increases indicate the activation of the plant defense system against salinity stress conditions. The results also showed that silicon nano-chelate treatment under salinity stress reduced dry and fresh weights of roots and shoots. These two compounds additionally influenced the content of catalase activity, ascorbate peroxidase, and superoxide dismutase content in shoots. Simultaneously, the silicon and silicon nano-chelate treatment under salinity stress reduced the dry and fresh weight of roots and shoots, catalase activity, and ascorbate peroxidase. Therefore, the results obtained in this study generally showed that silicon under salinity stress increased plant growth and positively affected the activity of its antioxidant system. But silicon nano-chelate not only did not improve plant performance but also reduced its growth.

Keywords: *salinity stress, silicon, wheat (Shiroodi cultivar), antioxidants, potassium*

1. INTRODUCTION:

High soil salinity is one of the factors limiting crop yields worldwide, which is one of the main problems of the agricultural sector, especially in arid and semi-arid regions. Almost all arid and semi-arid lands in the world and about fifty percent of irrigated lands deal with salinity stress (Jafarzadeh and Aliasgharzad, 2007; Dadkhah, 2011; Fateh and Alimohammadi, 2011). In Iran, saline lands are 18.5 million hectares (Qadir *et al.*, 2008). Due to the limited quality of water resources for the irrigation of crops globally, saline water for agriculture is inevitable. In such circumstances, one of the influential factors in the production and exploitation of saline soil and water is salt-tolerant plant cultivars. The literature is enriched with data on the effect of salinity on the growth and yield of crops and the identification of new plant genotypes with a high tolerance to salinity (especially bread wheat) (Sharma and Minhas, 2005; Letey and Feng, 2007).

Bread wheat is a critically important food plant globally and is the leading food source in arid and semi-arid regions. In these areas, water scarcity is the primary cause, and soil salinity is the secondary factor in reducing plant growth and grain yield (Dreisigacker *et al.*, 2008; Montenegro *et al.*, 2017). The destructive effects of salinity stress, due to the reduction of osmotic potential in the root environment and the impact on plant water balance and reduction of osmotic pressure, have been reported by many researchers at different stages of bread wheat growth (Sairam and Tyagi, 2004; Parihar *et al.*, 2015).

Crops grown in arid and semi-arid regions are often exposed to adverse environmental factors such as drought or high soil salinity. Salinity stress causes morphological, physiological, and biochemical changes in plants (Amirjani, 2010). Salinity reduces the water potential around the roots and reduces the plant water absorption capacity. Besides, with increasing salinity in the root environment, the

uptake and transfer of toxic ions to plant tissues increases, which leads to a decrease in the uptake of essential elements, disturbance of ion balance, and toxicity due to the accumulation of sodium and chlorine ions. Under saline conditions, potassium uptake by root cells is reduced by competition with sodium (Sairam and Tyagi, 2004; Rabie and Almadini, 2005; Tuteja, 2007).

The extent of these changes depends on the salinity level and its application duration (Li *et al.*, 2015). The typical effect of most saline soils is growth inhibition, which can have many causes. Still, this inhibition is often associated with high concentrations of Na+, Cl^- ions, and deficiency of K+ ions (Zhang *et al*., 2010). The indirect effect of salinity on plant growth is to reduce the plant water content. As salinity increases, the potential of soil water decreases. In general, the presence of salt in the soil solution reduces the osmotic potential of the soil. It creates water stress, impairs the uptake of sufficient water for plant growth, reducing growth rate and altering metabolic processes. Leads in plants (Hu *et al.*, 2012). Increasing sodium reduces other cations in the plant and upsets the cation balance of the plant, which in turn reduces the amount of calcium, magnesium, and potassium in the plant (Yan-de *et al*., 2007). At high salinity levels, the amount of potassium ions decreases due to replacement by sodium ions, which in addition to disturbing the ionic balance, also disrupts cellular metabolism. The potassium functions in the plant include colloidal effect, increase hydration, activation of photosynthetic enzymes, osmotic regulation, and its role in opening and closing pores. Potassium ions accumulate in the stomatal cells, causing the pores to open. As potassium escapes from the orifice, the orifices close. This role of potassium is due to the change in the protective cells due to the change in the osmotic potential in these cells (Kafi *et al.*, 2019).

Reactive oxygen species (ROS) are considered a major cell destruction source under biological and abiotic stresses (Candan and Tarhan, 2003). ROS forms oxygen reduction produced in biological processes such as light respiration, photosynthesis, and respiration (Uchida *et al*., 2002). To produce water in these processes, four electrons are needed to reduce oxygen. Still, in ROS, the transfer of one, two, and three electrons to O^2 usually results in superoxide, hydrogen peroxide, and hydroxyl radicals, respectively (Mittler, 2002). These types of oxygen are highly toxic and react with biological molecules such as lipids, proteins, and nucleic acids, causing lipid peroxidation, protein denaturation, and DNA damage (Quiles and López, 2004).

Silicon (Si) is the second most abundant soil element but is not considered an essential component of many plants. Many studies have recently shown that treating plants with Si can dramatically reduce biological and non-biological stresses such as heavy metal stress, salt, drought, cold, and frost and have beneficial effects on growth and plant production (Hu *et al.*, 2012; Kafi *et al.*, 2019; Elsheery *et al.*, 2020).

Recently, the reducing role of silicon in salinity stress has been considered. Besides, some studies have shown that silicon reduces salinity in various plant species such as barley (Ghorbanpour *et al.*, 2020), corn (Marulanda *et al.*, 2010), and wheat (Abbasi *et al.*, 2018). Silicon increases salinity stress tolerance by increasing photosynthetic activity, improving water status, stimulating the antioxidant system, and decreasing sodium uptake or potassium H + -ATPase-dependent increase in shoots (Chalmardi *et al.*, 2014). It has also been shown that silicon may activate superoxide dismutase, peroxidase, and catalase. The reduced malondialdehyde concentration in barley, tomato, and corn has been reported (Jafarzadeh and Aliasgharzad, 2007; Kafi *et al.*, 2019).

The use of nano-chelates has been considered by many agricultural researchers, although their mechanism of action is not well understood (Haghighi *et al.*, 2012). Si nano-chelates has been reported to increase nitrate reductase activity in soybean (Kafi *et al.*, 2019). Studies have also shown that 1 mM Si nano-chelates has a beneficial effect on reducing the harmful effects of salinity stress on tomato seedling growth and germination characteristics. Using 1 mM, Si nano-chelates combined with 25 mM sodium chloride increased seed germination and growth (Li *et al.*, 2015).

This study aimed to increase the resistance of wheat plants to salinity stress by evaluating the effect of silicon and silicon nano-chelate on increasing salinity stress resistance in wheat plants. In this regard, this plant salinity resistance under the application of silicon and silicon nano-chelate is examined and compared.

2. MATERIALS AND METHODS:

The plant material utilized in this was Shiroodi wheat cultivar, which was purchased from Karaj Seed and Plant Breeding Institute. Pots were filled with perlite and cocopeat and then transferred to the hydroponic environment in the

research greenhouse of the Payame Noor University of Najafabad.

2.1. Plant cultivation

First, a mixture of cocopeat and perlite was made in a one-to-one ratio to prepare the required substrate, and then soaked the seeds for 24 h. To prepare the environment, the tray was first filled with a mixture of coco peat and prepared perlite, sown the wheat seeds, then covered it with the prepared mixture and sprinkled water every day. Two weeks after planting, the plants were transferred to a hydroponic environment. Initially, four types of nutrient media were created: complete nutrient solution as a control, silicon-containing nutrient solution, silicon nano-chelate nutrient solution, and a solution containing silicon and silicon nano-chelate.

In each container, two plants were cultivated, and all the containers were connected to the air pump and aerated. Within a month, the containers were filled with the appropriate solutions, and once a week, the plants were sprayed with about 5 mL of iron chelate (to strengthen the growth). Plants were treated with 150 mM NaCl after 1 month and collected two weeks after treatment.

2.2. Measuring the fresh and dry weight of roots and shoots

After separating the roots and shoots, their weights were measured with a digital scale with an accuracy of 0.001 g. the shoots and roots were wrapped in aluminum foil and dried in an oven at 70°C for 48 hours until their weight stabilized. Then the dry weight of the samples was measured.

2.3. Superoxide dismutase (SOD)

This enzyme activity was determined based on its ability to inhibit the photochemical reduction reaction of nitroblue tetrazolium (NBT) at 560 nm based on Giannopolitis and Ries (1977). The reaction solution consisted of 50 mM sodium phosphate buffer (pH = 7.5), 13 mM methionine, 0.1 mM Na-EDTA, 75 µM nitroblue tetrazolium (NBT), 75 µM riboflavin and 100 µl of the plant extract. The reaction solution was exposed to fluorescent light for 14 minutes, and its absorbance was read at 560 nm. The basis of NBT conversion to formazan is in the presence of light and the appearance of purple dye. In the presence of the enzyme, superoxide dismutase inhibits the reaction and reduces the dye intensity. The difference between the control solution adsorption and the solution containing the extract indicates the enzyme superoxide dismutase reaction inhibition. Each unit is equal to the amount of enzyme that inhibits NBT light reduction by 50%. Therefore, this enzyme activity was calculated in units of enzyme per milligram of protein (Unitmg-1protein).

2.4. Catalase activity (CAT)

Catalase activity was assessed considering the rate of H_2O_2 decomposition by measuring the absorption changes at 240 nm (Beers and Sizer, 1952). The composition of the reaction mixture was 0.05 M phosphate buffer (pH = 7), 3% oxygenated water, and 5 µl of enzyme extract. The enzyme activity is expressed as each micromole of decomposed H_2O_2 per minute per milligram of protein.

2.5. Ascorbate peroxidase (APX)

Mhadhbi *et al.* (2004) method was used to measure the APX enzyme. Potassium phosphate buffer (0.05 M) (pH = 7), oxygenated water 0.1 mM, ascorbate 50 µM, and 10 µl of enzyme extract were mixed at 4 °C, and absorption changes at 290 nm were read. Enzyme activity was calculated in terms of each micromole of oxidized ascorbate per minute per milligram of protein.

2.6. Mineral elements

To determine the element content of the leaves, after washing and drying at 70°C for 48 hours and preparation, the samples were digested by oxidation method using 96% sulfuric acid, salicylic acid, oxygenated water, and selenium (Mozafari and Ghaderi, 2018). Weigh 0.3 g of the plant sample, and after transferring to the digestion tube, 2.5 ml of the acid mixture was added to the samples and shaken gently. After 2 hours, the digestion tubes were placed on the stove at 100°C for 2 hours. Afterward, 3 ml of oxygenated water were added to the tubes, and after each addition of oxygenated water, the tube was carefully shaken to react with the oxygenated water. Then raised the temperature to 330°C. The digestion process was completed when the color of the extract became colorless or yellow (this process lasted two h). After cooling, removed the tubes from the oven, added 48.3 mL of distilled water, and strain it after stirring. Potassium was measured by a flame photometer (Jenway C/PFPY) and iron measurement by atomic absorption spectrometer (Shimadzu AA-670) (2).

2.7. Statistical analysis

The experimental design used in this study was a completely randomized design with three replications. Statistical analysis of data was performed using one-way analysis of variance by SPSS software version 18. Duncan's test was employed to compare the mean of treatments at the error level of 0.05.

3. RESULTS AND DISCUSSIONS:

3.1.Biomass

Crops are exposed to salinity stress in many arid and semi-arid areas. The cause of this stress is in the salinity of water or soil and the method of irrigation (Parvaiz and Satyawati, 2008; Ashraf, 2010). Salinity stress severely reduces root and shoot growth in susceptible plants (Fateh and Alimohammadi, 2011). There is ample evidence that when silicon is available to plants, it plays a significant role in growth, mineral nutrition, mechanical strength, and resistance to various stresses (Hu and Schmidhalter, 2005).

The results obtained in this study showed that salinity stress had no significant effect on biomass production (fresh and dry weight). On the other hand, the use of nano-chelate significantly reduced the fresh and dry weight of the plant shoots (3.8±0.61 and 0.9±0.07 g) compared to the control, but on the contrary, silicon significantly increased the fresh and dry weight of the shoots (11.58±1.32 and 2.21±0.01 g, under 150 mmol/L salt; Figure 1a and c). Simultaneous application of silicon and silicon nano-chelate has also significantly reduced the fresh and dry weight of shoots at the level of 5% (6.25±0.93 and 2.73±0.16 g under 150 mmol/L salt) compared to the control (8.63±019 and 0.64±0.03, fresh and dry weight, respectively).

The salinity stress reduced root fresh and dry weight. The silicon nano-chelate application caused a significant reduction in root fresh and dry weight (0.263±0.58 and 4.03±0.38 g) compared to control, but silicon did not have a substantial effect on root fresh and dry weight (9.08±1.08 and 0.443±0.08; Figure 1b and d). Si and Si chelate simultaneous application significantly reduced the fresh and dry weight of roots (5.88±0.26 g). Under salinity stress, silicon nano-chelate decreased, and silicon alone increased the fresh (3.87±0.86 and 9.08±1.08 g, respectively) and dry (0.196±0.41 and 0.443±0.08 g, respectively) weight of roots. Simultaneously applying these two substances in these conditions according to the control caused a significant reduction in fresh root weight (4.78±0.84 g, P = 0.05).

In contrast, in the root dry weight (0.263±0.07 g), no significant effect was observed. Studies by Haghighi *et al.* (2012)showed a decrease in dry weight of roots and stems of four sugarcane genotypes in the presence of sodium chloride due to sodium ion toxicity. They reported that silicon reduced the harmful effects of sodium chloride by reducing absorption and adsorption of Na+ from roots to shoots and improved sugarcane growth (Chen *et al.*, 2010). In the present study, a decrease in fresh and dry weight of roots and shoots of wheat seedlings due to salinity was observed, and the treatment of wheat seedlings with silicon and improved stress conditions and relatively increased fresh and dry weight roots and shoots. In Sorghum bicolor, it was observed that the use of silicone increased the fresh weight of shoots and roots by 19.2% and 10% compared to salinity stress, which is consistent with the results of the present study (Yin et al., 2013). The positive effect of silicon on the fresh and dry weight of shoots in *Cucumis sativus* L. has also been reported (Amirossadat *et al.*, 2012). Investigation on sweet potato plants under salinity stress showed that silicon added to food improves growth inhibition caused by salinity stress (Haghighi and Pessarakli, 2013).

3.2. Antioxidants

The overall activity of catalase (CAT) in the absence of salt stress is only affected by Si nano-chelate (21.7±1.81, Figure 2a). In the salt-stressed wheat seedlings, CAT content increased markedly by 28.83±1.32 (µmol/min/gr FW), a little higher than the combination of Si and Si nano-chelate (27.65±3.15 µmol/min/gr FW, Figure 2a). Seedlings received Si only, indicated the lowest CAT content. The response of ascorbate peroxidase (APX) to Si and Si nano-chelate application was additive. That is, nano-chelate and Si treatment without salinity stress (0.221±0.03 and 0.14±0.01 µmol/min/gr FW, respectively) induced APX content significantly (>5%, Figure 2b). Under salinity stress, the APX content increased particularly in control plants (0.341±0.04, µmol/min/gr FW). Its range also increased in the Si alone level (0.245±0.02 µmol/min/gr FW). In general, superoxide dismutase (SOD) did not respond positively to either Si or salinity stress (Figure 2c). The only significant increase was observed in Si nano-chelate treatment (16.9±2.24 µmol/min/gr FW). While when seedlings were exposed to a combination of salt stress and Si nano-chelate,

SOD content decremented notably (2.61±0.41 µmol/min/gr FW). These results suggest that silicon may protect plant cell membrane structure exposed to salinity stress and prevent its degradation. Therefore, the present study results show that the addition of silicon prevents the oxidative degradation of wheat seedlings exposed to salinity stress. In general, plants have been proven to protect themselves from salinity stress by preventing lipid peroxidation with antioxidant enzymes help (Parvaiz and Satyawati, 2008; Parihar *et al.*, 2015).

Superoxide dismutase, peroxidase, and catalase are the main antioxidant enzymes for scavenging free radicals. Superoxide dismutase probably plays a central role in defending against free radical toxicity (Ashraf, 2010). According to initial reports, in the wheat plant, under salinity stress, by increasing the enzymes superoxide dismutase, peroxidase, and catalase, a certain level of protection against degradation is provided (Chen *et al.*, 2010; L. Hu *et al.*, 2012; Li *et al.*, 2015). These results suggest that salinity stress can cause significant changes in antioxidant enzyme activities in free radicals metabolism (Sui *et al.*, 2010). In the present study, an increase in catalase activity under salinity stress and a decrease in this enzyme activity were observed with the application of silicon and silicon nano-chelate. Free radicals trigger catalase production, and a reduction in the amount of catalase indicates a decrease in free radicals production and thus a reduction of oxidative degradation (Rahneshan *et al.*, 2018). These results suggest that the enzyme catalase is activated by increasing the production of reactive oxygen species (ROS) and adaptation for maize seedlings under stress conditions. The application of silicon and silicon nano-chelate in stressed wheat seedlings reduces catalase activity by reducing the effect of salinity stress and free radicals. In sunflower under salinity stress treated with silicon and silicon nano-chelate, catalase enzyme activity was significantly reduced compared to seedlings under salinity stress (Marulanda *et al.*, 2010; Mozafari and Ghaderi, 2018).

3.3. Potassium and Iron content

Silicon has been reported to improve the nutritional balance of plants under stress (Nwugo and Huerta, 2011). The uptake and transfer of sodium to the roots are greatly reduced by adding silicon to salinity stress conditions. The increase in tolerance to salinity stress by silicon is attributed to the selective uptake and transfer of potassium and sodium by plants (Amirossadat *et al.*, 2012). However, in this study, the content of K in shoots increased only in Si (0.00988 mg/g DW). Whereas, in salt exposed seedlings, the overall content of K decreased significantly (Figure 3a and b) with a combination of salinity and Si+Si nano-chelate had the lowest amount of K (0.0027 mg/g DW, at 5% level). On the other hand, the content of K in root was opposite, in which under free salt treatment, the K content was kept considerably low with no significant difference with control. In seedlings exposed to salinity, Si and a combination of Si and Si nano-chelate with 2.2 and 2.7 mg/g DW exhibited the highest content of K. the Fe content in shoots did not differ with and without salinity (Figure 3 c and d). After control with 0.25 mg/g DW only Si nano-chelate (0.175 mg/g DW) exhibited an increase. In salt-stressed seedlings. However, Si and Si nano-chelate combinations were found to have the highest Fe content in shoots (0.275 mg/g DW). The root Fe content in Si nano-chelate only treatment indicated the highest Fe in shoots (0.237 mg/g DW). In seedlings treated with a combination of chelate and salt, the root Fe content (0.512 mg/g DW) was found to be significantly increased compared to other treatments. Silicon reduces the uptake and transport of heavy metals and salts from the root to the shoot by reducing apoplastic currents (Ma and Yamaji, 2006). Studies by (Liang *et al.*, 2006); Liang *et al.* (2007) have shown that the addition of silicon increases potassium transport and improves the K + / Na + ratio by activating the H + -ATPase pump. The potassium content of silicon-treated corn seedlings increased significantly relative to salinity stress, and silicon improved the salinity effect in the present study. Similar results have been observed in *Spartina densiflora* (Mesa-Marín *et al.*, 2020) and *Saccharum officinarum* L. (Ashraf, 2010).

