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ARMENIAN NATIONAL AGRARIAN UNIVERSITY

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АГРОНАУКА И ТЕХНОЛОГИЯ

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Հայաստանի ազգային ագրարային համալսարան

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Development and Justification of a Self-Regulating System for Adjusting the Angle of Rotary Tiller Blade with a Vertical Rotation Axis

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ABSTRACT

The article considers the problem of providing constant or almost constant values of the blade cutting angles during one rotation of the tiller rotor with a vertical rotation axis. Analytical expressions were obtained by analyzing the trajectory of the tiller knife/blade, which enable to determine the change of the blade cutting angles at a constant location and to define the patterns of this change in the ranges of (0...π) and (π...2π).

The derived expressions and conclusions make it possible to adjust the location angle of the knife, using a pattern, in case of which the cutting angles of the knife will remain unchanged during one rotation of the rotor. This circumstance is important from the prospect of ensuring a uniform working mode of the rotary tiller.

Introduction

Row-spacing activities are of key importance in terms of labor-intensive priority action among the overall agrotechnical measures implemented in the orchards and vineyards (Damanauskas, et al., 2019; Acharya, et al., 2019; Schjønning & Rasmussen, 2000). The latter accounts for the activation of plants and soils biological processes, an efficient fight against weeds and pests, and finally for the yield capacity and product quality (Monoenkov, 2017).

The mentioned activities are possible to carry out with increased quality and in compliance with the times set per agrotechnical conditions only through their complete mechanization process.

The operational practice of rotary tillers in orchards has indicated that machines with active working parts are more efficient (Monoenkov, 2017; Tarverdyan, et al., 2022; Tarverdyan, et al., 2023; Panov and Tokushev, 2005; Koval, 2010; Kupryashkin and Gusev, 2020).

The first rotary tillers with active working parts were designed in the middle of the 19th century. In 1850 the Englishman Hoskins published a scientific paper, where he proposed to cultivate the soil with rotary tools similar to a tiller.

However, only in 1900 in France, Armenian engineer Poghos-Nubar (Nubaryan) – Pasha developed and created a workable rotary tiller for cultivating cotton fields (Koval, 2010). In the following years, the operational practice of the rotary machines developed, tested, and implemented in different countries of the world provides an opportunity to assess not only their advantages but also their shortcomings, which is vital in the process of developing improved and upgraded machinery (Panov and Tokushev, 2005; Koval, 2010; Chatkin, 2008).

The energy consumption of technological procedures in the case of rotary tillers is mainly and primarily related to the geographic form, installation, and alternate arrangements of the blades. This has been confirmed by many studies (Panov and Tokushev, 2005; Koval, 2010; Konstantinov, 2019; Vorobyov and Marchenko, 1990; Chatkin, 2008; Akimov, 2018). The only way to solve the problem is the development of new rotary tillers, with the possibility of current adjustment of the shape, alternate arrangement, and parameters of the blades.

Taking into account the circumstance that the operational reliability of the existing machines is low, particularly in conditions of the soil types peculiar to the Republic of Armenia, the latter being distinguished with high content of stones and gravel, we have made an attempt to develop rotary tiller with vertical rotation axis possibly free from the above stated shortcomings upon the self-regulation of blade installation angle for the cultivation of inter-tree, inter-trunk, and inter-vine areas both in orchards and vineyards.

Materials and methods

Based on the formulated provisions and goals of the problem, it is necessary to first examine the trajectory of the blade points in the rotary tiller and the changing patterns of the velocity vectors. This is necessary, since the values of resistance forces and changing patterns affecting the blades throughout the operation of rotary tiller are determined by the mentioned factors. Only on the basis of these data, it is possible to solve the problem of optimizing the geometric and kinematic parameters of the blades, from the point of view of ensuring minimum energy consumption.

During the operation of the rotary tiller when the rotor rotates at an angular speed ω $(\varphi=\omega t)$ and makes a parallel movement in the direction of the x axis at a speed of V_p (Figure 1), an arbitrary point C of the rotary tiller draws an elongated cycloid (trochoid) if $\lambda=\frac{\omega R}{V_p}>1$ (R is the rotation radius of the C point of blade).

In parametric form, the equation of C point motion in the XOY coordinate system is as follows:

$$\left.\begin{aligned} x &= V_p t \pm R\sin\omega t,\\ y &= R\cos\omega t. \end{aligned}\right\} \qquad (1)$$

Let's consider the movement of blade attached to the rotor during one rotation and assume the conventional starting movement to be the moment when the cutting edge of the blade crosses the y axis (M point, Figure 1). The M point draws the MCANBF cycloid during one rotation, and since the blade arm is fixed to the rotary tiller disk at a constant angle γ with respect to the radius (OM), the cutting angles characterizing the technological process of soil cutting mass are constantly changing during the rotation.