4. CONCLUSIONS:

In this study, first-hand scientific results were generated in favor of silicon application to mitigate the effects of salinity stress on wheat seedlings and improve seedlings growth and survival under salinity stress. Also, it was observed that the ameliorating results of silicone were considerably higher than nano- chelate silicon. Therefore, it could be suggested to use silicon as an ameliorator of salinity stress effects. However, it's highly advised to use a higher range of salinity treatments and silicone concentrations to pinpoint the optimized level of silicone applicable for agricultural practice in farmlands dealing with salinity stress.

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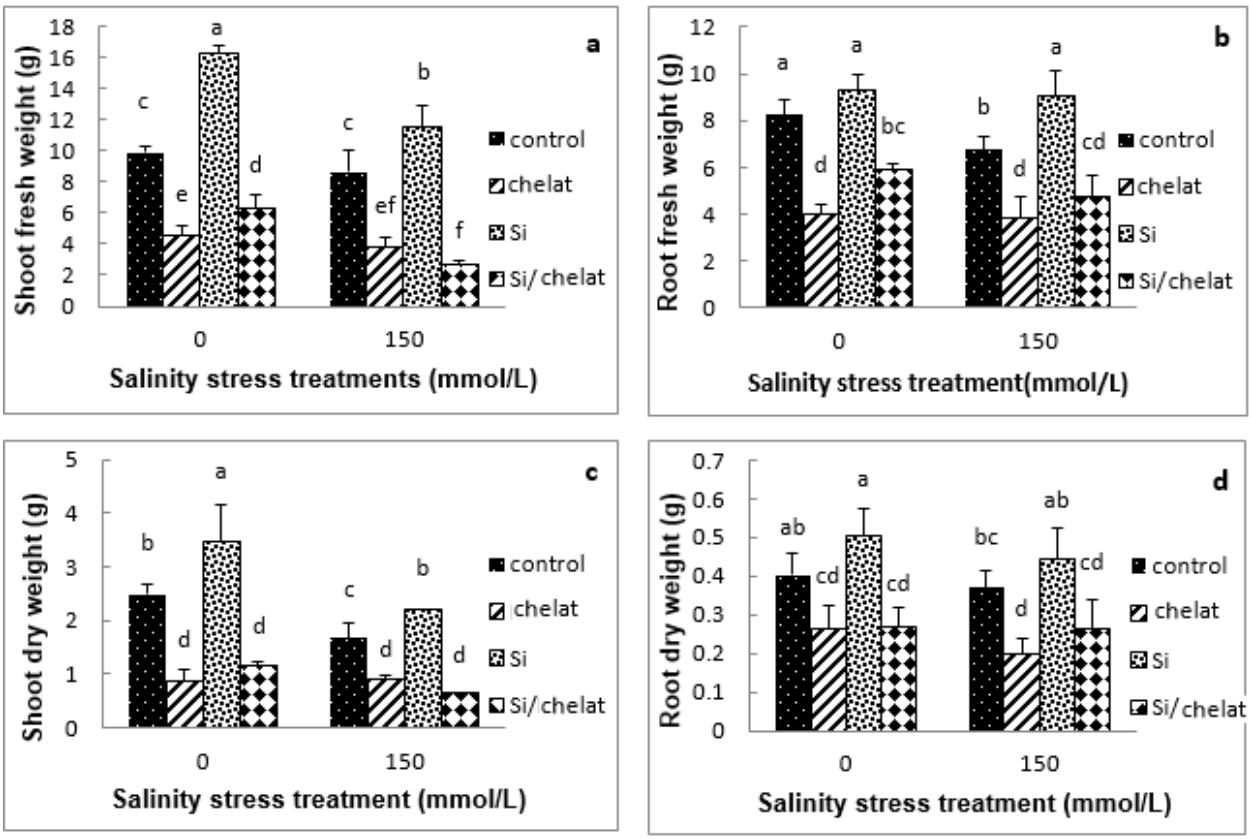

Figure 1. *Effect of Si (2 mmol/L), silicone nano-chelate (0.424 mg/L), salt (150 mmol/L) and their interaction on shoot fresh weight (a), shoot dry weight (c), root fresh weight (b), root dry weight (d). Columns with non-common letters indicate a significant difference between treatments based on Duncan's test ($p < 0.05$).*

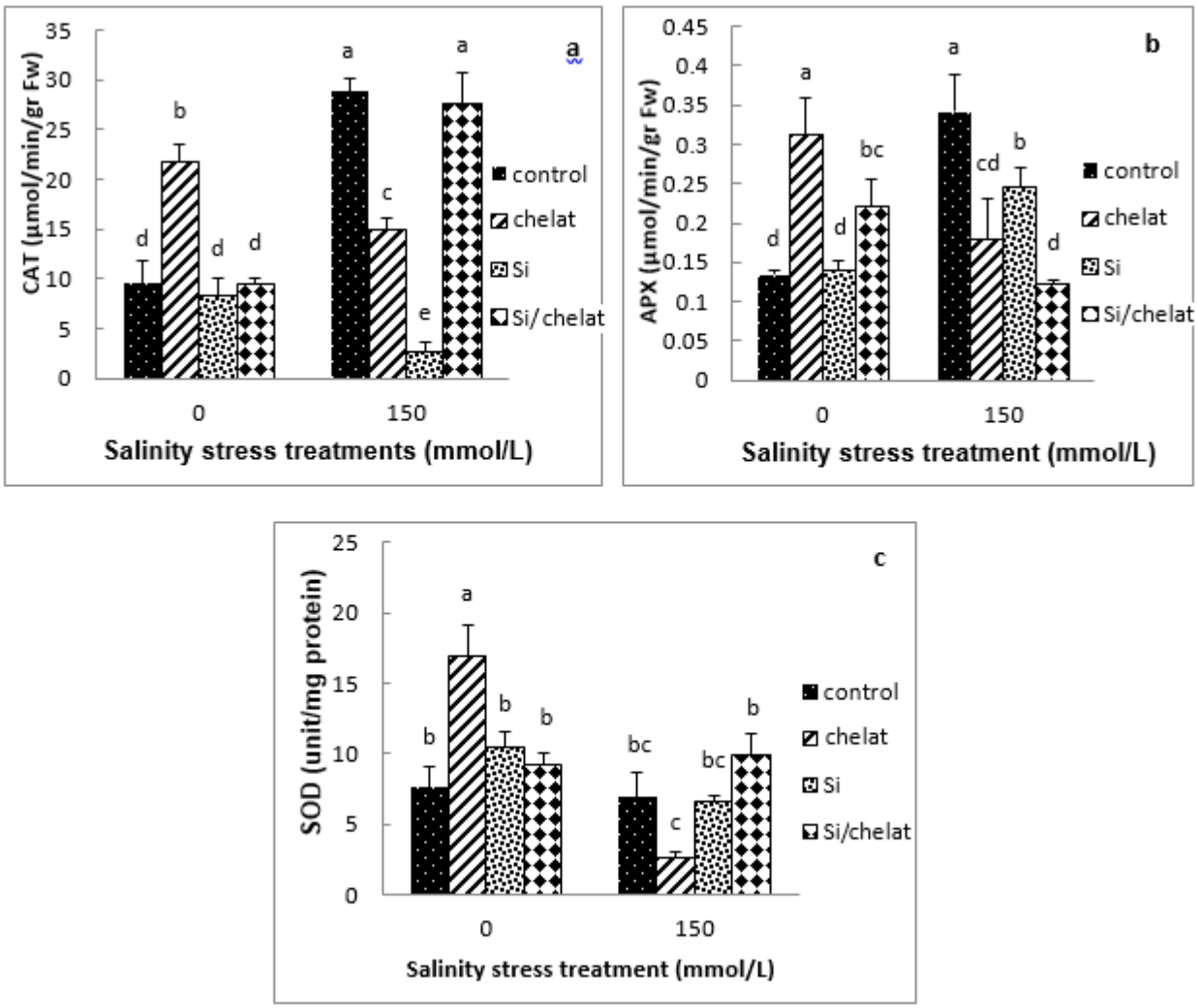

Figure 2. *Effect of Si (2 mmol/L), silicone nano-chelate (0.424 mg/L), salt (150 mmol/L) and their interaction on CAT (a), APX (b), and SOD (c). Columns with non-common letters indicate a significant difference between treatments based on Duncan's test (p <0.05).*

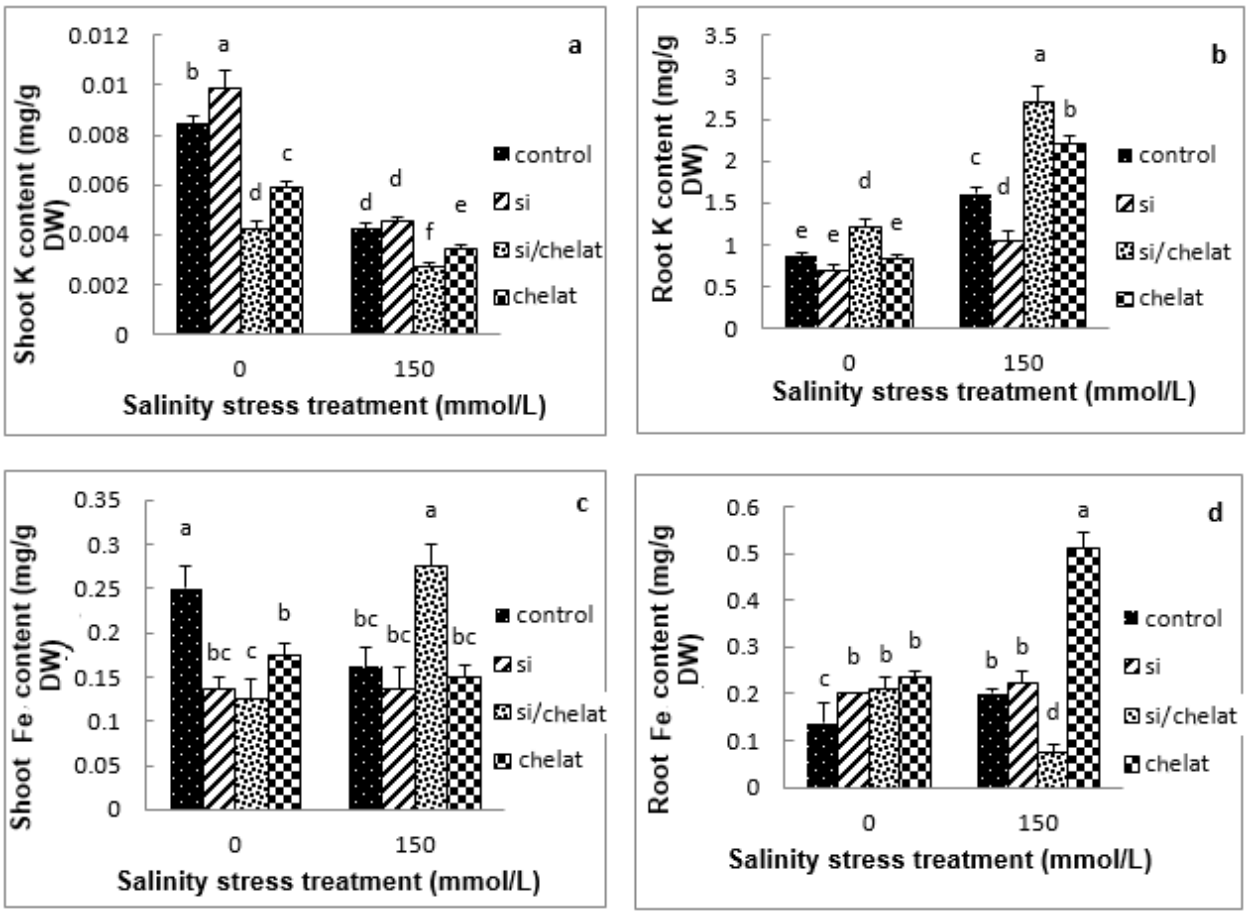

Figure 3. *Effect of Si (2 mmol/L), silicone nano-chelate (0.424 mg/L), salt (150 mmol/L) and their interaction on the content of K in shoots (a), the content of K in roots (b), the content of Fe in shoots (c) and content of Fe in roots (d). Columns with non-common letters indicate a significant difference between treatments based on Duncan's test ($p < 0.05$).*

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY

A COMPARATIVE STUDY ON THE RESPONSES OF TWO LINES OF SUGAR BEET (B*eta vulgaris* L) TO SALINITY

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ABSTRACT

Sugar beet is a crop able to resist high levels of soil salinity after emergence and establishment. Considering the significant difference in the effect of nitrogen forms on sugar beet performance under normal conditions, the form of nitrogen may affect the performance of sugar beet plants under abiotic stress, particularly salinity. Additionally, exploring the most appropriate type of nitrogen for sugar beet could mean optimizing sucrose content. Therefore, here using two lines, sugar beet was grown in pots (filled with 4 kg soil), salt-resistance (line 7233- p.29 x Mst), and salt-sensitive (line 3929-21939), the effect of two different forms of nitrate in the form of calcium nitrate (1 g per pot) and ammonium in the form of ammonium sulfate (1 g per pot) under normal and salt stress condition (40 Millimoles per liter sodium chloride) were evaluated. The result revealed the positive influence of nitrate over ammonium by indicating higher dry weight in both sensitive line: 19.2 and 13.6 g, and tolerance line: 20.4 and 13.6 g, respectively alone and in combination with salinity stress. Similarly, root yield levels positively influenced by nitrate treatment either alone or under salinity stress (sensitive line: 194.5 and 243.2 g and tolerance line: 207 and 249.5 g). The outcomes additionally showed the accumulation of proline aerial parts in both lines, and however, the proline accumulation of sensitive line was higher (3.9 mg/g dry weight). Moreover, induction of proline aggregation was considerably higher in nitrate nitrogen-treated sensitive line (9.3 mg/g dry weight). The absence of significant difference was obseved between nitrogen treatments in terms of extractable sucrose and root molasses sugar. Also, the root impurities increased in those treated with nitrate-nitrogen and salinity. It can be concluded that nitrate-nitrogen has improved the performance of both sugar beet lines against salinity stress, and its practical application is adviseable.

Keywords: *nitrogen, vegetative growth, root yield, extractable sucrose, salinity stress*

1. INTRODUCTION:

Sugar beet is one of the strategic industrial plants for sugar production. About 37% of the world sugar production is obtained from this plant (Bogetoft *et al.*, 2007; Rajaeifar *et al.*, 2019). In addition to producing sugar, molasses and aerial parts are also used for animal feed and fermentation products. In some countries, ethanol is also made from this plan pulp using bacteria (Zheng *et al.*, 2012; Habeeb *et al.*, 2017). Sugar beet is highly tolerant of cold, dry weather and salinity. To a large extent, this plant species has a halophytic behavior and can accumulate large amounts of sodium and chlorine ions (Zhou *et al.*, 2017; Skorupa *et al.*, 2019). The tolerance of sugar beet cultivars is varies greatly and with increasing environmental stress particularly salinity, its yield decreases (Hossain *et al.*, 2017). Sugar beets tolerate to salt is about 200 to 300 mM chlorine (Ulrich and Ohki, 1956; Gzik, 1996). In terms of salt tolerance, it is in the second place after halophytes.

Supplying good quality water in many parts of the world is fraught with limitations. In these areas, the water of the aquifers has sharply decreased due to over-exploitation for drinking, agriculture, industry, and green space, which has led to an increase in the salinity of these waters. For this reason, in the maintenance of agricultural areas, the tendency to use saline water to irrigate plants has increased, which in the long run can reduce the growth and yield of these plants (Ulrich and Ohki, 1956; Hossain *et al.*, 2017). The harmfulness of high salt concentrations to plants is due to the osmotic potential of water and the specific effects of ions on the protoplasm. Increasing the concentration of sodium and chlorine ions in the protoplasm disrupts the ionic balance and the particular impacts of these ions on membrane enzymes, resulting in photophosphorylation of the respiratory chain and

energy (Blumwald and Poole, 1987; Zhou *et al.*, 2017; Wang *et al.*, 2019).

It has been accepted that stress-induced ROSs are responsible for multiple damages to macromolecules and ultimately to cellular structure (Noreen and Ashraf, 2009) and need to be quenched to maintain normal growth. Ascorbate peroxidase (APX), catalase (CAT), and peroxidase (POD) along with low molecular weight scavengers such as ascorbate, glutathione, and proline act as the main defense against ROS produced in different parts of the plant cell. Catalase, which is located in peroxisomes, glyoxysomes, and mitochondria and is apparently absent in chloroplasts, mainly converts H_2O_2 from light or photorespiration to water and O^2 (El-Hendawy *et al.*, 2005).

Mechanisms that can be involved in plant salinity resistance are a) Lack of absorption or low uptake of salt into the plant; b) tissue resistance or tolerance; c) Accumulation of salt in vacuoles without interfering with physiological processes; d) Isolation of ions such as K+, Cl^-, Na^+ and SO_4 during root uptake and transfer to shoots; e) Various biochemical processes such as the production of some enzymes, hormones, antioxidants, etc. (Ahmad *et al*., 2019).

Increased nutrients may be associated with more or less plant tolerance to salinity stress, depending on the salinity level and abundance of nutrients in the culture medium (Mansour, 2000). Inadequate nitrogen content is considered a growth-limiting factor in plants. The addition of nitrogen fertilizer has been reported to reduce the harmful effects of salinity on some plants (El-Hendawy *et al.*, 2005). Salinity can also prevent the accumulation of nitrogen in plants. Jamil and Rha (2004); (Jafarzadeh and Aliasgharzad, 2007) reported increasing soil salinity reduces root yield in sugar beet. This decrease is quite evident when the salt content does not exceed half a percent of the soil. They also observed that when the percentage of exchangeable sodium (EPS) exceeds 10, the root yield decreases, and when it reaches 18, the root yield decreases by 50%.

In sugar beet, sucrose is transported from the cytosol to the vacuole during the proton-antiport-sucrose process, where it accumulates (Ghoulam *et al.*, 2002). This action is probably a function of the proton gradient, and it probably follows the feeding of the nitrogen form. Since the plant response to nitrate and ammonium nitrogen is different, this response may differ in saline and non-saline conditions. Because sugar beetroot should contain a high percentage of sucrose, study the effect of two nitrogen forms. Nitrate and ammonium prioritize plant growth, sucrose content, and proline content in two salinity-sensitive and sugar-tolerant sugar beet lines in saline and non-saline conditions (Ghoulam *et al.*, 2002; Abbas *et al.*, 2010; Dadkhah, 2011).