The angle of blade location in an arbitrary position A of the trajectory – the angle γ – formed by the back plane of the knife arm and the radius $O_4A=R$ in that position is constant and unchanged, β'_4 is the front cutting angle; it is the angle formed by the tangent t_A of the front plane of the blade and the tangent at point A to the circle with radius $O_4A=R$, ε' is the angle formed by the back arm of the blade and t_{Ac} tangent, β_4 is the real front cutting angle, it is the angle formed by the front plane of the arm and cycloidal tangent t_A across the A point, i is the sharpening point of the blade.

The diagram (Figure 1) attests, that

$$\beta'=\varepsilon'+i;\ \beta=\varepsilon+i;\ \beta=\beta'-\Delta\varepsilon;\ \varepsilon=\varepsilon'-\Delta\varepsilon, \qquad (2)$$

where $\Delta\varepsilon$ is the angle made by the cycloidal tangents (t_A and t_{Ac}) to the circle at the given point (A).

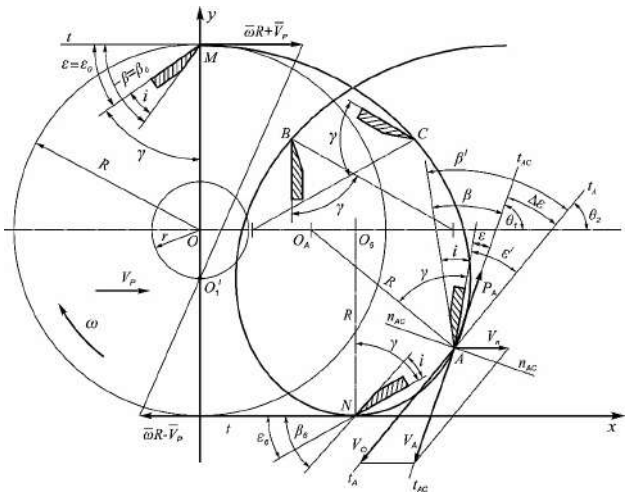

Figure 1. The changing scheme of the trajectory and cutting angles of the tiller's blade during one rotation of the rotor *(composed by the authors).*

The question under discussion aims to provide the most constant values of the blade cutting angles during one rotation of the tiller, since only then the equilibrium in the changes of moment loads generated from the factors of resistance forces applied to the rotor shaft can be provided. It is obvious that in this case the blade installation angle γ has to be changed or adjusted.

Usually, in case of similar problems, such a constant angle for the blade installation is identified, in which case the front and back cutting angles of the blade arm will receive optimal values from the point of view of the energy efficiency of the technological process during one rotation of the rotor. Based on the goal of the problem, let's try to solve the "inverse" problem and, based on the results, develop an adjustment system for the blade installation angle.

Since the installation and cutting angles of the blade arm are related to the angle *(Δε)* formed by the tangents to the cycloidal and circle of radius R at the given point of the trajectory, it is obvious that first the angles formed by the said tangents to the positive direction of x and patterns of their change during one rotation of the rotor should be determined.

Observing the movement of any point of the blade in a circle with radius R, we can write:

$$\left.\begin{aligned} x &= R\sin\omega t,\\ y &= R\cos\omega t. \end{aligned}\right\}. \tag{3}$$

Projections of the moving speeds of the point will be:

$$\left.\begin{aligned} V_x &= \frac{dx}{dt} = R\omega\cdot\cos\omega t\\ V_y &= \frac{dy}{dt} = -R\omega\cdot\sin\omega t \end{aligned}\right\}. \tag{4}$$

The angle formed by the tangent at any point on the circle will be determined as follows:

$$tg\theta_2 = \frac{dy}{dx} = -\frac{\sin\omega t}{\cos\omega t} = -tg\varphi. \tag{5}$$

The equation of a point movement with a real trajectory – a cycloid - is described by the expression (1), the components of the velocity will then be:

$$\left.\begin{aligned} V_x &= \frac{dx}{dt} = V_p \pm R\omega\cdot\cos\omega t\\ V_y &= \frac{dy}{dt} = -R\omega\sin\omega t \end{aligned}\right\}. \tag{6}$$

The equation of the tangent extended at any point of the cycloid will look like this:

$$tg\theta_1 = \frac{dy}{dx} = -\frac{R\omega\sin\omega t}{V_p \pm R\omega\cos\omega t}, \tag{7}$$

or $\theta_1 = -\operatorname{arctg}\left(\frac{\lambda\sin\varphi}{1\pm\lambda\cos\varphi}\right)$, where $\lambda = \frac{R\omega}{V_p}$ is the kinematic parameter of the rotary tiller.

From (5) $\theta_2=-\varphi$ hence the angle formed by the two tangents extended at the arbitrary A point of the trajectory – $\Delta\varepsilon_4$ - will be (Figure 1):

$$\Delta\varepsilon_4 = \theta_1 - \theta_2 = -arctg\left(\frac{\lambda\sin\varphi}{1\pm\lambda\cos\varphi}\right) + \varphi. \tag{8}$$

From the point of view of the relevancy of further analysis, the following can be determined from ΔAPK (Figure 1):

$$V_p^2 = V_0^2 + V_A^2 - 2V_0V_A\cos\Delta\varepsilon, \tag{9}$$

where $V_0=\omega R$ is the circumferential velocity of point A, V_A is the total velocity of A point, directed by the tangent to the cycloid at that point, the module of which is determined through the following expression:

$V_A = \sqrt{V_x^2 + V_y^2}$, or placing from (6) –

$V_A = \sqrt{V_p^2 + V_0^2 \pm 2V_pV_0\cdot\cos\varphi}$. Placing in (9) we'll get:

$$\cos\Delta\varepsilon = \frac{V_0^2 \pm V_pV_0\cdot\cos\varphi}{V_0\sqrt{V_p^2 + V_0^2 \pm 2V_pV_0\cdot\cos\varphi}} \text{ or,}$$

$$\Delta\varepsilon = arc\cos\left(\frac{\lambda\pm\cos\varphi}{\sqrt{1+\lambda^2\pm2\lambda\cdot\cos\varphi}}\right). \tag{10}$$

Deriving (10) expression per and equating to 0 we'll have:

$$\Delta\varepsilon' = -\frac{1}{\sqrt{1-\frac{\lambda\pm\cos\varphi}{\sqrt{1+\lambda^2\pm2\lambda\cos\varphi}}}}\cdot\left(\frac{\lambda\pm\cos\varphi}{\sqrt{1+\lambda^2\pm2\lambda\cos\varphi}}\right) = 0,$$

or $2(1+\lambda^2)\sin\varphi \mp 4\lambda\cos\varphi\cdot\sin\varphi - 2\lambda^2\sin\varphi \pm 2\lambda\sin\varphi\cdot\cos\varphi = 0$

$$\sin\varphi(1\pm\lambda\cos\varphi) = 0. \tag{11}$$

It follows from the expression (11), when when $\varphi=\{0;\ \pi;\ 2\pi\}$, $\Delta\varepsilon=\Delta\varepsilon_{min}=0$ and when $\varphi_1 = \arccos\left(\mp\frac{1}{\lambda}\right)$ and $\varphi_2=\varphi_1+\pi$, $\Delta\varepsilon$ acquires the maximum value:

$$\Delta\varepsilon = \Delta\varepsilon_{\max} = \arccos\frac{\lambda^2-1}{\lambda\sqrt{\lambda^2-1}}\cdot \tag{12}$$

Since during one rotation of the rotor $\Delta\varepsilon$ changes the sign $\Delta\varepsilon=\theta_1-\theta_2$ (Figure 1), in the range of $0\le\varphi\le\pi$ $\theta_1>\theta_2$, and in that of $\pi\le\varphi\le2\pi$ $\theta_1<\theta_2$, hence the changing pattern of $\Delta\varepsilon$ should be discussed in two variants:

$$\Delta\varepsilon = \pm\arccos\frac{\lambda\pm\cos\varphi}{\sqrt{1+\lambda^2\pm2\lambda\cos\varphi}}, \tag{13}$$

The variant with *"+"* has been analysed, while in case of *"-"* sign, when $\varphi=\{0;\pi;\ 2\pi\}$, we'll have:

$\Delta\varepsilon=\Delta\varepsilon_{max}=0$ and when $\varphi_1 = \arccos\left(\mp\frac{1}{\lambda}\right)$ and $\varphi_2=\varphi_1+\pi$

$$\Delta\varepsilon = \Delta\varepsilon_{\min} = -\arccos\frac{\lambda^2-1}{\lambda\sqrt{\lambda^2-1}} \text{ (obviously } \lambda\geq 1) \quad (14)$$

$\Delta\varepsilon=\pm f(\varphi)$ (diagrams are introduced in Figure 2).

Results and discussions

Based on the theoretical and practical research results related to the rotary machines with vertical rotation axis (Tarverdyan, et al., 2022, Tarverdyan, et al., 2023, Tarverdyan and Sargsyan, 2015), during the design of diagrams we have found it relevant to take the following geometrical and kinematical values as baseline data: $R=0.35$ m; $\omega=11s^{-1}$; $\lambda=3.85$; $V_p=1.0$ m/s.

The diagrams show (Figure 2), that the signs of the members in (13) expression $(\pm 2\lambda\cos\varphi)$ do not affect the modules of $\Delta\varepsilon_{max}$ and $\Delta\varepsilon_{min}$; only their values are derived from different values.