Having a critical role as a food plant, sugar beet salt stress tolerance should be evaluated specifically under different nitrogen forms. Thus, two lines of sugar beet receiving two forms of nitrogen, nitrate, and ammonium were investigated to understand the response of these cultivars and find whether nitrogen forms can influence the salt tolerance of sugar beet plant.

2. MATERIALS AND METHODS:

2.1. Plant material and pot experiment

A total of 48 pottery pots with a capacity of 5 kg of soil and a diameter of 25 cm were prepared and filled with 4 kg of soil, which was analyzed using conventional methods of the Water and Soil Research Institute, and its specifications are shown in Table 1. These pots were then placed outdoors and based on a completely randomized statistical design in 6 replications with salinity-sensitive diploid polygram seed (line 1962-2939) and salinity tolerant hybrid (line 7233-p.29 xMst) at depth 1 planted up to 2 cm and irrigated to saturation (Figure 1a). After complete germination of seeds and their growth, additional seedlings were kept in each pot, and only two seedlings were held in each pot (Figure 1b). Then nitrogen treatment was applied as follows. One gram of nitrogen from calcium nitrate was added to the soil of pots for nitrate-nitrogen treatment. One gram of nitrogen from ammonium sulfate was added to the soil of the pots for the treatment of ammonium nitrogen. Salinity stress was applied by adding salt to irrigation water. For this purpose, all seedlings treated with salinity were subjected to the same saline water stress (32 bar every 5 days with 250 ml of water containing 40 mM sodium chloride). The rest of the seedlings were irrigated with the same amount of unsalted water. The plants were harvested 167 days after sowing the seeds. During the experiment, pots were kept outdoor to simulate the field condition where average, maximum and minimum temperatures were 24ºC, 33ºC, and 16ºC. Thus, the first 0.5 g of the leaf was taken to measure the amount of proline and immediately transferred to the laboratory and frozen in liquid nitrogen at -196°C. The samples were then stored in aluminum foil at -20°C in the freezer. Also, the aerial part of the plant was washed entirely after harvest, and its dry weight was determined at 70°C

until reaching a constant weight. The roots were immediately harvested and immediately transferred to the Sugar Technology Laboratory of the Karaj Sugar Beet Research Institute for qualitative chemical analysis.

2.2. Proline measurement

Leaf tissue proline was measured based on the method developed by Bates *et al.* (1973). For this purpose, 0.5 g of fresh leaves first weighed and homogenized in 10 ml of 3% sulfosalicylic acid solution with quartz and filtered with Whatman 42 paper. Then, in 2 ml of the filtered extract using ninhydrin reagent and 2 ml of glacial acetic acid, proline measured as a solution in toluene appeared red. The amount of proline in the tested samples was calculated in micrograms per gram of leaf fresh weight.

2.3.Nitrate test

According to Wollring (1983) method, a one-millimeter thick segment was prepared from the sugar beet plant petiole and placed on a glass plate to perform the nitrate test. Then, a drop of 1% diphenylamine reagent was added to each segment, and another glass plate was placed on them so that the petiole cuts were placed between two layers of glass. In this case, two glass plates were pressed together to extract the nitrate-containing extract in the petiole pieces. After reaction with the mentioned reagent, this extract produced a blue color, which varied depending on the quantity of nitrate in the petiole tissue. The amount of dye produced was evaluated between zero and 6 (nitrate test index). Then, by measuring the amount of nitrate in the leaf tissue, the relationship between the nitrate test index and the amount of nitrate in the tissue was determined based on the prodecure developed by Cataldo *et al.* (1975) and the amount of nitrate in leaf tissue was calculated in micrograms per gram of dry weight.

2.4.Chemical analysis of root

From the harvested roots, after complete washing, root pulp was prepared by a sampling device. The root was analyzed, and its quality factors were measured by a model 3016 -D beta analyzer and a flame photometer. A beta-analyzer and a flame photometer measured the sugar content (SC, Riyahi and Sajadi, 1984) and impurities in the root (amounts of potassium, sodium, and residue nitrogen) (Allen *et al*., 1985). Other factors such as molasses sugar (MS) and sugar content (WSC) indirectly measured using the available experimental equations and information obtained from the factors mentioned above (Clarke *et al.*, 1991). Through equations 1-3 is possible to obtain the percentage of molasses sugar.

$$\% MS = 0/343 (k + na) + 0/094 (a - amion - n) - 0/29 \quad \text{(Eq. 1)}$$

$$\% MS = 0/175 K + 0/13 Na + 0/215 (a - a min o - n) \quad \text{(Eq. 2)}$$

In these equations, potassium and sodium, and residue nitrogen impurities are mill equivalents per hundred grams of sugar beet root. WSC or percentage of extractable sugar can be obtained from reducing sugar content and molasses sugar as shown in equation 3.

$$\% WSC = \% SC - \% MS \quad \text{(Eq. 3)}$$

2.4.1 Measurement of sugar content

The percentage of sugar content or grade of sugar beet includes the percentage of extractable sugar and the percentage of sugar present in the molasses. To measure the quality parameters in the root, root paste, and lead (II) acetate in the ratio of 26 g of paste and 177.77 cubic centimeters, respectively, were thoroughly mixed using automatic mixers, then filtered with paper number 42, and its extract was separated. Its sugar percentage was determined by the polarimetry method (Clarke *et al.*, 1991), which is based on the deviation percent of polarized light using a polarimeter (Rudolph Autopol V Polarimeter APV-6W, NJ, USA).

2.4.2 Determination of potassium, sodium, and residue nitrogen impurities

The amounts of potassium and sodium in the extract prepared from the root paste are measured by a flame photometer that compares the sample emission spectrum obtained from lithium—subsequently, its amount in milliequivalents per gram of the resulting paste calculated from the root. A beta-analyzer was used to measure the residue nitrogen. In this device, by mixing the extract and cooper reagent, color changes are made in equal proportions compared with existing standards and expressed as milliequivalents per hundred grams of root paste

(Allen *et al*., 1985).

3. RESULT AND DISCUSSIONS:

According to the results shown in Table 2, nitrate-nitrogen treatment in comparison with ammonium nitrogen has significantly increased the dry weight of the aerial part and also the fresh weight of the root part in both lines (Figure 2), which may be due to the higher photosynthesis activity in plants treated with (DIAS and Costa, 1983; Raab and Terry, 1994; Malnou *et al.*, 2008). Ammonium nutrition also causes ammonia to accumulate within plant tissue cells, resulting in disruption of cellular pH, inhibition of phosphorylation coupling, and reduced photosynthesis (Raab and Terry, 1994). A report by Rubin *et al.* (2009) on tobacco shows that ammonium nitrogen reduces plant growth by reducing cell number, cell size, and leaf area. However, the number of leaves was equal in both nitrate and ammonium nitrogen treatments. Such a result has been reported for previously reported in sugar beet cultivars (Raab and Terry, 1994; Brentrup *et al.*, 2001; Demiral and Gündüzoğlu, 2010).

Decreased growth due to ammonium nitrogen nutrition is also associated with ammonium toxicity. It causes phosphorylation uncoupling and reduces carbohydrate compounds due to excessive consumption of soluble sugars for NH4+ reduction and detoxification (Walch-Liu *et al.*, 2000; Bittsánszky *et al.*, 2015). However, nitrate ions as a moderator in osmotic conditions increase water absorption and increase plant growth (Cordovilla *et al.*, 1995). Salinity treatment with either nitrate and ammonium nitrogen has a different effect on plant growth. Consumption of nitrate-nitrogen with salinity compared to ammonium nitrate consumption alone has significantly reduced the aerial part dry weight and the root fresh weight in the salinity sensitive line. In the line tolerating salinity, this reduction was only observed for root fresh weight. Nonetheless, the application of ammonium nitrogen combined with salinity compared with ammonium nitrogen alone in both lines did not cause a significant reduction in shoot dry weight. Still, this effect on root fresh weight was noticeable.

The data in Table 3 show the nitrate levels of leaf tissue. In nitrate-nitrogen treatment 167 days after sowing, the amount of leaf nitrate in both lines is less than the amount of ammonium nitrogen. However, this difference is not significant, probably due to the leaching phenomenon and the removal of nitrate from the soil around the roots treated with nitrate nitrogen. Ammonium ions are stored in the soil around the roots and are converted to nitrate by nitrification. Therefore, these nitrates can be gradually given to the plant (Alexander, 1965; Zaman and Blennerhassett, 2010). Salinity treatment in each of the two forms of nitrogen significantly reduced nitrate in leaf tissue than non-salinity treatment.

To achieve high-quality sugar beet, the plant must be balanced in terms of nitrogen nutrition and not suffer from insufficiency or excessiveness. Achieving a balance between sufficient nitrogen at the beginning of the growing season and its deficiency at the end of the season is critical. This matter requires the management of nitrogen fertilization according to the amount of nitrogen in the soil and the amount of nitrate in the petiole tissue of the sugar beet plant (Brentrup *et al.*, 2001; Campbell, 2002; Malnou *et al.*, 2006). Table 3 shows a direct relationship between leaf nitrate content and the index obtained from the petiole nitrate test evaluation. In other words, the higher the amount of leaf nitrate, the higher this index. By performing a nitrate test on sugar beet petiole, it is possible to speculate the plant need for nitrogen by considering its vegetative growth period.

Wollring and Koehler (1989) organized nitrogen fertilization in cereals in a balanced way and according to the plant needs by performing this nitrate test. Table 3 indicates the correlation between the nitrate concentration of sugar beet leaf tissue and plant evaluation according to the nitrate test. Completing a rapid nitrate test makes it possible to understand the need for nitrogen in sugar beet during the vegetative growth period. Thus, identifying its immediate needs for nitrogen and preventing excessive nitrogen consumption reduces the percentage of sugar in sugar beet plants.

Salinity treatment in both lines (Table 4) has increased leaf tissue proline content regardless of nitrogen. The results also show that the accumulation of proline with salinity conditions in the salinity tolerant line is more than the salinity sensitive line. This result is consistent with Carillo *et al.* (2008), who reported that the accumulation of proline in plants can indicate salinity tolerance and that salt-tolerant species accumulate more proline.

The results also show (Table 4) that the salinity tolerant line under salt stress has a higher proline accumulation when nitrate nitrogen is used compared to those under ammonium nitrogen

treatment. This observation is possibly due to the lower growth rate of sugar beet in ammonium nitrogen nutrition conditions. Sumithra *et al.* (2006) found that the activity of enzymes involved in proline synthesis is higher in the vegetative stage. Therefore, any factor that affects the vegetative growth of the plant limits the synthesis of proline. Because the growth of sugar beet in ammonium nitrogen-fed conditions was less than that of nitrogen-nitrogen-fed conditions, the decrease in proline content may be related to the effect of nitrogen form.

From the information presented in Table 5, it could be inferred that there is no significant difference in the amount of sucrose, extractable sugar, and molasses sugar of root tissue between the two forms of nitrogen. Salinity treatment has significantly reduced the percentage of extractable sugar and significantly increased the percentage of molasses sugar.

Root impurities include potassium, sodium, and residue nitrogen (amino nitrogen) presented in Table 6. The amount of sodium and potassium in the roots in both lines differs from both forms of nitrogen. They were not significant, but nitrogen in nitrate-nitrogen treatment is higher than ammonium nitrogen. Salinity treatment increased root sodium regardless of the nitrogen form, while it has no significant effect on root potassium in both lines. The combined use of salinity and nitrone nitrate has led to a substantial reduction in the amount of nitrogen in the salinity-sensitive line and a significant increase in the salinity tolerant line than the nitrate-nitrogen treatment alone. On the other hand, consumption of ammonium nitrogen with salinity compared to ammonium nitrogen alone has increased the amount of residue nitrogen in both sugar beet lines.

4. CONCLUSIONS:

In nitrate-nitrogen treatment, the total amount of root impurities in both lines is significantly higher than in ammonium nitrogen treatment. Additionally, salinity treatment in both nitrogen conditions has increased the number of impurities. This increase was noticeably higher in ammonium nitrogen consumption when compared with nitrate nitrogen. Increasing the impurities of sugar beet extract may reduce the percentage of extractable sugar and increase the molasses sugar during the sucrose crystallization process. Given the significant results of this study, it's highly recommended further experiments be conducted in this area, particularly by increasing the levels of salinity stress using different lines of sugar beet.

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6. OPEN ACCESS:

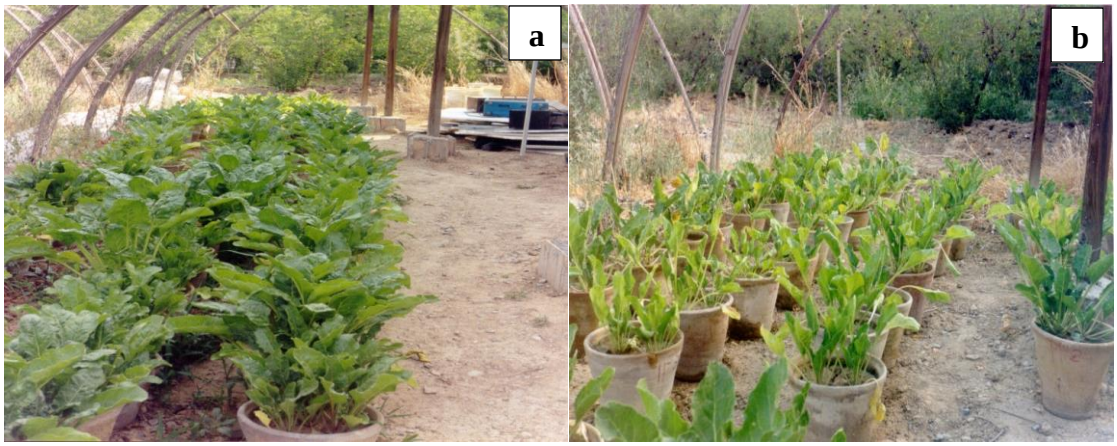

Figure 1. *Sugar beet plants in pottery pots before (a) and after (b) thinning.*

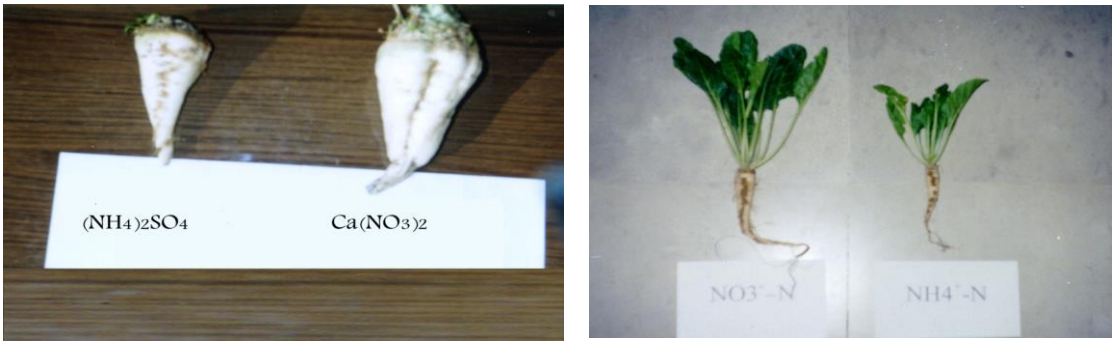

Figure 2. *The effect of nitrogen forms, nitrate, and ammonium on the growth and root yield of sugar beet.*

Table 1. *The characteristics of the soil of pots used in this study.*

Organic carbon (%)	**Neutralized compounds (%)**	**Electrical conductivity (S/m)**	**saturated soil paste acidity (pH)**	**Clay (%)**	**Silt (%)**	**Sand (%)**
1.2	16.6	3.2	7.7	28	33	39
	Micronutrient (mg/kg)					
Cu	Zn	Mn	Fe	Absorbable potassium (mg/kg)	Absorbable phosphorus (mg/kg)	NH_4-N (mg/kg)	NO_3-N (mg/kg)
1.4	7.8	13.1	12.8	440	61.4	40.3	50.9
Cations and Anions (mEq/L)of saturated soil paste)							
		Na^+	Mg^{2+}	Ca^{2+}	SO_4^{2+}	Cl^-	HCO_3^-
		7.0	8.4	32.2	29.0	7.6	3.6

Notes: Milliequivalents per litre (mEq/L); Siemens per meter (S/m);

Table 2. *Effects of N-forms and salinity on biomass production of two lines of sugar beet.*

Root Fresh Weight (g)	Shoot Dry Weight (g)	Shoot Fresh Weight (g)	Treatments
Sensitive Line			
243.2 d	19.2 d	178.4 d	NO_3-N
141.4 a	13.6 a	122.7 a	NH_4^+-N
194.5 c	17.0 c	158.3 c	NO_3-N and NaCl
163.7 b	13.7 a	123.9 a	NH_4^+-N and NaCl
Tolerant Line			
249.5 d	20.0 d	181.9 d	NO_3-N
143.1 a	13.7 a	123.9 a	NH_4^+-N
207.5 c	18.0 cd	167.3 cd	NO_3-N and NaCl
168.6 b	13.8 ab	125.5 ab	NH_4^+-N and NaCl

Table 3. *Leaf nitrate concentration and Nitrate-test index in the petiole of sugar beet lines under salinity stress and two forms of nitrogen.*

Tolerant		Sensitive			
Leaf Nitrate density (mg/kg)	**Nitrate-index***	**Leaf Nitrate density (mg/kg)**	**Nitrate-index***	**Treatment**	**Growth stage (days)**
132 f	2.8 c	128 d	2.8 c	NO_3-N	**96 days**
100 d	2.0 b	94 c	1.9 b	NH_4^+-N	
120 e	2.4 b	121.0 d	2.5 bc	NO_3-N and NaCl	
95 c	1.9 b	86 c	1.7 b	NH_4^+-N and NaCl	
307 i	5.0 d	324 h	6.0 d	NO_3-N	**131 days**
252 h	4.6 d	220 f	4.3 d	NH_4^+-N	
291 f	5.0 e	307 g	5.9 d	NO_3-N and NaCl	
192 g	4.0 d	174 e	3.8 cd	NH_4^+-N and NaCl	
48 b	1.0 a	28 b	0.8 a	NO_3-N	**167 days**
51 b	1.1 a	33 b	1.0 a	NH_4^+-N	
26 a	0.6 a	6.0 a	0.3 a	NO_3-N and NaCl	
23 a	0.6 a	14 a	0.5 a	NH_4^+-N and NaCl	

*Nitrate index was measured visually according to the intensity of color produced in the reaction which is from 0 to 6. Note: mean values with the same letters indicate homogeneous subsets for $P \leq 0.05$ according to Duncan's test. Means in the same column with different letters differ at $P \leq 0.05$.