Thus, in case of "+" sign of the mentioned members, when the expression is also with "+" sign: $\Delta\varepsilon_{min}=0$, when $\varphi=\{0;\pi;\ 2\pi\}$ $\Delta\varepsilon_{max}=15^0$ when $\varphi\approx\{105^0 \text{ and } 255^0\}$; (ABCDE curve in Figure 2).

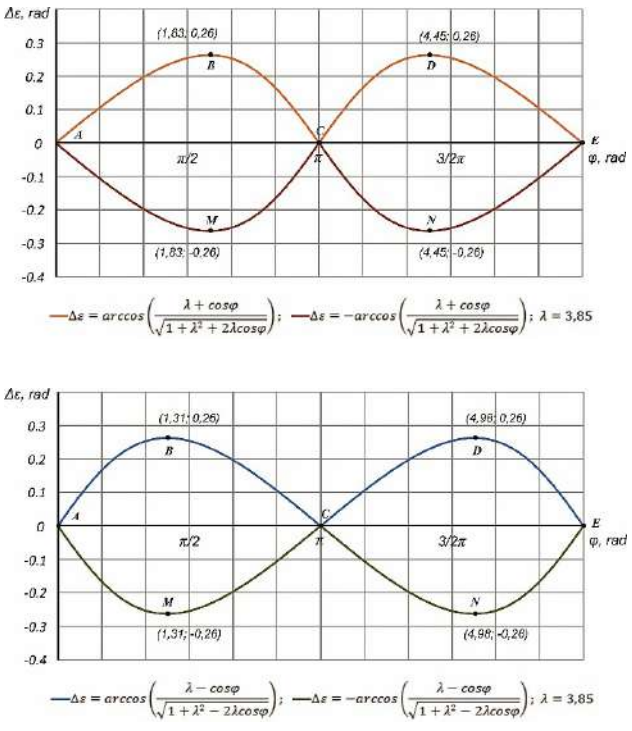

Figure 2. Diagrams of $\Delta\varepsilon=\pm f(\varphi)$ function throughout single rotation of the tiller's rotor $(\gamma=const)$ *(composed by the authors).*

In case of "-" sign of the expression (AMCNE curve in Figure 2a) $\Delta\varepsilon_{max}=0$, when $\varphi=\{0;\pi;\ 2\pi\}$ and $\Delta\varepsilon_{min}=-15^0$, when $\varphi\approx\{105^0 \text{ and } 255^0\}$.

In case of the sign "-" of $\cos\varphi$ and $2\lambda\cos\varphi$ members when the expression is with "+" sign: $\Delta\varepsilon_{min}=0$, when $\varphi=\{0;\ \pi;\ 2\pi\}$ and $\Delta\varepsilon_{max}=15^0$, when $\varphi\approx\{75^0 \text{ and } 285^0\}$ (ABCDE curve in Figure 2b), in case of "-" sign of the expression: $\Delta\varepsilon_{max}=0$, when $\varphi=\{0;\pi;\ 2\pi\}$ and $\Delta\varepsilon_{min}=15^0$, and $\varphi\approx\{75^0 \text{ and } 285^0\}$ (AMCNE curve in Figure 2 b).

The real change of the cutting angle in the tiller's blade during one rotation of the rotor $(\overline{\Delta\varepsilon})$ is described in the range of $0\leq\varphi\leq\pi$ with ABC curve, whereas in $\pi\leq\varphi\leq 2\pi$ range - with CNE curve or in the range of $\pi\leq\varphi\leq\pi$ - with AMC curve, while in $\pi\leq\varphi\leq 2\pi$ range - with CDE curve (Figure 2), depending on the direction of rotor's rotation. This statement is of vital significance in view of the objective of the problem discussed.

The changing pattern of the cutting angle in the rotor's blade depending on the kinematic parameters of the unit gives a ground to induce important judgements from the practical perspectives: the uniformity of the work in the rotary tiller with vertical rotation axis can be ensured, particularly in case of relatively high values of kinematic parameters, but their structural features and the requirements for the implemented technological processes do not allow the kinematic parameters to be increased beyond the size determined for practical considerations. Therefore, the only way to solve the problem is to adjust the blade location during the work.

The relationships between the blade location, cutting angles and their changes have the following form:

$$\gamma = \frac{\pi}{2} - \beta + i - \Delta\varepsilon \quad (15)$$

This relationship is well-known and has been always dsicussed from one perspective: γ and ψ are constants; the changing parameters of β and $\Delta\varepsilon$ during single rotation of the rotor in case of different values of γ and i have been studied (Panov and Tokushev, 2005; Kupryashkin and Gusev, 2020; Chatkin, 2008).

In the current case the changing parameter of γ is considered, in case of which the cutting angle β keeps the optimal constant or almost constant value during single rotation.

Placing $\Delta\varepsilon$ value in (15), we'll get the following from (13):

$$\gamma(\varphi) = \frac{\pi}{2} - \beta + i \mp \arccos\left(\frac{\lambda \pm \cos\varphi}{\sqrt{1+\lambda^2 \pm 2\lambda\cos\varphi}}\right),$$

i is the sharpening angle of the blade, it is constant in each specific problem, we assume that β is also constant as a precondition and hence assigning $\frac{\pi}{2} - \beta + i = K$ we can write:

$$\gamma(\varphi) = K \mp \arccos\left(\frac{\lambda \pm \cos\varphi}{\sqrt{1+\lambda^2 \pm 2\lambda\cos\varphi}}\right). \qquad (16)$$

The extreme points of $\gamma=f(\varphi)$ function are determined in the same way, as in case of $\Delta\varepsilon$ investigation.

It follows from $\gamma'(\varphi)=0$ condition, that $\gamma(\varphi)_{\min}^{\max}$ points are determined from the conditions of $\begin{cases}\sin\varphi = 0\\ \cos\varphi = \mp\frac{1}{\lambda}.\end{cases}$

It is relevant to track the changing regularities of γ depending on the rotor's rotation angle (φ) by the above stated example identifying also the optimal value of K constant based on the results of theoretical and practical research conducted in the previous years (Tarverdyan, et al., 2022; Tarverdyan, et al., 2023; Panov and Tokushev, 2005; Kupryashkin and Gusev, 2020; Chatkin, 2008).

Let's assume that $\beta=28^0;\ i=20^0$, in case of those values $K=1.43$ rad. The dependence graphs (diagrams) $\gamma=f(\varphi)$ have been designed for the same variants and cases, as in the case of $\Delta\varepsilon=f(\varphi)$ diagrams.

The value of blade installation angle (γ) in case of $\varphi=\{0;\pi;2\pi\}$ values $\gamma_0=K=1.43$ rad,, in (16) expression in case of "+" sign in $\cos\varphi$ and $2\lambda\cos\varphi$, $\gamma_{max}=1.693$ rad ($\approx 97^0$), $\gamma_{min}=1.1697$ rad $(66^052')$, when respectively $\varphi=\{1.834$ rad $\approx(105^0)$ and 4.45 rad$\approx(255^0)\}$.

In case of "-" sign: the values of γ_{max} and γ_{min} are the same as in the previous case.

In all cases in the considered example $\Delta\gamma=\pm 0{,}263$ rad, which is equal to $\pm\Delta\varepsilon$.

The results indicate that upon the regulation of the installation angle (γ) of the tiller's blade, it is quite possible to ensure a constant value for the blade cutting angle during a single rotation of the rotor. It should be taken into consideration that if the selected rotation direction of the rotor is in the way that $\Delta\varepsilon=f(\varphi)$ dependence is described with $A'B'C'N'E$ curve (Figure 2), then the regulation of γ should be implemented through curve (Figure 3), considering the manifestation of the functions in the ranges of $0\le\varphi\le\pi$ and $0\le\varphi\le 2\pi$, depending on the signs of $\cos\varphi$ and $2\lambda\cos\varphi$ members.

In case of self-regulating blade of the rotary tiller it is necessary to ensure the $\gamma_{\min} = \frac{\pi}{2} - \Delta\varepsilon_{\max}$ condition for the effective soil treatment (Panov and Tokushev, 2005), upon $\gamma_{min}>\Delta\varepsilon_{max}$. Assuming that in the boundary case $\gamma_{min}=\Delta\varepsilon_{max}$, considering (14) expression and modifying (16) expression, we can get:

$$K \pm \arccos\left(\frac{\sqrt{\lambda^2-1}}{\lambda}\right) = \pm\arccos\left(\frac{\sqrt{\lambda^2-1}}{\lambda}\right), \qquad (17)$$

wherefrom $K = \pm 2\arccos\frac{\sqrt{\lambda^2-1}}{\lambda}$ ("-" sign doesn't have any sense, since $K>0$) and hence from the first expression the minimum boundary value of λ will be:

$$\frac{\sqrt{\lambda^2-1}}{\lambda} = \cos\frac{K}{2} \text{ or } \lambda_u = \frac{1}{\sin\frac{K}{2}}. \qquad (18)$$