Table 4. *Effect of N-forms on leaf proline content in sugar beet lines under salinity stress.*

Proline density (mg/g fresh weight of leaves)		Treatments
Torelant	**Sensitive**	
3.6 a	3.9 a	NO_3-N
3.2 a	3.8 a	NH_4^+-N
6.5 b	9.3 c	NO_3-N and NaCl
5.9 b	7.2 b	NH_4^+-N and NaCl

Notes: mean values with the same letters indicate homogeneous subsets for P ≤ 0.05 according to Duncan's test. Mean in the same column with different letters differ at P ≤ 0.05.

Table 5*. Effects of N-form on root qualitative properties in two lines sugar beet*

Molasses sugar (%)	Extractable sugar (%)	Sucrose (%)	Treatments	Lines
3.0 bc	11.2 bc	14.2 b	NO_3-N	Sensitive Line
2.5 ab	11.8 bc	14.3 bcd	NH_4^+-N	
4.00 d	8.1 a	12.0 a	NO_3-N and NaCl	
3.00 bc	12.8 bc	15.8 cd	NH_4^+-N and NaCl	
2.5 ab	11.5 bc	14.1 b	NO_3-N	
1.8 a	13.8 c	15.6 cd	NH_4^+-N	Tolerant Line
3.1 bc	12.6 bc	15.7 cd	NO_3-N and NaCl	
2.5 ab	13.4 bc	16.0 d	NH_4^+-N and NaCl	

Notes: mean values with the same letters indicate homogeneous subsets for P ≤ 0.05 according to Duncan's test. Mean in the same column with different letters differ at P ≤ 0.05.

Table 6. *Effects of N-form and salinity on K+, Na+ and residue nitrogen in sugar beetroots*

Impurity content (milliequivalent per 100 g of sugar beetroot)					
Total residues	**Residue nitrogen**	**Na+**	**K+**	**Treatments**	**Lines**
4.4 cd	4.9 d	2.3 bc	6.00 bc	NO_3-N	**Sensitive Line**
3.3 b	1.6 a	1.8 a	6.1 bc	NH_4^+-N	
4.5 d	1.6 a	5.2 d	6.7 c	NO_3-N and NaCl	
4.1 cd	3.5 c	3.03 bc	5.6 bc	NH_4^+-N and NaCl	
3.4 bc	2.8 b	4.5 abc	4.9 a	NO_3-N	
2.4 a	1.3 cd	1.9 ab	3.8 a	NH_4^+-N	**Tolerant Line**
3.4 cd	3.9 cd	2.5 abc	6.5 bc	NO_3-N and NaCl	
3.3 b	2.4 ab	2.5 abc	5.1 bc	NH_4^+-N and NaCl	

Notes: mean values with the same letters indicate homogeneous subsets for P ≤ 0.05 according to Duncan's test. Mean in the same column with different letters differ at P ≤ 0.05.

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY

EVALUATION OF THE EFFECTIVENESS OF TREATMENT OF THE ORAL MUCOSA WITH PHYTO-OINTMENT BASED ON PHYTOECDYSTEROIDS

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ABSTRACT

An indicator of the health of the human body in the state of the oral mucosa. Mechanical and chemical factors constantly influence it. At the first stage of the study, a comparative analysis of the frequency of various forms of traumatic injuries of the oral mucosa was carried out. The distribution of patients into groups was also carried out, depending on the medicinal product used. Then clinical examinations were carried out. After that, the therapy of traumatic erosive and ulcerative lesions of the oral mucosa began. The developed method for treating traumatic lesions of the oral mucosa with medicine based on phytoecdysteroids provides for eliminating the traumatic factor, applying ointment based on phytoecdysteroids to the dried out focus twice a day. The use of phyto-ointment leads to complete repair of traumatic erosive and ulcerative lesions of the oral mucosa on average by the eighth day from the start of treatment; a similar effect with the use of "Solcoseryl dental adhesive paste" is achieved by the tenth day, and the gel "Cholisal Dental" - at a later date, which is confirmed in this study. The most significant positive effect on the level of quality of life associated with the effectiveness of treatment of traumatic erosive and ulcerative lesions of the oral mucosa in comparison with the dental “Solcoseryl dental adhesive paste” and the gel “Cholisal Dental” is exerted by phyto-ointment, where a decrease in the total points was recorded. When conducting routine examinations of patients, it is necessary to pay attention to the oral mucosa damage. Moreover, in treating traumatic injuries of the oral mucosa, it is recommended to use phyto-ointment, which contains phytoecdysteroids.

Keywords*: oral mucosa, phytoecdysteroids, erosive and ulcerative lesions, trauma to the oral mucosa*

1. INTRODUCTION

The condition of the oral mucosa is an indicator of the health of the human body. The mucous membrane of the mouth is often constantly exposed to various influences beyond physiological parameters (Utyuzh *et al*., 2020).

The mucous membrane of the mouth is continuously exposed to various stimuli. Factors such as mechanical and chemical affect the mucous membrane even when eating. However, due to the enormous strength of immunity: local and general, these factors do not exert much harm since the mucous membrane has good regenerative and protective properties (Ajayi *et al*., 2019, Bokov *et al*., 2020).

According to the mechanism of action and the nature of the course, acute and chronic mechanical trauma is distinguished. Acute trauma mostly occurs in the presence of a strong short-term blow, once, for example, with trauma by foreign metal objects. In most cases, children have an acute mechanical injury, which can be caused by a blow, biting the tongue when falling, and damage to the mucous membrane by stabbing or cutting objects, such as toys. Acute trauma provokes a local tissue defect and is also accompanied by bleeding, swelling, and pain, which in turn can increase during conversation and eating (Zibareva *et al*., 2017; Dikhit *et al*., 2018).

In chronic mechanical injury, the stimulus is weak, but the effect is longer. The chronic effect can be manifested by biting the mucous membrane with teeth, trauma with lamellar

prostheses, dental defects with caries, as well as poor-quality filling. At the site of injury, swelling and redness occur. Then this area is transformed into erosion and then into a decubital ulcer (Olennikov *et al*., 2017).

Erosions are superficial defects of the epithelial layer without the inclusion of the underlying connective tissue base. Their size, shape, and localization often coincide with the shape and size of the source of injury (Jurenka *et al*., 2017).

Secondary infection of wounds causes ulcers or cracks that do not heal for a long time. Limited inflammation and infiltration of the lamina propria of the mucous membrane occur around the wound. An uninfected wound epithelializes rather quickly. Ulcers can appear due to trauma of any kind and against the background of autoimmune infectious diseases (Kaçar *et al*., 2013; Gafurovich *et al*., 2020).

An ulcer is defined as a more profound defect in the mucous membrane that extends beyond the epithelial layer. The difference between erosion and an ulcer is that a scar remains after the ulcer heals. Moreover, a dehydrated exudate - crust may appear (Rahman *et al*., 2017).

It is noted that the development of chronic mechanical trauma is accompanied by the development of local congestive hyperemia and edema. At this point, erosion may occur. In the future, a pressure ulcer is a single, painful pressure ulcer surrounded by an inflammatory infiltrate, with a fibrinous plaque at the bottom. The mucous membrane around is hyperemic and painful. Regional lymph nodes are enlarged, painful on palpation. With a prolonged course, the edges and base of the ulcer become denser (Reaper *et al*., 2017).

The anatomical structure of the gum is divided into several parts, the marginal part or gingival margin; the alveolar gum or attached; the papillary part or gingival papilla. Furthermore, the papillary and marginal shell in the soy turn is transformed into the gingival margin or free gum (Tarkowská *et al*., 2020).

The movable part of the gum is more susceptible to injury, as opposed to its fixed part. With the subsequent development of inflammation, violations of the integrity of the epithelium of the oral mucosa are infected. If the oral hygiene is unsatisfactory and the traumatic factor has a long-lasting character, then the mechanism of chronic inflammation begins (Timoshin *et al*., 2019; Yumashev *et al*., 2020).

The question of how quickly and fully therapeutically eliminate the traumatic complications of the oral mucosa with the development of new, more effective therapeutic agents, which in particular have a combined - analgesic and regenerative ability, is acquiring a rather high significance (Miclín *et al*., 2014; Yusupova *et al*., 2019).

Treatment of mechanical injury involves the mandatory elimination of the traumatic agent, anesthesia, treatment of the area of the injured mucous membrane with antiseptic solutions (Kim *et al*., 2013, 2014; Kuznetsova *et al*., 2018).

To correct pathological conditions in oral mucosa diseases, drugs of both synthetic and natural (including plant) origin are used. Herbal remedies, unlike synthetic ones, have several advantages: a mild effect, low toxicity, activation of the functions of not only the immune but also the nervous and endocrine systems due to the presence in their composition of a complex of biologically active substances that affect the body as a whole (Wang *et al*., 2017; Dinan *et al*., 2020).

Due to the urgency of finding new ways to treat traumatic injuries of the oral mucosa, we set out to develop a new treatment algorithm using a drug that would provide a powerful analgesic and epithelial effect without having negative effects.

As a promising basis for such a drug, in particular, ecdysteroids can be considered substances that are structurally identical or close to the true hormones of molting and metamorphosis of insects. In mammals and humans, these compounds have anabolic and wound-healing effects. A. Butenand and Carlson first obtained these hormones in 1954 from silkworm cocoons. In addition to insects, ecdysteroids have now been found in plants (phytoecdysteroids) belonging to more than 100 families from the divisions Polypodiophyta, Pinophyta, and Magnoliophyta. Screening studies have shown that these hormones are present in about 400 plant species. The largest detections of ecdysteroids were recorded in ferns and gymnosperms. Phytoecdysteroids are also found in some representatives of red algae and fungi.

Phytoecdysteroids are also responsible for the biological activity of some species of medicinal plants used in medicine of many peoples of the world: Achyranthes fauriei and Cyathula capitata (go-shitsu) — in ancient China, Ajuga iva (chenjoura) — in North Africa, Pfaffia iresinoides (suma) — in Latin America, Serratula coronata

(serpia) — in Siberia, Silene tatarica and Oberna behen (shlachkan turun) — in the European North near the Komi-Zyryans. Ecdysteroids also enter the human body with fairly common food plants, such as spinach and rice quinoa. The literature notes the fact that ecdysteroids are found in plants in negligible amounts. And only in some species, the amount of these hormones reaches 2%-3%.

The main therapeutic effect of ecdysteroids is a significant stimulation of anabolic, mainly protein-synthetic processes, which is supplemented by the possibility of their long-term use in combination with the absence of any negative manifestations.

In the works of S. O. Volodina (2012), the universal hormone-like role of phytoecdysteroids is described. They have the ability to alter the body's homeostasis by affecting cell growth, differentiation, and programmed cell death. In this regard, a wound-healing effect is also assumed. It is also indicated on the processes of protein synthesis in the body. In experiments, it was proved that phytoecdysteroids do not cause any negative effects on the part of androgen-dependent organs. In contrast to true steroid hormones, they do not exhibit androgenic, thymolytic, or antigonadotropic effects (Kharazmi et al., 2014; Plakhova et al., 2020).

For many years, experimental and clinical studies of drugs based on phytoecdysteroids have been conducted at the basis of the Ryazan State Medical University. There are data on the use of this active substance in patients in dentistry, ophthalmology, gastroenterology. A positive therapeutic effect was also obtained in the treatment of burns of the skin and mucous membranes. The study of phytoecdysteroids in the course of these studies revealed a multi-sided positive effect on the body without showing toxic effects (Sevbitov et al., 2020).

Thus, based on the indicated list of necessary properties of a new drug, the most rational is to manufacture it based on a drug collection that has analgesic, antibacterial, anti-inflammatory, and anabolic effects in combination with acceleration and optimization of regeneration processes.

Such a medicinal collection, which has the necessary properties, can be presented in the form of a combination:

- marigold flowers (*Calendula officilalis* L.) - anti-inflammatory, antimicrobial, decongestant, antitoxic, hyposensitizing, reparative, antiviral effect;

- herbs of the drooping gum (Silene nutans L.) - analgesic, anti-inflammatory, antitoxic and hemostatic effect;

- herbs of meadowsweet (Latin: Filipéndula ulmária) – antibacterial effect and promotes epithelization of trophic ulcers on the legs, wounds, and burns;

- rhizomes and roots of Leucea safflower (lat. Rhapónticum carthamoídes), rich in ecdysteroids that have an anabolic effect.

Among other things, a significant advantage of the proposed herbal collection is the availability and relative cheapness of its components. Its natural biologically active substances are widely used in the form of dietary supplements, some of them are included in the official pharmacopoeia.

The method of treatment developed by us on the basis of this phytomasy has significant prospects in the treatment of erosive and ulcerative lesions of the oral mucosa of traumatic etiology (Patent No. 2577240 of 09.12.2014)

In connection with the above, the search for a new modern method of treating traumatic erosive and ulcerative lesions of the mucous membrane is an important area of therapeutic dentistry and orthodontics.

This study aims to increase the effectiveness of treating erosive and ulcerative lesions of the oral mucosa.

2. MATERIALS AND METHODS

The study involved 110 patients with erosive and ulcerative lesions of the oral mucosa. The local ethics committee (protocol № 05-16) approved the study of Sechenov University. All patients signed voluntary informed consent for the study before the study. The distribution of patients into groups was carried out depending on the type of drug used (Table 1).

Table 1. *Principle of group formation in the study*

No of group	Medicine name	Number of observations
1	Phyto-ointment	38
2	“Cholisal Dental”	35
3	“Solcoseryl dental adhesive paste”	37

Therapy of traumatic erosive and

ulcerative lesions of the oral mucosa was carried out after a clinical examination, photographing the lesion, and cytological examination.

For treatment, phyto-ointment with phytoecdysteroids (group 1), gel “Cholisal Dental” (Jelfa SA, Poland) with a predominant analgesic effect (group 2), and dental paste “Solcoseryl dental adhesive paste”, (Legacy Pharmaceuticals Switzerland, GmbH), mainly with an effect that stimulates regeneration (group 3).

Phyto-ointment was developed jointly by the teams of the Department of Pharmacognosy with the course of botany at the Ryazan State Medical University and the Department of Propedeutics of Dental Diseases of the Sechenov University. The developed phytopreparation has the following composition: 40% alcohol (1: 5) tincture of resinous herbs and rhizomes with roots of leuzea, marigold flowers (calendula), meadowsweet herb taken in mass parts (3: 1: 1) respectively - 30 ml; petrolatum, anhydrous lanolin taken in parts by weight (3: 2) respectively - 68.0 g; essential clove oil - 1.0 g; eucalyptus essential oil - 1.0 g.

"Cholisal Dental" - a medicine for treating ulcerative necrotic and trophic lesions of the oral mucosa. It has a pronounced anti-inflammatory and analgesic effect at the site of application. Cholisal Dental composition: water, propylene glycol, hypromellose, methyl parahydroxybenzoate, peppermint oil, propyl parahydroxybenzoate, disodium edetate, aprotinin, sodium hydroxide. It is quickly absorbed from the surface of the application; the active substances of the drug penetrate to the nerve endings and are held there for a long time. According to the pharmacological action, it is active against Gram-positive bacteria, to a lesser extent Gram-negative, as well as fungi and viruses, i.e., it has an active antimicrobial effect.

“Solcoseryl dental adhesive paste” represents such drugs that stimulate regeneration and have a cytoprotective, membrane-stabilizing, wound-healing effect. Solcoseryl dental adhesive paste in the area of application may cause partial taste changes and swelling. Composition of "Solcoseryl dental adhesive paste": deproteinized dialysate from the blood of healthy dairy calves (Bos Taurus), chemically and biologically standardized (in terms of dry matter), polidocanol 600, preservatives, methyl parahydroxybenzoate (E218), propyl parahydroxybenzoate (E216), auxiliary substances: sodium carboxymethyl cellulose - 22.875 mg; peppermint oil - 2.925 mg; menthol - 0.075 mg, paste base: (sodium carboxymethyl cellulose, gelatin, pectin, polyethylene 350,000, liquid paraffin) - 960 mg.

After the elimination of the traumatic factor, local application of these medicines was carried out. The erosive and ulcerative areas were dried with a cotton swab, on which the medicinal preparation was applied 2 times a day until the complete epithelialization of the traumatic injury.

The distribution of acute and chronic traumatic injuries by observation groups is presented in Table 2.

Table 2. *Distribution of the frequency of acute and chronic erosive and ulcerative lesions of the oral mucosa by observation groups*

No of group	Medicine name	Number of observations	
		Acute	Chronic
1	Phyto-ointment	34	4
2	“Cholisal Dental”	33	2
3	“Solcoseryl dental adhesive paste”	34	3
TOTAL		101	9

Control examinations of patients were carried out at least once every 2-3 days. Cases with severe pain symptoms were observed daily. The treatment was discontinued when complete healing of traumatic erosive and ulcerative lesions of the oral mucosa was established, that is, on the 8-10th day.

All patients were surveyed using a special questionnaire, “Oral Health Impact Profile” (OHIP-14), before and after traumatic injury treatment. The questioning was used to determine the level of quality of life before and after treatment (Appendix 1) (Sevbitov *et al*., 2020).

The obtained results were processed in the IBMSPSS program, version 21.0.

3. RESULTS AND DISCUSSION:

The degree of edema and hyperemia of the wound surface in patients decreased with time at different rates, depending on the applied therapeutic agent.

The disappearance of signs was evidenced by the indicator of the intensity of hyperemia. In group 1 - by the 6th day of observation, in group 2 - by the 10th day, and in

group 3 - by the 8th day. The final disappearance of edema signs in group 1 was on the 8th day of observation and in 2 and 3 - on day 10.

Simultaneously, the statistical significance ($p \leq 0.05$) of the difference between similar average intensity indicators in groups 2 and 3 relatives to the corresponding values in group 1 was recorded starting from the 4th day of observation and further.

The highest rates of decrease in the average point indicators of the intensity of hyperemia and edema were noted in group 1, the lowest - in group 2. Intermediate values of the corresponding indicators were recorded in group 3.

Thus, of the three considered medications, signs of hyperemia and edema in the zone of erosive-ulcerative traumatic lesions of the oral mucosa are most effective and quickly eliminates ointment with phytoecdysteroids.

Compared to the initial data, the most pronounced changes in the indicators of the quality of life associated with dental health following the treatment results were stated in the scale "Problems in eating".

Similar but slightly less pronounced changes were recorded following treatment results in the scale "Problems of everyday life".

The sum of average points on this scale in group 1 before treatment was 18.0 points, in group 2 - 18.2, and in group 3 - 17.6. As a result of treatment, the corresponding indicators in group 1 were determined at the level of 10.4 points, in group 2 - 14.3, and group 3 - 12.8. Thus, as a result, the sum of the average points characterizing the scale "Problems of everyday life" at the end of treatment decreased.

Changes in the indicators of the quality of life associated with dental health determined by the treatment results in the scale "Communication problems" are recorded in the minimum range. The initial sum of mean points on this scale in group 1 before treatment was 4.7 points and in group 2 and 3 - 4.8. At the end of treatment, the corresponding indicators in group 1 were determined at the level of 4.3 points, in group 2 - 4.7, and in group 3 - 4.4. Thus, the sum of the mean points characterizing the scale "Communication problems" at the end of treatment decreased compared to the initial indicator.

4. CONCLUSIONS:

It was concluded that the healing time of erosive and ulcerative lesions of the oral mucosa is faster when using phyto-ointment based on phytoecdysteroids than when using “Cholisal Dental” and “Solcoseryl dental adhesive paste” ($p < 0.05$)

An insignificant dependence of changes in the quality of life indicators concerning the methods and outcome of treatment, traumatic erosive and ulcerative lesions of the oral mucosa was established.

Changes in the indicators of the quality of life associated with dental health, determined by the treatment results, compared to the initial values were found most pronounced in group 1, where a decrease in the total score from 35.6 to 20.2 (by 43, 3%).

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6. OPEN ACCESS

Appendix 1. Oral Health Impact Profile (OHIP-14) (Slade G.D., Spencer A.J., 1994)

Question	Very often	Rarely	Usually	Almost never	Never
	5	4	3	2	1
1. Have you had trouble pronouncing any words because of problems with your teeth, mouth or dentures?					
2. Have you felt that your sense of taste has worsened because of problems with your teeth, mouth or dentures?					
3. Have you had painful aching in your mouth?					
4. Have you found it uncomfortable to eat any foods because of problems with your teeth, mouth or dentures?					
5. Have you been self conscious because of your teeth, mouth or dentures?					
6. Have you felt tense because of problems with your teeth, mouth or dentures?					
7. Has your diet been unsatisfactory because of problems with your teeth, mouth or dentures?					
8. Have you had to interrupt meals because of problems with your teeth, mouth or dentures?					
9. Have you found it difficult to relax because of problems with your teeth, mouth or dentures?					
10. Have you been a bit embarrassed because of problems with your teeth, mouth or dentures?					
11. Have you been a bit irritable with other people because of problems with your teeth, mouth or dentures?					
12. Have you had difficulty doing your usual jobs because of problems with your teeth, mouth or dentures?					
13. Have you felt that life in general was less satisfying because of problems with your teeth, mouth or dentures?					
14. Have you been totally unable to function because of problems with your teeth, mouth or dentures?					

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY

EVALUATION OF IMMUNE SYSTEM STIMULATION WITH VACCINE PREPARED AGAINST INDUCED BREAST CANCER IN ALBINO MICE

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ABSTRACT

This study was designed to prepare vaccines. Cancer vaccines promote the destruction of cancer cells, and the cancer cells contain special antigens on their surface when the vaccine is given, it acts as an antigen to activate the immune system. The IL-2 stimulates the response to a type of cells called T-cell, and outer membrane vesicle (OMV) targeting cancer cells by stimulating the immunity to respond with it two types including innate and adaptive immunity, that lead to stimulating the immune system to reduce mammary adenocarcinoma induction in lab mice using T4-1 cell line breast cancer by taking blood and serum to evaluate the immune system efficacy. The tumor induction success by monitoring mice body weight loss showed the lost weight began in the third week after tumor induction, so 23.2 g at first and second week to 14.35 g at the end of the fourth week, whereas the control animals were weighed 32.37 g. The immunity system efficacy results appear a difference in blood and serum parameters after cancer induction. The result shows an increase in total WBC and monocytes (5900, 0.2 cells/mm respectively) but non significantly decreased in neutrophils and lymphocytes count (2.6, 5.9 cells/mm, respectively). Therefore, the first and second doses of vaccines increased the antibody and complement of the immune system compared with control. While Eliaza data for cytokines profile referred to elevated IL2 (26.5 pg/ml) in the serum of vaccination mice but only significantly decreased in IL6 and IL-22 amount (20.4 and 19.6 pg/ml respectively) comparing with control.

Keywords: ***anti-tumor cytokines, murine cell line, immunotherapy, IL2, outer membrane vesicles***

1. INTRODUCTION

The common treatment methods are surgical treatment, radiotherapy, and chemotherapy (McCune, 2018). Most of the malignant growth drugs are exceptionally toxic. Therefore, the need to search for a new drug is required, preventing or slowing cancer progression and is less toxic and safer (Aziz *et al*., 2017). A non-specialist reader could understand the significance of the presented results. Another restorative methodology is to treat malignant growth through bacterial outer membrane vesicles (OMV) as therapeutic agents to treat cancer via immunotherapy. The ability of bacterial outer membrane vesicles to effectually make lasting anti-tumor immune reactions can destroy recognized tumors without distinguished adversative properties. Furthermore, steadily controlled bacterial outer membrane vesicles collect in the tumor tissue and help produce anti-tumor cytokines (Kim *et al*., 2017).

This system includes supporting and remaining so far weak immune response against cancer cells (Xu *et al*., 2019). These days, immunotherapy is acknowledged as the fourth mainstay of standard cancer treatment (McCune, 2018). In the malignancy immunotherapy field, the cancer vaccine inspires the most excitement. They can effectively produce a resistant reaction explicit to tumor cells (TCs) and a dependable immunological memory (Rosenberg *et al*., 2004). The cancer vaccine helps re-teach the immune system to recognize and eliminate tumor cells (Lasaro *et al*., 2010). There is proof that both

humoral and cell-mediated reactions are significant in anticancer immunotherapy. Antibodies can viably dispose of malignant growth cells through immune response intervened cell cytotoxicity (Karagiannis *et al*., 2013). Expanded anticancer humoural reactions are associated with diminished cancer repeat and enhanced survival (Vermaelen, 2019). For the readiness of complete cancer cell vaccines, cancer cells from cell lines or essential tumors can be utilized (Ning *et al*., 2012). Moreover, malignant growth cells can be given by the patient (autologous) or a contributor (allogeneic) (Li *et al*., 2009).

At the point as soon as a cell cancer dies, it very well may be evident by (immunogenic) or without (nonimmunogenic) inspiring, an immune reaction in contrast to its dead cell antigens, in cancer cell vaccine produce, immunogenic cell death (ICD) is the target. An immunogenic death relies upon ICD-instigating improvement (Kroemer *et al*., 2013). Different ICD- inducing motivations have been defined, and their crucial characteristic is the capability to make expression and release of a damage-associated molecular pattern (DAMP) from the cells they were killed. The DAMPs that are expressed and discharged by the cancer cells afterward, ICD can cooperate with design recognition receptors on several immune cells affording to a distinct spatiotemporal design. DAMPs tie to pattern recognition receptors transports around the coming of cytokines and chemokines by the immune cell. Therefore, APCs are animated to effectively take up and progress tumor antigens and cross-prime T cells (Kepp *et al*., 2011). Different elements can impact the adequacy of DAMPs (Krysko *et al*., 2012). The death-inducing stimulus, the created DAMPs themselves, the DAMP location, and the mix of cancer and host-related components affect the immunogenicity of the entire cancer cell vaccine. The malignant growth cell has to express the DAMPs, which thus must bind immune cells and not cancer cells (Cicchelero *et al*., 2014). For sure, the binding of DAMPs to toll-like receptors (TLRs) expressed on malignant growth cells can advance cell endurance and chemoresistance (Krysko *et al*., 2012). Conversely, the patient must be immunodeficient. So line with this theory. This research aimed to assay the efficacy of the death cell line with the OMV vaccine on mice with induction breast cancer (Kroemer *et al*., 2013).

2. MATERIALS AND METHODS

2.1. Extraction of outer membrane vesicles (OMVs) from *E. Coli*

The *E. coli* were grown up overnight in LB medium with carbenicillin (50 µg/mL). Later overnight culture, the *E. coli* solution (200 µL) was washed away with new LB media, and new Luria-Bertani media with the antibacterial drug (carbenicillin, 20 mL) was added. The solution was put in an incubator at 37 °C with strong shaky (200 rpm) awaiting the optical density reached OD578 nm 0.7. Formerly, Isopropyl β-D-1-thiogalactopyranoside (IPTG, 1 mM) was added to cells, and the solution was incubated for 1 h at 30 °C with strong shaking (200 rpm). The collected E. coli cells were put off with 0.2 M Tris–HCl buffer (pH 8.0) and lysed by lysozyme (200 µg/mL in an ending concentration with 20 mM sucrose and 0.2 mM EDTA) for 10 min at RT. Formerly, PMSF (1 mM) and aprotinin (20 µg/mL) were additional. The OMs of E. coli cells were secluded by adding a similar capacity of extraction buffer (2% Triton X-100, 50 mM Tris–HCl, 10 mM $MgCl_2$) with DNAse (10 µg/mL). Afterward, incubation for 30 min on freeze, the lysate was centrifuged at 4,000 rpm for 5 min. The OM-containing supernatant was centrifuged at 18,000 rpm for 10 min and rinse away with PBS 2 times (Park *et al*., 2015).

2.*2.* Cell lines:

4T1 murine breast cancer cell line, It was received from the consultation office in the medicine college Babylon university that made in National cell bank-Pasteur Institute (NCBI), Iran, and cultured in RPM1-1640 medium enhanced with 20%FBS (Sigma –Aldrich), 2 mmol/L GlutaMax,100 µ/ml penicillin, and 100 µg/ml streptomycin.

2.3 Vaccine preparation*:*

To generate breast cancer vaccine, 4T1 cell line was incubated in whole media enhanced with 200 ng/ml IL2 (Elabscience, USA) through 24 h, γ-irradiated (150 Gy) on snow for killing cells, then wash away 3 times in PBS and resuspended in PBS for inoculation. Then 0.05 µmol/l/L doxorubicin (DOX) and 5 µg/ml OMVs were additional to the ending 24 hours of culture and store at 4 °C (Park *et al*., 2015).

2.4. Experimental Design:

In the experimental design, mice (n=30) were assigned randomly to 3 experimental groups:

***2.4.1.Cancer implantation*:** (25 mice), 4T1 Murine breast cancer cell line were harvested at the mono confluent stage and wash away. Subcutaneous implantation 5×105 cells in 100µL of RPM1-1640 were inoculated in the back area of mice (Hunn *et al*., 2012).

Two doses of immune suppressants (hydrocortisone) were given to mice, one before 24 h of tumor implantation and the other after 24 h after tumor implantation. All animals give gentamycin orally in drinking distilled water for 24 hours after implantation. Period to sign look was definite as a period to mass loss further than 10% obvious interactive signs (reduced activity, hunching).

2.4.1. First group: Vaccination group (15 mice of cancer implantation): This group was injected intraperitoneally with 200µL of a vaccine in PBS weekly for two months.

2.4.2 Second group: Cancer implantation positive group: remained 10 mice with cancer implantation preserved without a vaccine.

2.4.3. Third group: Control group (5mice): This group was injected intraperitoneally with 200µL of PBS weekly for two months.

All groups were killed after two months. Analysis of cytokine released: All animals autopsied for blood analysis. The blood samples were put in an anticoagulant tube for measuring the WBC and differential count by hematology analyzer system (Lab. tech, Korea). The serum was taken for quantities estimation of IL15, IL22, and IL-2 were performed by ELISA, which based on using the cytokines in Rate sera, plasma, and other body fluids. This test has been achieved according to the manufacturing company (Elabscience, USA).

2.5 Ethical Approved

This study was appropriate by the ethical and research committee of the College of Science / Kufa University / Iraq. protocol number (15788/RE)

2.6 Arithmetical study

The results are obtainable as income and statistical with standard error (S.E.) and analyzed using a one-way analysis of variance (ANOVA) test, using Graph pad prism 35.04. $P < 0.05$ was considered significant.

3. RESULTS AND DISCUSSION

3.1 Induction Tumor in Albino Mice:

Mass Loss Convinced by Tumors. Assorted murine transplantable tumors were examined for their faculties to make mammary adenocarcinoma tumors in mice founded on how much consider had been absorbed during tumor growth. The lost weight started in the third week after tumor induction, so 23.2 g at the first and second week to 14.35 g at the end of the fourth week while the control animals were weighed 32.37 g (Figures 1 and 2)

The result approves with (Tanaka *et al*., 1990), who discussed that later 3 weeks of tumor induction, the adipose tissue was virtually completely depleted and compositional analysis of the body components further confirmed that this substantial tissue wasting was due to the damage of whole-body fat and slim body mass but not that of water content. Implanted tumor models have been informed to result in key features of anorexia, heaviness loss, and loss of lean and fat mass, and increased energy spending (Marks *et al*., 2003). Among the features used to evaluate animal models is anorexia leading to losing weight. Dependent on the model used, this decrease in food intake occurs following 4–14 days of tumor burden, resulting in at first a slight decrease in food intake before revealing a severe decrease in food intake shortly afterward (Valenzano *et al*., 2005).

Figure 1. *Death albino mouse after a month of cancer induction by Murine breast cancer 4T1 cell line*

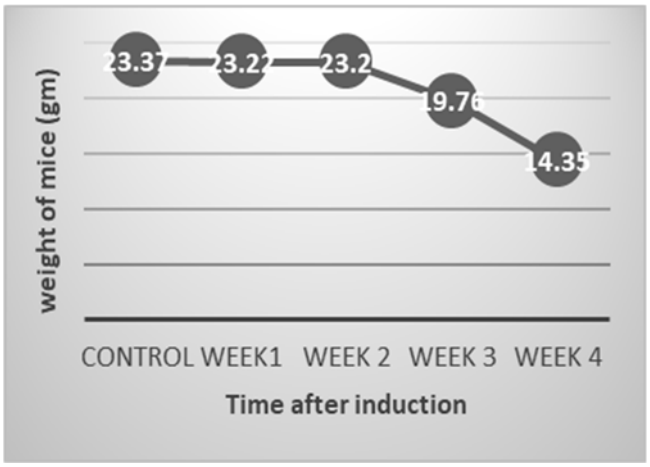

Figure 2. *Weight monitoring in cancer induction mice group*

3.2 Immunological parameters profile

When one month of murine tumor stimulation by T4-1 cell line, groups of induction mice were killed for assaying the immunity system ability. The results showed a decrease in hematology parameters. In the long run, a decrease in total WBC and monocytes but non significantly decreased in neutrophils and lymphocytes count.

Furthermore, there a significant decrease in humoral immunity parameters presented in IgG, IgM, C3, and C4 that is assaying by immunodiffusion agar. While Elizas data for cytokines profile referred to elevate of IL22 and IL6 in the serum of stimulation mice but only significantly decreased in IL2 amount (Table1). The reasons for these results related to exposing the animals to immunosuppression drugs dexamethasone previously tumor stimulation that causes the decreased immunity for a short period until development tumor that too contributed in this decreasing through that tumors are skilled at flaking external antigens or down-regulating expression of key particles essential for associates by immune cells (Whiteside, 2006). Here means tumors can escape the host's immune reaction by the existence of deprived stimulators of T cells or reduced goals for tumor-specific T cells (CTL). The appearance of molecules such as TAA, HLA class I molecules, or antigen processing mechanism components (APM) is generally miserable planned or altered in tumor cells (Ferris *et al*., 2005)

This variety shows that tumors are very skilled in their capacity to weaken host immune reactions which by an extensive collection of biological effector molecules such as many different adjacent receptor systems, small molecular types, cell enzymes, soluble cell components and cytokines / chemokines, This variety made cancer cells weakens the immune system by suppressing insoluble immune immunity (TGFβ, IL-10, ROS, enzymes, inhibitory lignans such as FasL or TRAIL) that tumor cells or additional cells release into the micro-tumor environment, Suppressive cell groups, i.e., regulatory T cells (CD4 + CD25) or marrow-derived marrow cells have established a major role in depressing the regulation of host anti-tumor immunity [20] and there are several mechanisms for performance these complicated functions: by increasing cAMP, increasing or Programmed cell death by FAS is stopped by planar regulation NO, has pro-oxidant activity, increases cAMP levels, reasons programmed cell death in NK cells, inhibits tumor CTL (Uotila, 1996). prevents T cell responses weaken T cell functions, reduces expression ζ series (Rodriguez *et al*., 2004), prevents ointment and grenase expression MRNA; stops multiplying of lymphocytes (Mocellin *et al*., 2004), inhibits making of IL-1_, IFN-_, IL-12, and TNF_ (Vicari and Trinchieri, 2004; Young, 2004), promotes macrophage expansion linked with immunosuppressive tumors (McKallip and Ladisch, 1999), prevents proliferation of IL-dependent lymphocytes 2 or instigates apocalyptic signs (Aparicio-Domingo *et al*., 2015)

About the raised levels of IL22 and IL6 in tumor stimulation mice related, several populations of immune cells at a site of inflammation create IL-22. Producers are αβ T cells classes Th1, Th22, and Th17 along with γδ T cells, NKT, ILC3, neutrophils, and macrophages. IL-22 incomes influence non-hematopoietic cells – chiefly stromal and epithelial cells (Khosravi *et al*., 2018) IL-22 natural action is begun by binding to a cell-surface complex composed of IL-22R1 and IL-10R2 receptor chains and additionally planned by contacts through a solvable binding protein, IL-22BP, which dividends sequence likeness by an extracellular region of IL-22R1 (sIL-22R1). IL-22 and IL-10 receptor chains play a portion in cellular directing and signal transduction to selectively recruit and normalize immune reactions (Aparicio-Domingo *et al*., 2015). The role of IL-22 in cancer is composite, and cancer-promoting and restrictive functions of IL-22 have been termed. The exact details why IL-22 can be a barricade in contradiction of cancer progress then below sure situations are likewise capable of helping tumor growth are indefinite (Hernandez *et al*., 2018).