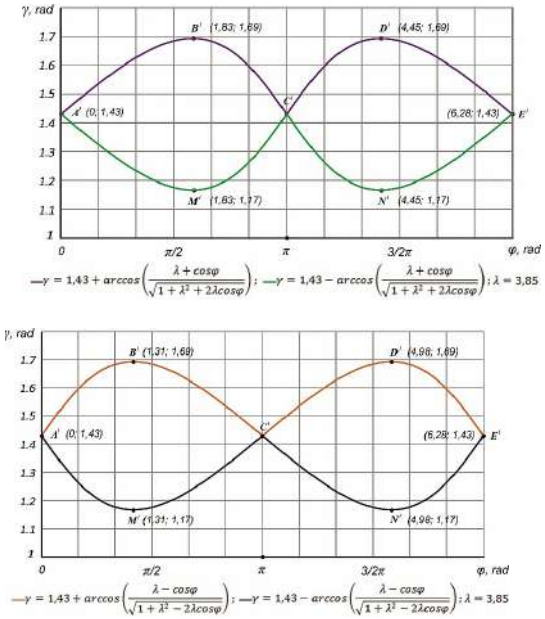

Figure 3. The diagram of $\gamma=f(\varphi)$ function during a single rotation of the rotor in case of constant cutting angles *(composed by the authors).*

It is obvious that for the selected K constant the real kinematical parameter (λ) of the rotary tiller should be higher than the boundary value (λ_u) of the parameter estimated via (18) expression.

Identifying and accepting the kinematical, geometrical and optimal parameters of the blade installation angles of the rotary tiller - $R;\ V_p;\ \omega;\ \lambda;\ \gamma;\ \beta;\ \varepsilon;\ i$ – necessary to regulate (change) the γ according to (16) expression, so that β could stay possibly constant.

After setting up the changing function of the blade installation angle (γ), related to $\beta=const$ condition, the next important step is the implementation of γ changing pattern during a single rotation of the rotor. The regulation of the blade installation angle (γ) is possible to carry out

through different technical solutions, particularly through mechanical copying with the impact of resistance forces using inertial and elastic elements (there will be a separate reference to this issue in the near future).

In all solution options, in addition to $\gamma_{(\varphi)}=f(\varphi)$ function, it is necessary to also have the variation graphs of velocities and accelerations for the blade arbitrary point depending on the rotation angle of the rotor (φ).

The velocity graph (Figure 4) has been designed per (6) expressions, whereas that of acceleration – per the (20) expressions presented below.

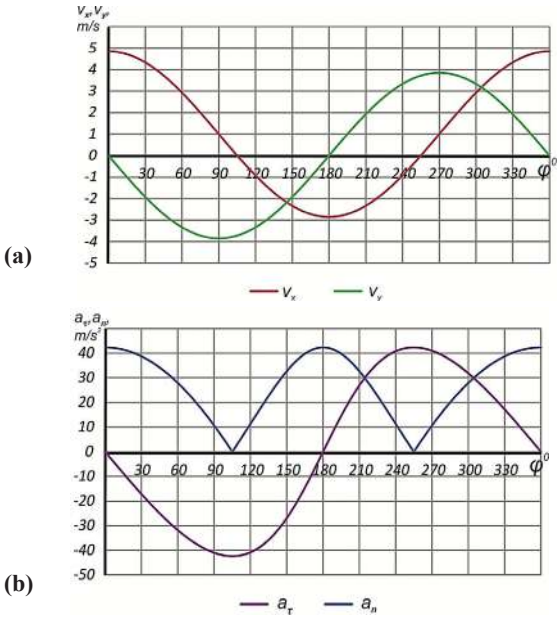

Figure 4. Variation graphs of velocity(v_x,v_y) (a) and acceleration (a_τ,a_n) (b) components of the fixed point in the rotor's blade*(composed by the authors).*

Since the rotor rotates at a constant angular speed $\omega=const,$ there is, therefore only centripetal acceleration $a_n(\omega)=\omega^2R,$ which, however, has both normal and tangential components to the cycloid trajectory.

$$\left.\begin{aligned} a_\tau &= R\omega^2 \sin\theta_1 \\ a_n &= R\omega^2 \cos\theta_1 \end{aligned}\right\}, \qquad (19)$$

where θ_1 is the angle formed by the tangent drawn to the real trajectory of the fixed point of the blade and x axis with positive direction in the case of an arbitrary rotation angle φ of the rotor. Considering that according to the (7) expression:

$\theta_1 = -arctg\left(\dfrac{\lambda\sin\varphi}{1\pm\lambda\cos\varphi}\right)$, hence, the acceleration components will be:

$$\left.\begin{aligned} a_\tau &= R\omega^2 \sin\left[-arctg\left(\dfrac{\lambda\sin\omega t}{1\pm\lambda\cos\omega t}\right)\right] \\ a_n &= R\omega^2 \cos\left[-arctg\left(\dfrac{\lambda\sin\omega t}{1\pm\lambda\cos\omega t}\right)\right] \end{aligned}\right\}, \qquad (20)$$

Another important factor in the regulation process of blade installation angle is the disclosure of variation patterns of resistance forces. Accepting that the resistance force of the blade is directed to the total velocity of the blade (V_A) along the vector's impact line and has the opposite direction (Tarverdyan, et al., 2023; Akimov and Konstantinov, 2018; Konstantinov, 2019) we can draw out the torque value applied to the rotor's shaft.