Though IL-6 is a multifunctional cytokine that was initially categorized as a regulator of

immune and inflammatory responses; still, raised expression of IL-6 has been noticed in multiple epithelial tumors (Kishimoto, 2005), IL-6 signing has also been involved in tumorigenesis (Jung *et al*., 2015) Yet, the nature of IL-6's contribution in cancer has been fairly contentious, such as dichotomous roles for IL-6 in both tumor-promoting and -oppressive doings have been described. For example, IL-6 signaling has been connected to together pro-and antiapoptotic action in breast cancer cells (Conze *et al*., 2001; Alkayaty *et al.,* 2017), since normal and tumor tissue from the identical patient exposed that IL-6 mRNA is stated at meaningfully advanced stages in mammospheres resulting from tumor tissue. In accumulation, spheroids cultured from the MCF-7 breast cancer cell line also enclosed high IL-6 levels, and action with IL-6–blocking antibodies repressed spheroid creation. In height, expression of IL-6 was too distinguished in basal-like breast carcinoma tissues, which are supplemented in mammosphere and stem cell markers. (Dittmer *et al*., 2020)

3.3 Evaluation of Immunological parameters after vaccination:

The results showed that later vaccination indicated elevated in all tested parameters Total WBC, Monocytes, neutrophil, and lymphocytes (9.700, 0.5, 3.1, and 6.2 cells/mm respectively). The highest increase of IgG, IgM, C3, C4, and IL-2 (65.7, 16.6, 14.7, 9.7 cells/cu.mm and 26.5 pg/ml respectively), while a decrease of IL-6 and IL-22 (20.4 pg/ml and 19.6 pg/ml respectively). Alkayaty *et al.* (2017) suggested that the mixture of interleukin-2, tumor cell, and VOM vaccine induces long-lasting anti-tumor immunity that can protect a system against tumor recurrence; also, it can preserve a modulate host immune system and increased phagocytic activity. The contact between neutrophils and the mixture has the probability of being helpful in the treatment of cancer disease. Neutrophils have been reflected the "kamikaze" cells that reach initial at the place of damage and sacrifice themselves capable of using an anti-tumor action straight a diversity of mechanisms that include phagocytosis secretion of Tumor Necrosis Factor-α TNF-Related Apoptosis-Inducing Ligand; TRAIL, anti-microbial protein or antibody-dependent cell-mediated cytotoxicity (Zhang *et al*., 2019). Stimulation of cytotoxic T-cells (CTL) is an important factor of the immunological response to cancer cells. CTL is created by cancer in treating lymphocytes. It distinguishes TAAs, which currently on the MHC Class I molecules on the tumor cell surface. Later CTL and recruit the programmed-cell death of the malignant cells will stimulate the Fas/FasL pathway (Zilio *and* Serafini*,* 2016).

Weiner *et al*. (2010) found the binding IgG molecules to the cell surface primes to high-affinity binding of C1q to the Fc domain, trailed through stimulation of C1r enzymatic action then subsequent stimulation of downstream complement proteins. The end of this cascade is creating holes by the membrane attack complex (MAC) on the tumor cell surface and following tumor cell lysis. Also, the making of the extremely chemotactic complement molecules C3a and C5a leads to the recruitment and stimulation of immune effector cells, such as macrophages, neutrophils, basophils, mast cells eosinophils. Cross-linking of FcγRs on these cells helps ADCC and tumor cell obliteration next tumor cell lysis. Antigen-presenting cells can present tumor-derived peptides on MHC class II molecules and promote CD4+ T cell stimulation. Moreover, in a progression identified as cross-presentation, tumor-derived peptides can be accessible on MHC class I molecules, causing the stimulation of CD8+ cytotoxic T cells (Roopenian and Akilesh, 2007).

OMVs of *E. Coli* can cooperate with pattern recognition receptors, such as TLR4 to encourage cytokines and chemokines. OMV might stay accepted via DCs that are directed to the enrolment of immune cells, then motivated antigen-presenting cells (APC) concluded (TLR) documented (PAMPs). This progression heightened T helper cell creation (with Th1 and Th2) and completely improved cellular and humoral immunity. Also, OMVs can too circulate interested in non-immune cells and weight on MHC class II molecules. Stimulated antigen-presenting cells rapid MHC class II molecules that relate with the T cell receptor (TCR) on CD4+ T cells to initiative antigen-specific T cell responses, ensuing in T helper cell propagation, thus producing antigen-specific antibodies in many tissues (Zhang *et al*., 2019) (IL-2) stimulates the development and action of T cells. IL-2 prevents the progress and production of cancer cells and creates additional observable to the immune system. The medication encourages the growth of killer T cells and additional immune cells. IL-2 does not spasm cancer cells straight – it supports the immune system do the profession by improving the capability of white blood cells, called T cells, to target and destroy cancer cells. It created that Blocking IL-1 activity by IL-1R antagonist or anti-IL-1R neutralizing antibody directed to a decrease in the number of IL-22 generating cells associated

with reducing tumor development decrease (Markota *et al*., 2018). (IL-2) stimulates the development and action of T cells.IL-2 stops the growth and increase of cancer cells and makes them more visible to the immune system. The drug encourages the development of killer T cells and other immune cells. IL-2 does not directly attack cancer cells, so when stimulating the immune system, IL-2 helps do the work (Jubori *et al*., 2018) by improving the capability of certain leukocytes called T cells to destroy cancer cells. The blocking of the IL-1 axis will disrupt IL-22 making and reduction its effect on cancer cells leading to improved tumor control (Voigt *et al*., 2017)

It is attractive to venture that obstruction of IL-1 reduced IL-22 production in these patients and eventually stopped or slowed cancer growth and increase. Likewise, an additional way of manipulating the IL-1and IL-22 axis would be to avoid the processing of IL-1β by exact inhibitors of the IL-1-processing mechanism such as the inflammasome. The growth of inflammasome inhibitors is presently started for the treatment of inflammatory diseases. These combinations might also show of use in cancer treatment as soon as their in vivo efficiency in reducing IL-1β production has been proved (Markota *et al*., 2018)

Another way to target IL-22 in cancer is direct inhibition to signs of IL-22-IL-22-R1, using antibody neutralization or IL-22-BP. Targeting chemokines that penetrate cell producing IL-22 or by inhibiting transcription factors that induction IL-22 signaling (Sabat *et al*., 2014). IL-21 is a type I cytokine with close homology to IL-2, IL-4, and IL-15 that shares with these cytokines and IL-7 and IL-9 the γc receptor subunit cytokine-specific-receptor (Gajewski and Hodi, 2011).

4. CONCLUSIONS:

The use of cancer immunotherapy agents to stimulate the immune system to identify and attack malignancies has provided new potentials for active cancer treatment. Additional cytokine IL2 with OMV to completely dead cancer cell line for generating cancer vaccine product showed substantial improvement in the efficient immune system parameters so, Combination therapy might be a promising therapeutic strategy to treat cancer in the future. Something in the related field.

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6. OPEN ACCESS

***Table 2*.** A table showing the immunological parameters of the mice in which the cancer was induced and the mice that received the vaccine

Parameters	**mean**					
	Control	**±Sd**	**Cancer induction**	**±Sd**	**Cancer vaccine**	**±Sd**
Total WBC	7.800	±0.2	5.900	±1153.25	9.700	±1374.77
Monocytes	0.4	±0.2	0.2	±0.15	0.5	±0.28
neutrophil	2.9	±0.2	2.6	±1.33	3.1	±1.43
lymphocytes	6	±2.64	5.9	±1.94	6.2	±2.12
IgG	57.3	±1.51	49.6	±9.95	65.7	±5.001
IgM	12.1	±1.05	8.7	±0.90	16.6	±1.82
C3	10.3	±1.18	6.7	±1.80	14.7	±2.56
C4	6.4	±1.56	4.2	±1.65	9.7	±2.95
IL-22	15.6	±2.38	23.1	±3.75	19.6	±3.22
IL-6	18.9	±3.37	27.8	±1.95	20.4	±2.47
1L-2	17.4	±2.90	15.6	±3.82	26.5	±3.84

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY

EXPERIMENTAL SUBSTANTIATION OF MANURE FRACTIONATION ON PIG FARMS USING A SPIRAL-SCREW MECHANISM

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ABSTRACT

The main vector of mechanization and automation of livestock farming at the present stage of technological development of producers is the improvement of resource-saving technologies and technical devices that enables agricultural producers to produce relatively expensive and high-quality equipment to improve conditions for the animals. This article was considered a method for effective fractionation of manure on pig farms to further obtain humus for soil fertilization. The optimal conditions for the performance of the presented gadget were identified, namely: the time spent by the manure mass in the rotor is 0.1, with a separation factor of 170 to 180, the partition for the filter is made of metal sheet with holes whose diameter varies from 0.8 to 1.5 mm and a thickness of no more than 1 mm. The device presented in the manuscript has several advantages in the form of automation, low energy consumption and cost, novelty, and high efficiency.

Keywords: *Humus, pig farming, fractionation, manure, centrifuge, spiral*.

1. INTRODUCTION

At the moment, there are a large number of machines and devices for processing and using manure mass in the world. The concept of processing of lint-free liquid material (manure) it consists of the following main stages: separation, decontamination, and purification of liquid material from light particles.

A spiral-screw manure-dewatering device (Isaev Yu. M., Gubaidullin Kh. Kh., Shigapov I. I. 2016) has been developed, which is designed to separate manure with a moisture content of more than 97 %. The device for manure dewatering consists of supporting struts and a housing (Figures 1 and 2), inside which there is a pipe fixed in the bearing unit on both sides and a pulley that drives the helical mechanism utilizing an electric motor through a V-belt drive. A bushing with a cup is rigidly attached to the pipe by a cotter pin connection, on the outside of which a transporting spiral-screw working body is attached with a bracket. There is a hopper for loading and a branch pipe for unloading the dehydrated product in the upper and lower parts of the housing, a tightly pressed spiral with a gap, and in the lower part in the center a drain pipe.

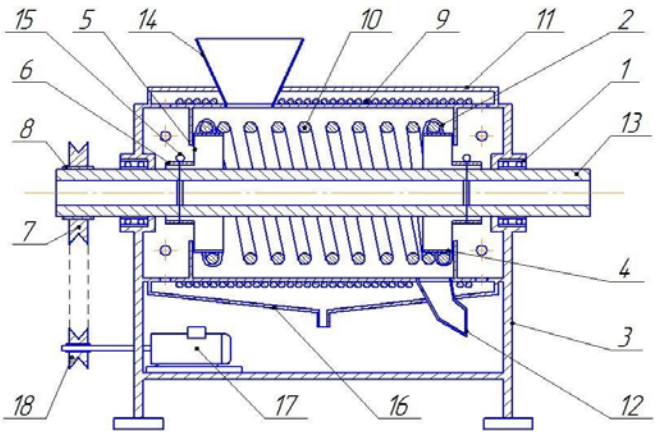

Figure 1. *Diagram of a helical-screw manure dewatering device where 1 – bearing; 2 – bracket; 3 – bearing; 4 – cup; 5 – disk; 6 – sleeve; 7 – driven pulley; 8 – fastening of a pulley; 9 – spring; 10 – working on; 11 – casing; 12 – the discharge outlet for the dewatered*

manure; 13 – pipe; 14 – the hopper; 15 – pin; 16 – discharge pipe for the liquid; 17 – motor; 18 – drive pulley.

The main working body of the technical means in the device recommended by us for removing water from liquid and semi-liquid manure (cattle, pig) and other contaminated liquids of organic origin is a spiral screw (Zuev V. A., Kovalev V. A. 1976.), (Kapustin V. P. 1997) (Figure 1). The helical screw is driven in rotational movement of the shaft–pipe (7) through a pulley (8), the shaft tube rests on two the bearing unit (1), the bearing device is mounted on a common frame (3) on the shaft are two cups (4) by disks (5), and bushing (6) mounted on the shaft by cotter pins (11). The glasses are inserted inside the spring (10), and the coils are tightened rigidly to the glasses with brackets (2), two fasteners for each glass at an angle of 180 degrees. Overall dimensions and dimensions of parts are selected based on the parameters of the technological process (volume of work, the percentage reduction in manure moisture, degree of clarification of the resulting liquid). Figure 3 shows the following layout dimensions: shaft-pipe length (1650 mm), spring length (1200 mm), outer spring diameter (300 mm), device height in the center of the spring– (750 mm). A general view of the layout of the spring attachment to the stakan is shown in Figure 3.

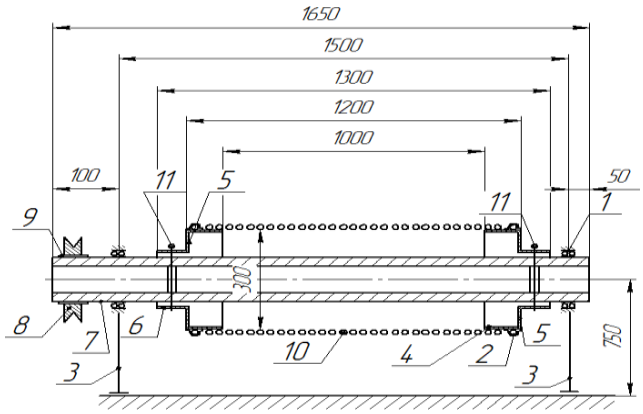

Figure 2. *The general arrangement of the helical screw where 1-bearing; 2 – bracket for pressing the wire to the cup; 3 – support; 4 - cup; 5-disk; 6 - bushing; 7-pipe (shaft); 8 – pulley; 9-fixing the pulley; 10-spring; 11-cotter pin.*

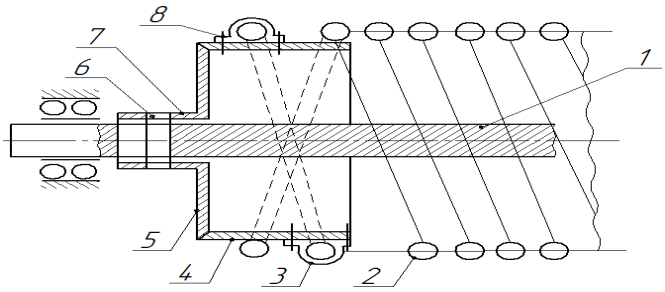

Figure 3. *Arrangement of node A: 1-shaft; 2-spring; 3-bracket; 4-cup; 5-disk; 6-pin; 7-bushing; 8-bolts tightening the wire bracket to the cup.*

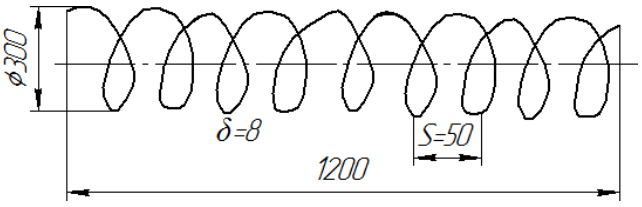

Figure 4. *Spring: art. 65 g, i = 24, length of dense winding a)нн =192 mm + 24 = 216 mm.*

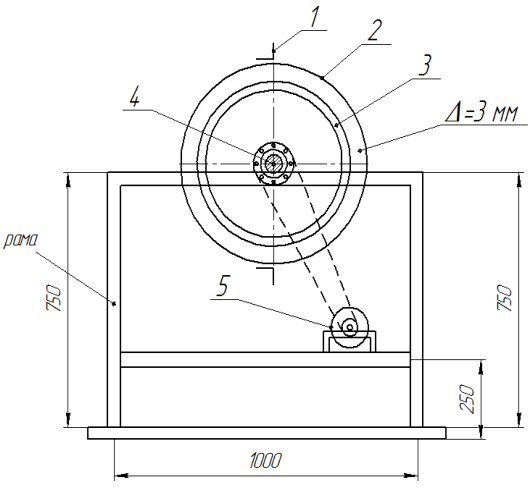

Figure 5. *Layout scheme of the spiral drum: 1–corners; 2-drum; 3-spring; 4-pulley; 5 – electric motor.*

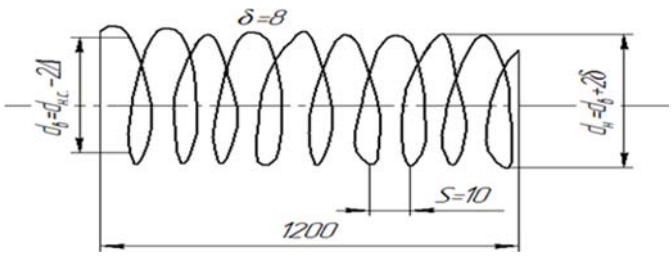

Figure 6. *Spiral drum (art. 3): i = L/S = 1200/10 =120; ℓ1в=πdn=3,14·30=94,2 cm; the total length of the wire p= ℓ1в ·I = 0,942·120 = 113м; G = 0,4·113 = 45,2 kg; length thick coiling PL = 0,8·120= 96 cm (δ = 6).*

The above data follows that only this centrifuge is capable of separating the initial manure mass without initial preparation into liquid and solid material that meets agrotechnical and veterinary requirements.

The technological process of separating liquid manure mass allows reducing transport and handling operations by 70% by using pipelines, reducing losses of manure mass, nutrients, and eliminating contamination of the territory (Yu. A. Kirov, F. G. Zabirov, D. N. Kotov 2012). This technology allows reducing the volume of expensive concrete manure storage facilities, simplifying working conditions by using mechanized and automated equipment at all stages, as well as the final technological processes for applying manure to the soil. (Shigapov I. I., Artemyev V. G., Kadyrova A.M. 2012)

2. MATERIALS AND METHODS

When developing the working body, it is necessary to identify its main design and operating parameters and their impact on the flow and uniformity of the filtration process. In this case, it is necessary to identify the relationship between physical and mechanical properties and manure mass supply.

Based on the analysis of the patent review and scientific and technical literature (Yu. a. Kirov, D. R. Costa-Rin, T. Yu., Kozlov, D. N. Kotov, S. V. Eremin), the results of initial experimental studies, the factors that mainly affect the uniformity of filtration by the working body of the filter device are determined.

In the scientific and technical literature, data on the impact of parameters such as the rotation speed of the screw working body n and the pitch of the spiral s on the supply of manure mass by the working body is insufficient. This was the basis for the use of first-order orthogonal planning and applying a full-factor experiment of type 2^2, the purpose of which is to vary two variable factors at two main levels.

The mathematical model uses an algebraic polynomial of 1 degree:

$$y = b_0 + \sum_{i=1}^{k} b_i x_i \quad \text{(Eq. 1)}$$

where *x* is the factor affecting the process;

k – number of factors;

i - factor number;

b_i – coefficient of the regression equation of the corresponding *i*-th factor;

b_0 – free term equal to the response at *xi* = 0;

y – output parameter of the process.

The calculated coefficients of the mathematical model are entered in a table called the planning matrix, which is shown in Table 1.

When conducting experiments, certain conditions were observed since the planning of a multi-factor experiment is carried out when all factors change simultaneously. In the course of the conducted experimental studies, the results were obtained, which are summarized in Table 2, which are necessary for screening out insignificant factors with the help of an experimental setup.

In the experiment, were adopted on the following variables:
α – the angle of inclination of the casing to the horizon; *s* – pitch propeller; *n* – frequency of rotation of the working body; d_{in} – inner diameter of the working body; d_h – internal diameter of the casing. These factors have a significant impact on the process of moving manure mass by the screw working body.

3. RESULTS AND DISCUSSION

The density of liquid biological waste from pig farming varies, depending on the moisture content percentage in them. The different speed of movement of liquid and solid particles (dispersion medium and dispersed phase) in the process of separating pig biological waste is due to the different densities of these particles. Thus, light

particles, because of centrifugal separation, quite quickly fall on the filter surface, clogging it, which creates an additional obstacle to the passage of the dispersion medium.

Experimentally, it was found that the temperature and percentage of moisture content in the initial biological waste of pig farming directly affect the dynamic viscosity of the clarified liquid fraction.

With increasing humidity of the filtrate (w), its viscosity (μ) decreases significantly; this dependence is shown in Figure 7.

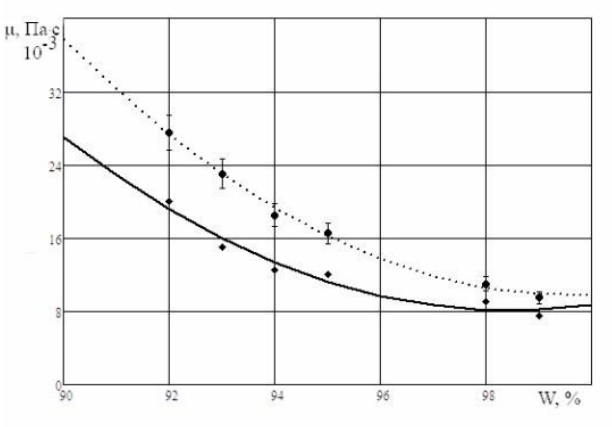

Figure 7. *Change in filtrate viscosity μ depending on the percentage of moisture content in it. W.*

From this dependence (Fig. 7), it can be seen that the range of changes in viscosity is extensive. This is due to the presence of colloidal particles and salts in the clarified fraction of liquid manure, and when the liquid fraction of manure is diluted with water, the percentage of suspended particles per unit volume will decrease. This leads to a decrease in viscosity.

Analyzing the dependence presented in figure 8, it can be said that when the temperature of the clarified manure liquid increases, the viscosity decreases, which leads to an increase in filtration units.

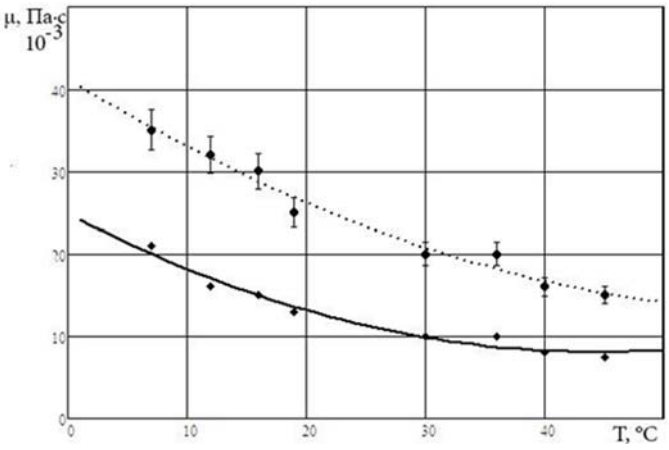

Figure 8. *Graph of changes in the viscosity (μ) of clarified liquid manure from its temperature (T) °C (humidity of biological pig waste is 1-W= 97.2%, 2-W = 92.7%)*

Pig manure is not uniform in its fractional composition. Thus, experimental studies suggest that the dispersed phase has the form of fibers, the length, and diameter of which, respectively, are in the ranges of 0.25 to 10 mm and 0.1 to 0.5 mm (Figure 9).

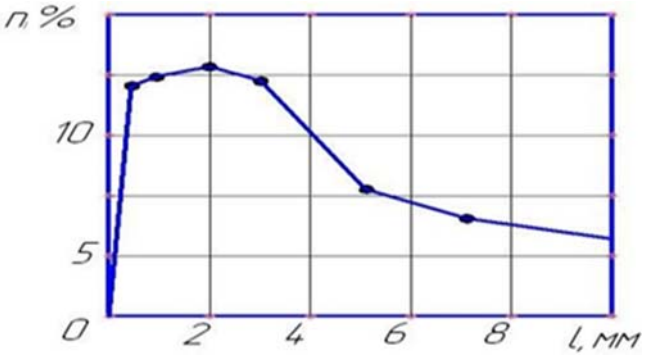

Figure 9. *Distribution of fractional sizes of the dispersed phase of pig manure.*

Having analyzed the dependence shown in Figure 9, it can be said that there is a clogging of the pores between the dispersion medium and the fibers in the liquid waste of pig life. As a result, the pig manure contains a lot of liquid.

Summing up the above, it can be said that due to the fibrous structure of the biological waste of pigs, there is a "soiling" of the filter surface, which in turn significantly reduces the productivity of the installation. This, in turn, leads to the need to extract fibers from its surface.

The graph of the dependence (Fig. 10) shows that if the percentage of moisture increases to 72% to 87%, the coefficient of friction of the dispersed phase on the inner surface of the

housing decreases in biological waste from pig farming. This is due to the occurrence of liquid friction. Figure 10 also shows that the coefficient of friction of the dispersed phase on the steel surface of the casing is higher (here, the manure fibers cling to the roughness of the steel casing, and in some places, there is friction between the fibers) than the coefficient of friction on a smooth polyethylene surface.

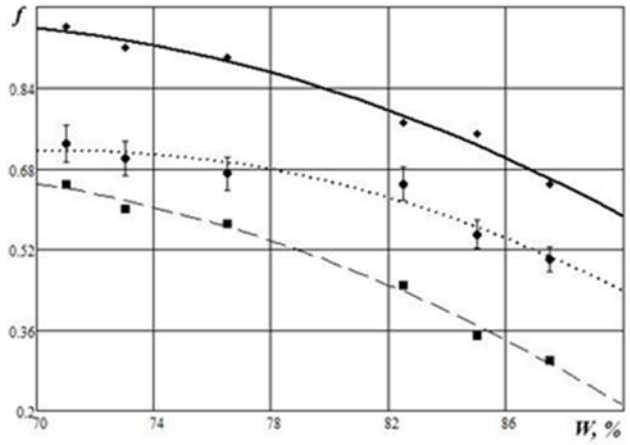

Figure 10. *Change in the friction coefficient f of the solid phase from humidity W at a specific pressure N = 600 PA: 1 – on the filter spiral; 2 – on steel; 3 - on polyethylene.*

As a result of laboratory and experimental studies, a regression equation was obtained that allows describing the change in the friction coefficient (f) of the solid phase of pig manure depending on the percentage of moisture content and the forces of adhesion on the friction surface (F):

f = 9.89·10 -6WF– 9.18·10-4 W2 + 0.132W – 2.58·10-7F2+ 6.85·10-4 F – 3.842 (insert equation according template)

The optimum coordinates are also obtained, and the response surfaces of the change in the friction coefficient (f) of the solid phase of pig manure depending on the percentage of moisture content and the adhesion forces on the friction surface (F) are constructed, as shown in Figure 11 (Put “Figure 11A and 11B” the figures need to be identified).

Analyzing the surface cross-sections (Figure 11), it can be said that with the humidity of biological pig waste W = 72.3% and the coupling forces on the friction surface F = 57.913 N, the highest value of the coefficient of friction is achieved.

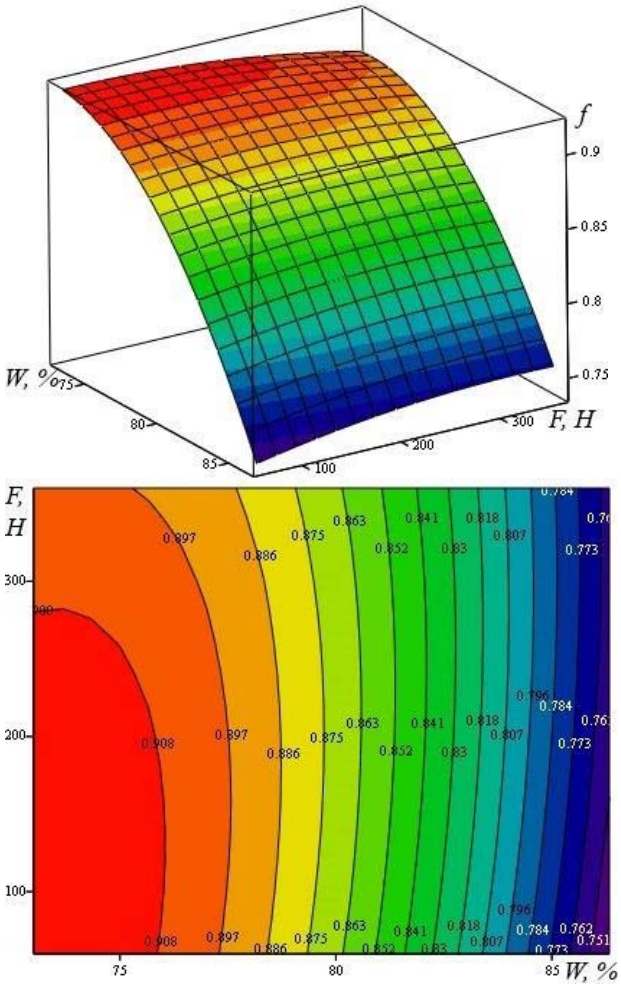

Figure 11. *Cross-section of the surface of changes in the friction (f) coefficient of the solid phase of pig manure depends on the percentage of moisture content and adhesion forces on the friction surface (F).*

Because of the analysis of the dependence of the sliding coefficient f of the solid fraction on the speed of movement *v* (Figure 12), it can be said that with an increase in the speed of movement of the manure mass, the friction coefficient is also increased. This is explained by the fact that, with an increase in the speed of movement of the solid fraction of manure over 0.5 m/s, liquid friction practically ceases (since the speed of liquid intake is lower than the speed of solid fraction intake), intermolecular forces of coupling increase.

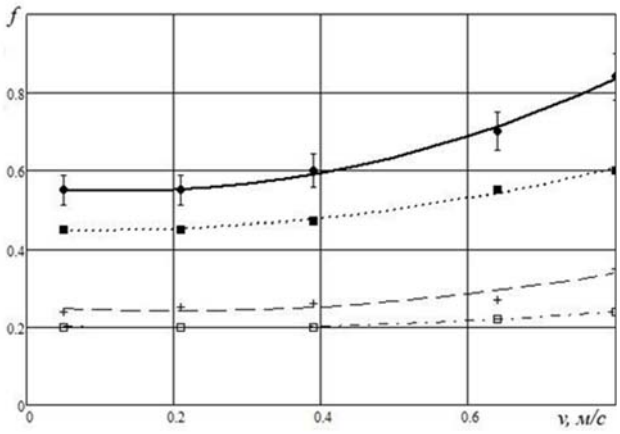

Figure 12. *Dependence of the sliding coefficient f of the solid fraction on the speed of movement v: 1, 3 – on the filter partition; 2, 4-on the steel.*

Based on the obtained experimental data, dependencies of the change in the stickiness of the dispersed phase of pig and fresh pig manure on the percentage of moisture contained in them are constructed (Figure 13). From which it can be seen that the stickiness of fresh pig manure is 660 N/m^2, which in turn is twice as much as the stickiness of the dispersed phase of pig manure (1250 N/m^2) with a humidity of 82%. This phenomenon is explained by a decrease in the total number of colloidal particles in the dispersed phase, which have a high degree of stickiness.

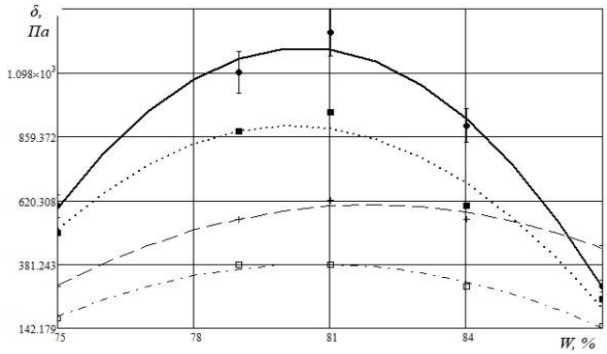

Figure 13. *Dependences of changes in the stickiness of the dispersed phase of pig and fresh pig manure on the percentage of moisture contained in them: 1, 2 –manure mass; 3, 4 – filtered sediment; 1, 3–for steel; 2, 4 – for plastic.*

Experimental studies have shown that with the humidity of biological waste from pig farming in the range of 78 to 83%, the highest degree of stickiness is observed, which in turn is the main factor in reducing the productivity of the centrifuge for separating biological waste from pigs.

Based on the experimental studies described above, the design of the spiral screw was selected, which reduces the degree of stickiness and theoretically calculating its performance, taking into account the speed of movement of the manure material and the amount of pressure from the casing. Also, it was found that the pitch (S) and angle (a) of the helix lift have a significant influence on the movement of the manure mass. So the angle (a) of the lifting of the helical line, in order to coordinate with the friction forces of the dispersed phase (where the coefficient of friction on the steel is f = 0.7 to 0.6, then φ =30 to 35°) must be equal to ≤ 30°. Also, to increase the ability of the spiral screw to capture biological waste from pig farming, it is necessary to increase the roughness of the working surface of the screw.

To coordinate the obtained theoretical and experimental data on the velocity *v,* taking into account *π* the rotation frequency n of the helical working body for different travel speeds.

For a spiral with a diameter $d_n = 90$ mm and a wire diameter δ = 8 mm, we obtain the dependence $\upsilon(n)$ (Figure 14).

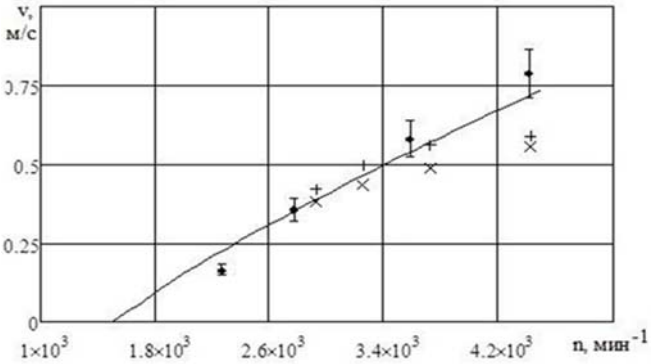

Figure 14. *Dependence of the axial velocity U of the material on n the rotation frequency n of the helical device at s = 80 mm (solid line– theoretical dependence).*

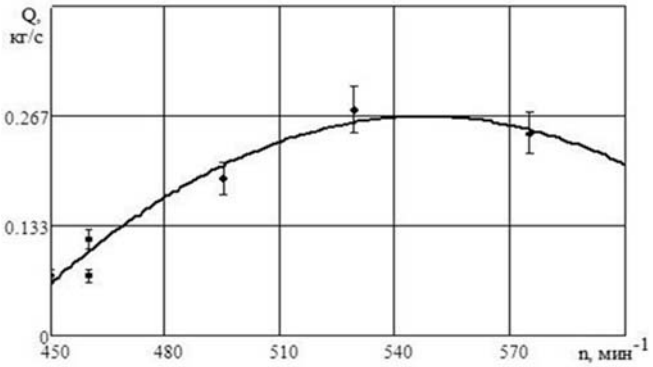

Figure 15. *Dependence of feed Q(n) of the helical device on the rotation speed of the working body on the horizontal plane.*

Figure 15 shows the dependence of the feed *Q(n)* of the helical device on the speed of rotation of the helix along the horizontal plane with parameters: p = 512 kg/m^3; v V = 0.39 PA·C; δ = 8 mm; s = 80 mm; d_n = 90 mm. The solid line represents the theoretical dependence, and the dots represent the experimental values.

The conducted studies show that for a liquid fraction with a viscosity v of V = 0, 39 PA·s, and a density of p = 512 kg/m^3, the coincidence of the results of the theoretical dependence U (n) with the experiment is observed throughout the test site. For manure mass with higher viscosity and density, the results are well consistent with the theoretical dependence until the set speed of rotation 500 min^{-1}, and then there is a discrepancy. This is explained by the fact that at the maximum rotation speeds of the helical working body, the coefficient of resistance must be specified, considering the specific conditions of the research being conducted.

This graph shows that the experimental data are consistent with the theoretical dependence, which confirms the movement of the manure mass in the complex process of rotation of the helicoidal working organ in the channel.

According to the results of the experiment, a regression equation was obtained, which makes it possible to show the changes in the dependence of the feed Q of the helicoidal working body on its rotational speed and the linear speed of movement u of the device itself on a flat floor surface accurately:

Q = 8.17x10^{-4} nu – 1.7x10^{-5}n^2 + 0.02 n – 9.82 u^2 + 3.16 u – 5.43 (insert equation according template)

Because of the model calculation, sections of the presented response surface are made to figure 4.10 (Figure 4? Or 10? or 14? it is not clear). The maximum feed Q = 0.288 kg/s when moving, obtained by the traditional optimization method applied at a rotation *speed of* n = 562 min^{-1}, the linear speed of movement W = 0.184 m /s.