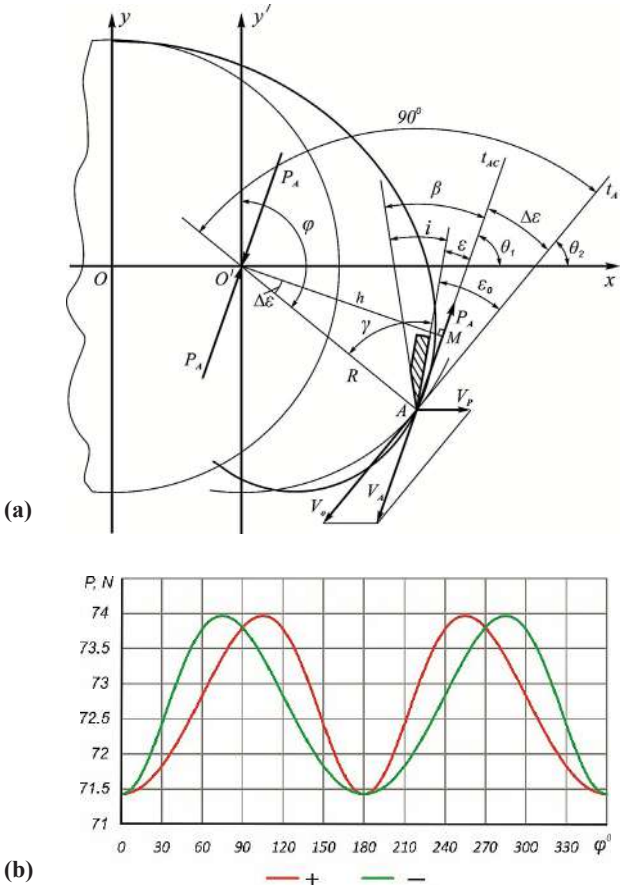

Figure 5. The diagram of determination of resistance moment applied to the tiller's rotor shaft (5a) and the graphs of variations of resistance force applied to the tiller's blade (5b).*(composed by the authors).*

$M_r=P_A\cdot h$, where $h=R\cdot cos\Delta\varepsilon$, is the arm of force couple (Figure 5a). By placing the h value, considering the (10) and accepting, that the external moment (M) applied to the rotor shaft balances the resistance moment, for P_A force we can have the following:

$$P_A = \frac{M}{R \cdot \cos\left[\arccos\left(\frac{\lambda \pm \cos\varphi}{1+\lambda^2 \pm 2\lambda\cos\varphi}\right)\right]} = \\ = \frac{M\sqrt{1+\lambda^2 \pm 2\lambda\cos\varphi}}{(\lambda \pm \cos\varphi)\cdot R}. \quad (21)$$

Variation graphs for P_A are introduced in Figure (5b). The changing pattern of P_A during a single rotation period of the rotor should serve as a background for γ regulation with the account for the impact of resistance forces and selection of the blade's geometric form.

Conclusion

As a result of analysis of the blade trajectory in the rotary tiller with vertical rotation axis several analytical expressions have been derived, which describe the changing patterns of the blade cutting angle during a single blade rotation; besides, it has been identified that the axis perpendicular to the movement direction of the unit devides the trajectory into two domains in which the expressions for the determination of the cutting angle changes are different.

The derived expressions enable to solve the problem of keeping the cutting angles in a constant state throughout a single rotation period of the rotor by means of regulating (self-regulation) the blade's installation angle, which is of utmost significance from the prospect of ensuring uniform working mode for the rotary machine.

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A New Approach to Spirulina Cultivation

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ABSTRACT

This paper presents an overview of a novel approach to spirulina microalgae cultivation. The most optimal design of a photobioreactor with compact dimensions and, at the same time, a large production area is proposed. The research areas that can significantly impact increasing spirulina cultivation productivity are outlined. Results of studies confirm the relatively high productivity of spirulina cultivation, which is more than 30 g/l of dry biomass per 1 sq. m per a day.

Introduction

Nowadays, microalgae are gaining popularity in worldwide market, including spirulina and Arthrospira genera. The relevance of research related to the cultivation and use of spirulina and its processed products is due not only to the content of many valuable substances such as proteins, vitamins, and some essential minerals (www.fdc.nal.usda.gov) but also to the possibility of their use in many areas of human activity such as agriculture, medicine (Nawal, et al., 2022), food industry (www.fao.org), crop production, environmental protection, etc. (Muzafarov and Taubaev, 1984).