Investigating the dependence of the specific energy consumption N (kW·s/kg) of the transported manure mass on the rotation speed of the helical screw p (min^{-1}) and linear travel speed u (m/c), we have built a regression equation showing the model changes:

N = 0.118 nu – 3.1x10^{-5} n^2 + 3.5x10^{-3} n + 79.6 u^2 – 110 u + 16.9.(insert equation according template)

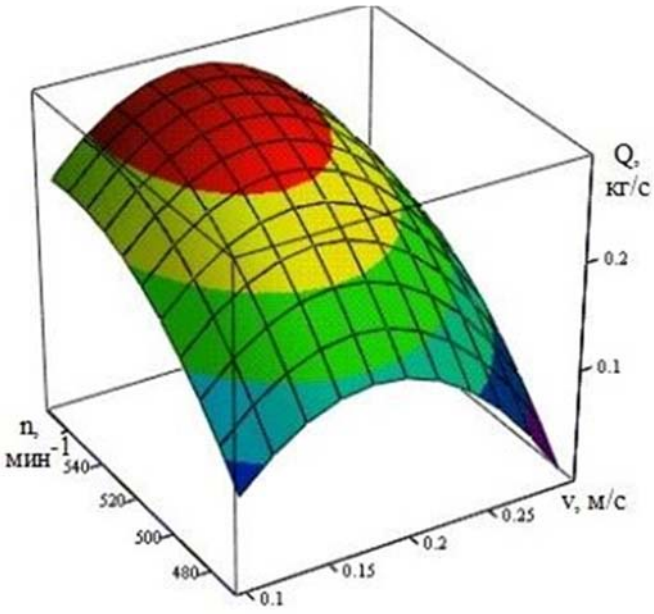

Figure 16. *Response surface of the regression dependence of the feed of the helicoid device Q (kg/s) on the speed p(min^{-1}) and linear displacement u velocity (m/s) ρ = 512 kg/m^3.*

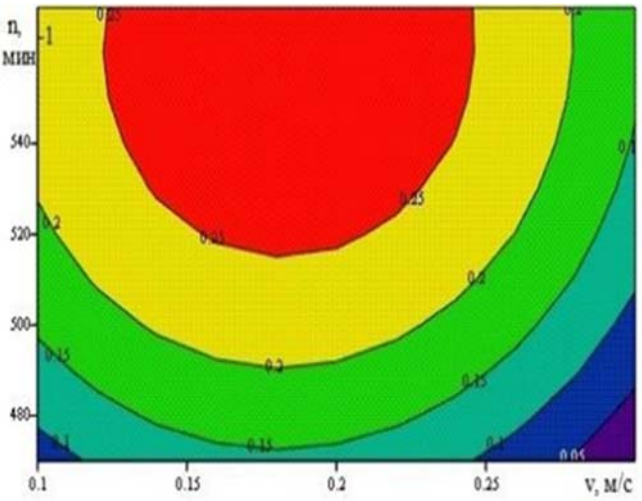

Figure 17. *Experimental dependence of the feed of the helicoid device Q (kg/s) on the rotation speed p (min^{-1}) and the linear velocity of movement u (m /s) ρ = 512 kg/m^3.*

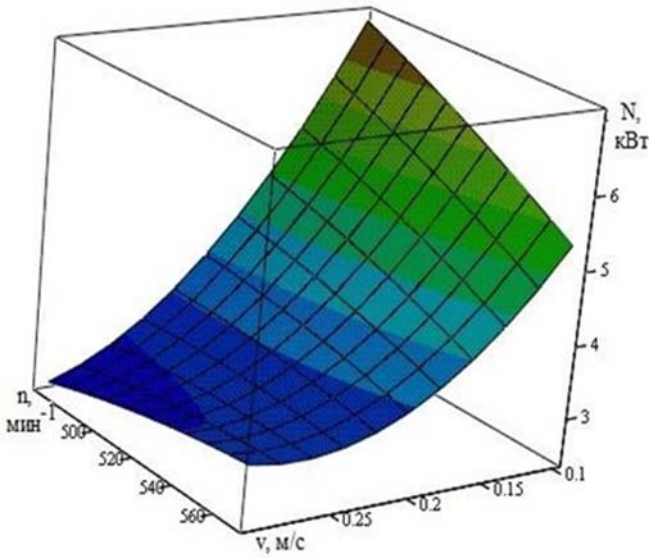

Figure 18. *The response surface of the dependence of the specific energy consumption of the device N (kW. s/kg) on the rotational speed p (min-1) and the linear displacement velocity u h (m/s) p = 512 kg/m3.*

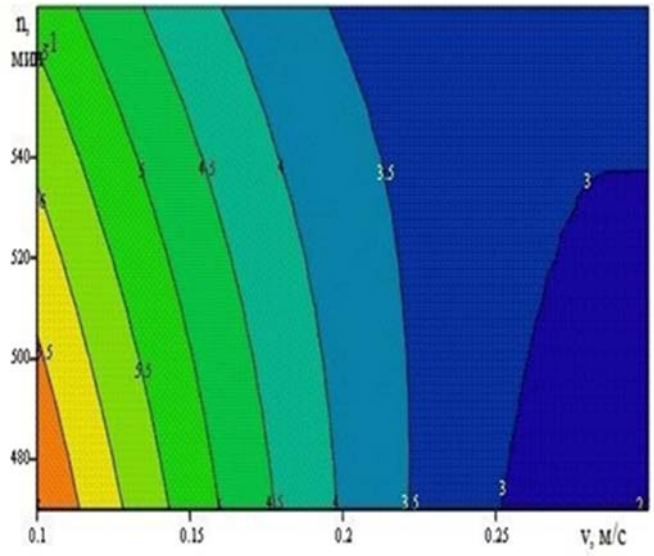

Figure 19. *Experimental dependence of the specific energy consumption of the n-helical device (W.s/kg) to the rotational speed n (min-1) and the linear velocity of movement u (m/s) ρ = 512 kg/m3.*

The specific energy consumption at minimum values calculated by the traditional optimization method is achieved at a rotational speed *of n* = 570 min-1, and a linear displacement speed *u* = of h = 0.27 m/s. Combining the results for specific energy consumption and feed, we will obtain optimal performance indicators of a vertical helicaldevice. For a material with a viscosity of V = 2.36·PA·s and a density *of p* = 916 kg/m3 at *n* = 563 to 570 min1 and a linear displacement velocity *of V* = 0.184 to 0.27 m/s. *Q* = 0.288 kg/s; *N* = 3.1 kW.s/kg.

Based on this, the optimal design and operating parameters of the working body are found, which has the necessary supply of liquid material, taking into account the minimum value of specific energy consumption.

In experimental studies, we obtained regression equations, showing the nature of the change accurately based on the flow *Q* of the working body to the rotational speed of spiral *n* and the linear travel speed *u* of the device on a flat floor surface:

$$Q = 8.17x10^{-4}\ nu - 1.7\cdot10^{-5}\ n^2 + 0.02\ n - 9.82\ u^2 + 3.16\ u - 5.43.$$

Based on the regression equation, the response surface shown in figure 20 was constructed. The graph shows that the maximum feed *Q* = 0.373 kg/spri movement, obtained by the traditional optimization method, with optimal values such as: rotation *speed n* = 536 min-1, linear velocity of movement *u* = h = 0.141 m/s.

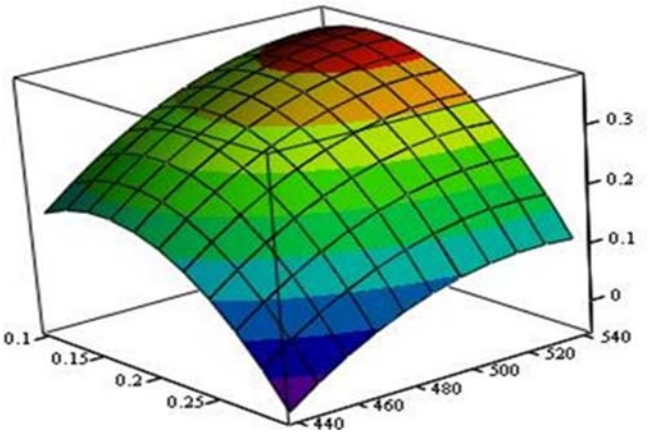

Figure 20. *Surface response regression curves of the helical feed device (kg/s) speed n (min-1) and linear carotidartery u(m/c)ρ = 916 kg/m3.*

The evaluation of the efficiency of the helical device for feeding will not fully reflect the characteristics.

Following the obtained values, we calculated a regression equation that characterizes the changes in the dependence of the specific energy consumption *N* (kW.s/kg) of the material being moved on the speed of rotation of the helical screw *n* (min-1) and linear velocity of movement *of the object* (m/s):

$$N = 0.039\ nu + 3.6x10^{-5}\ n^2 - 0.05\ n - 11.7\ u^2 - 18.9\ u + 20.1.$$

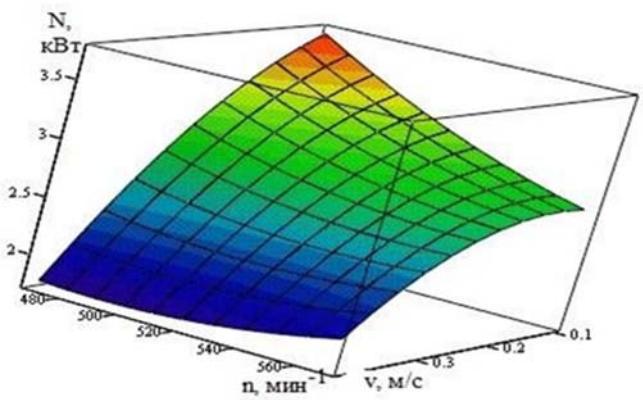

Figure 21. *Response surface of the dependence of the specific energy consumption of the device under development N (kW.s/kg) on the rotational speed n (min^{-1}) and the linear velocity of movement υ(m/s)ρ = 916 kg/m^3.*

The minimization of specific energy consumption values revealed by the traditional optimization method is found at a rotation frequency *of n* = 570 min^{-1}. A linear displacement velocity *υ* = of h = 0.27 m/s. Combining the data on specific energy consumption and on the supply, we get the permissible values for the operation of the helicoidal device along the plane. For a material with a viscosity vof V = 2.76 * 10^{-} 6м^{6m2}/ s (confused, please improve expression) at *n* = 536 min^{-1} and a linear displacement velocity *υ* = of h = 0.141 m/s, *Q* = 0.373 kg/s; *N* = 0.754 W.h/kg. Based on the results, the design operating parameters of the installation were calculated using a helical working body along the plane, which performs the highest supply of liquid fraction at low values of specific energy consumption.

Figure 22 shows the productivity dependence on the separation factor of the centrifuge, which is very difficult to analyze since an increase in the rotor speed gives, on the one hand, an increase in the specific filtration surface and an increase in the filtration pressure affecting the unit volume of treated manure, and on the other hand, a decrease in the duration of suspensions in the rotor (Text which has no punctuation and long sentence). From the obtained figures, it can be seen that the separation factor of the filter centrifuge increases sharply with its performance [31, 33].

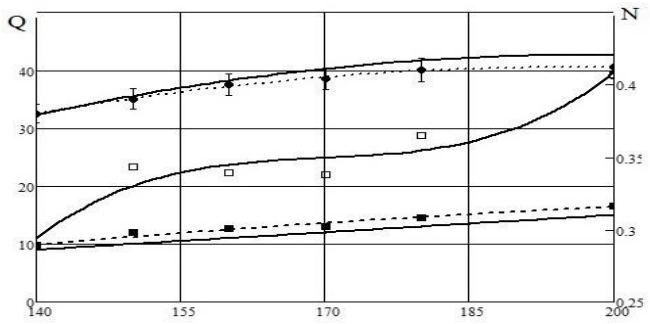

Figure 22. *Dependence of productivity Q and power N_C on the factor of separation of the centrifuge: calculated theoretically.*

The optimum for the separation factor can be considered from 160 to 190. With its subsequent increase, the productivity growth process decreases, and according to the observations made, removing sediment significantly worsens. Experimental studies have allowed us to determine the filter centrifuge performance in theoretical terms; however, its real performance is much lower than the theoretical one. The actions of factors can be taken into account by the coefficient K. The value of K obtained experimentally is in the range of 1.2 to 1.3. The experimental results Found agree well with the data calculated theoretically. This reflects the reality of technological parameters calculation of the filter centrifuge according to the theoretical expressions (Shigapov I. I., Gubaidullin Kh., 2012.),(Shigapov I. I., Kadyrova A. M. 2012.).

To calculate engineering parameters, it was necessary to know the values R of the AF, n, t, RK, and L due to the design feature of the centrifuge and the values that depend on the properties of the manure mass. The following two tasks must be performed: calculate the performance of a filtering centrifuge with known values when processing any material, or find design values based on a given performance and characteristic of the initial suspension.

Production studies on finding the filter centrifuge energy indicators have found confirmation of mathematical equations for calculating the required energy. It is established that a significant part of the necessary energy (up to 40%) is spent on removing sediment.

4. CONCLUSIONS:

1. The obtained results of experimental studies of the filter centrifuge found their confirmation of the theoretical assumptions about 2 filtration zones, which are carried out only with thin-layer filtration. During the second stage, all the free moisture leaves. In this case, the specific resistance of the sediment remains almost unchanged.
2. In the developed filter centrifuge, humidity W_{0C} = 80 to 82% was achieved due to centrifugal forces. In the dung mass of cattle, the solid fraction was practically absent of free moisture, resulting in a dried consistency and had a flowability at the slope angle of 70-80 degrees.
3. To calculate the parameters of the filter centrifuge, use the values of the physical and mechanical properties of liquid manure mass in liquid form from cattle in the following ranges: the density of dry matter from 1250 to 1300 kg/m^3; the density and viscosity of the filtered liquid, respectively, 1005...1020 kg/m^3 and 0.008 to 0.025 PA·s. The value of the coefficient of friction of the sediment on the filter surface is 0.9 to 1.2, the stickiness of the sediment is 300 to 600 PA.
4. The main parameters that affect the filtration process due to centrifugal forces are the moisture content of the initial suspension, centrifugation, the thickness of the filtered sediment layer on the filter, and the separation factor.
5. Studies have shown that during the operation of the centrifuge, optimal values were identified that have an impact on productivity: the time of manure mass in the rotor is 0.1, with a separation factor of 170 to 180, the partition for filtering is made of a metal sheet with holes whose diameter varies from 0.8 to 1.5 mm and a thickness of no more than 1 mm.

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6. OPEN ACCESS

Table 1- matrix of experiment planning 2^2 (Build the table. It is not acceptable to table this way)

Number of experiment	Variable factors			Results of experiments			
	x_0	x_1	x_2	y_1	y_2	...	y_i
1	+1	-1	-1	y_{11}	y_{21}	...	y_{i1}
2	+1	-1	+1	y_{12}	y_{22}	...	y_{i2}
3	+1	+1	-1	y_{13}	y_{23}	...	y_{i3}
4	+1	+1	+1	y_{14}	y_{24}	...	y_{i4}

Table 2 - levels of variables

Variable factor, *x*	Upper level Level (+1)	Lower level Level (-1)
Rotation speed of the working body *n*, min $^{-1}$	35	15
Inner diameter of the outer casing *Dk*, mm	36	28
Outer diameter of the helical working body *dh*, mm	26	20
Wire diameter *dp*, mm	18	15
Length of the helical working body *L*, mm	400	100
helix Pitch of the working body *s*, mm	24	6
The angle of inclination of the casing to the horizon *α*, deg.	45	0

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The authors should include Abbreviations and Nomenclature listings when necessary.

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INTRODUCTION: The introduction must clearly state the problem, the reason for doing the work, the hypotheses or theoretical predictions under consideration, and the essential background. It should not contain equations or mathematical notation. A brief survey of the relevant literature so that a non-specialist reader could understand the significance of the presented results. A good introduction should ideally have 3-5 well-explained paragraphs and should finishing pointing out the AIM of the study.

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rules for Methods sections are:

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- The study system should be clearly described. In medicine, for example, researchers need to specify the number of study subjects; how, when, and where the subjects were recruited, and that the study obtained appropriate 'informed consent' documents; and what criteria subjects had to meet to be included in the study.
- In most cases, the experiments should include appropriate controls or comparators. The conditions of the controls should be specified.
- The outcomes of the study should be defined, and the outcome measures should be objectively validated.
- The methods used to analyze the data must be statistically sound.
- For qualitative studies, an established qualitative research method (e.g. grounded theory is often used in sociology) must be used as appropriate for the study question.
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CONCLUSION: Summarize the data discussed in the Results and Discussion or Findings section showing the relevance of the work and how different it is from other researches. Also, point out the benefits and improvements that can be observed to develop new science standards that can change something in the related field.

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REFERENCES: References should be cited in the text using the **name-and-year system (Author, year) (APA FORMAT).** Alternatively, the author's surname may be integrated into the text, followed by the year of publication in parentheses. **Examples:** Grasslands are regarded as important

foraging areas for many insectivores in Europe, such as birds (Vichery, 2001; Barnet *et al*., 2004), bats (Guttinger, 1997) or amphibians and reptiles (Langton and Burton, 1997). However, the knowledge of the overall arthropod availability in such grasslands is scarce, since many studies about insect populations concentrate on extensive grasslands on poor, dry or wet soils include only few species or systematic groups (Ellgsen *et al*., 1997; Gibson *et al*., 1992; Hansel and Plachter, 2004; Manhart *et al*., 2004; Kruess and Tscharntke, 2002a, b; Wingerden *et al*., 1992; Sjodin, 2007a, b; Perner *et al*., 2005). Carbon dioxide produced by the combustion of biodiesel can be recycled by photosynthesis, thereby minimizing the impact of biodiesel combustion on the greenhouse effect (Korbitz, 1999; Agarwal and Das, 2001).

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2. Mitchell, J.A. (2017). Citation: Why is it so important. *Mendeley Journal, 67*(2), 81-95. Retrieved from https://www.mendeley.com/reference-management/reference-manager
3. Karthiga, N., Rajendran, S., Prabhakar, P., Rathish, R.J. (2015). Corrosion inhibition by plant extracts - An overview. *Int. J. Nano. Corr. Sci. Eng, 2*(4):31-49.
4. Akbulut, S., and Bayramoglu, M.M. (2013). The Trade and Use of Some Medical and Aromatic Herbs in Turkey. *Ethno Med, 7*(2): 67-77.

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ВИРТУАЛЬНАЯ КОНФЕРЕНЦИЯ 2021

НАУЧНАЯ КОНФЕРЕНЦИЯ

ДЕКАБРЬ 2021

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სამეცნიერო კონფერენცია

დეკემბერი 2021

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GÜNEY BREZİLYA KİMYA DERGİSİ

SANAL KONFERANS 2021

BILIM KONFERANSI

ARALIK 2021

2021 GÜNEY BREZİLYA KİMYA DERGİSİ KONFERANSI Güney Brezilya Kimya Dergisi, Periódico Tchê Química ve diğer ortaklar tarafından Güney Brezilya Kimya Dergisi'nin neredeyse 30. yılını kutlamak için düzenlenen kültürel bir etkinliktir.

Konferansın amacı, bilim yazarlara poster sunumu, tam makale yayını, genişletilmiş özet yayın ve önceden kaydedilmiş bir poster sunumu (5 dakikaya kadar) gibi çeşitli bilimsel iletişim biçimlerini sunabilecekleri bir yer sağlamaktır.

PERIÓDICO

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY
ESTABLISHED IN 1993

Journal of Law, Public Policies and Human Sciences
Established in 2020

www.sbjchem.com

Resmi diller İngilizce ve Portekizce

Deadline: 15th October 2021.
Conference date: 15 - 17 th December 2021.
http://www.deboni.he.com.br/sbjchem/new/conference.htm

SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY
CONFERÊNCIA VIRTUAL 2021

CONFERÊNCIA CIENTÍFICA

DEZEMBRO 2021

A CONFERÊNCIA 2021 do SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY é um evento cultural organizado pelo SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY, o Periódico Tchê Química, e outros parceiros para comemorar os quase 30 anos do SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY.

O objetivo da conferência é fornecer aos autores um local para apresentar diversas formas de comunicação científica, como a apresentação de pôster, a publicação do manuscrito completo, a publicação de resumos expandidos e uma apresentação pré-gravada do pôster (de até 5 minutos).

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Idiomas oficiais: Inglês (USA) e Português (BRA).

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CONFERENCIA VIRTUAL 2021 DE LA
REVISTA DE QUÍMICA DEL SUR DE BRASIL

CONFERENCIA CIENTÍFICA

DICIEMBRE DE 2021

La CONFERENCIA VIRTUAL 2021 DE LA REVISTA DE QUÍMICA DEL SUR DE BRASIL es un evento cultural organizado por la Revista de Química del Sur de Brasil, el Periódico Tchê Química y otros socios, para celebrar los casi 30 años de la Revista de Química del Sur de Brasil.

El objetivo de la conferencia es proporcionar a los autores un lugar para presentar sus comunicaciones científicas en múltiples formas, como la presentación de pósters, la publicación de un manuscrito completo, la publicación de un resumen ampliado y/o presentación de un póster pregrabado (hasta 5 minutos de duración).

PERIÓDICO

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