Spirulina is a blue-green algae belonging to the oscillatoraceae-class of cyanobacteria. It is about 500 million years old and is one of Earth`s first photosynthetic life forms. A detailed mention of spirulina dates back to 1964 when J. Leonard discovered an aboriginal tribe in the African forests near Lake Chad that ate spirulina cakes. Unlike other African inhabitants, they had a long life expectancy, well-preserved teeth, and thick hair, and they rarely got sick. In 1974 spirulina was recognized by the World Food Conference of the UN as possibly the most healthy food for the future. Spirulina production for food purposes is gaining momentum in overpopulated countries such as China and India. In these countries, spirulina is officially recognized as the “national food” (www.un.org).

Taking into account the drawbacks of cultivation of spirulina in different types of photobioreactors, in particular equipment complexity and reliability and relatively low efficiency and productivity, we conducted R&D to determine the optimal structure of photobioreactors and designed and constructed the first modular prototype model, which was successfully tested

and showed promising results. The core objective is to have developed technology for the production of spirulina with a productivity of more than 20 g/l of dry biomass per 1 sq. m a day.

Materials and methods

The current research and experimental works were carried out in the R&D department of Smart Technology Systems, JC Armenian Branch. This department is equipped with laboratory and production facilities such as press filters, centrifuges, and water treatment units based on reverse osmosis and deionization sections. The R&D object was the design and creation of an industrial-scale modular prototype photobioreactor for spirulina cultivation based on affordable and technically feasible materials with a productivity of more than 20 g/l of dry biomass per 1 sq. m a day (Meshcheryakova et al., 2015), which is higher than in conventional cases.

It was considered that the existing traditional methods for cultivating spirulina biomass have several drawbacks; in particular, they are not environmentally sound or cost-effective. Also, it's worth noting that the industrial large-scale production of phototrophic microorganisms, in general, faces problems related to the control, monitoring, and aseptic cultivation of technological processes at all stages, which complicates the production of the desired product with a predetermined biochemical composition as it also depends largely on lighting conditions (Bondarev and Muzafarov, 2016). The design of the proposed photobioreactor system is carried out to ensure the optimal light intensity for the microalgae cultivation, as it is one of the key factors in photobioreactor design together with nutrients and pH (Rajendran and Anderson, 2014).

From the beginning, there were several geometrical types of photobioreactor cells, such as spheric, cuboid, cylindrical, rhomboid, and other forms. Based on that, the existing operating photobioreactors are diverse, including: 1. Tubular photobioreactor; 2. Flat-panel photobioreactor; 3. Bubble columnar (bubbling) photobioreactor, and 4. Film photobioreactor.

The most important aspect of any photobioreactor is to provide efficient light intensity. In particular, the photobioreactor design should provide intense and uniform lighting for the culture. It is very important to use a combination of artificial and natural light sources here; otherwise, the cost of the resulting product will be uncompetitive. To uniformly supply light to each cell in the required amount, it is convenient to use sources evenly distributed in the culture liquid. Microalgae use light as an energy source for the synthesis of protoplasm. The microorganism growth rate is maximum when intensive light saturation is reached and decreases if it is less. As mentioned, light can be of different natures (natural or artificial), intensities, and wavelengths, and these parameters can improve or worsen growth conditions.

The listed photobioreactors, despite their design, do not always meet the desired requirements since they do not have a compact size or a sufficiently extended layer of two-sided lighting for a liquid mass of up to 10 cm. Large dimensions worsen not only the light intensity but also heating, cleaning, sterilization, harvesting, occupying a large area, etc.

The experience showed that the cuboid type of photobioreactor cell is preferable as it has several advantages: it is technically easy to carry out, there is sufficient light penetration or intensity across the photobioreactor cell, efficient distribution of carbon dioxide and salts in the whole volume, which are key factors for microorganism growth (Zarrouck, 1966) together with temperature control of the photobioreactor cell's liquid (Badger and Price, 2003), easy maintenance, etc. The growth process in the proposed photobioreactor system was carried out in 2021 inside the industrial building of the company in Yerevan, in multiple rounds to get accurate results and also to handle any problems that occurred during technological processes. For high spirulina productivity in a photobioreactor system, the following conditions are required: carbon dioxide supply, water supply, optimal temperature, nutrients supply, efficient light intensity, culture density, pH levels, gas exchange, and aeration. Fully grown, concentrated spirulina cultures are required for inoculum preparation and culture maintenance. The chosen spirulina mother culture must have a high proportion of coiled filaments and clear green color. In the technological line, mixing, aeration, centrifugation, and filtration are applied (Rajendran and Anderson, 2014).

Results and discussions

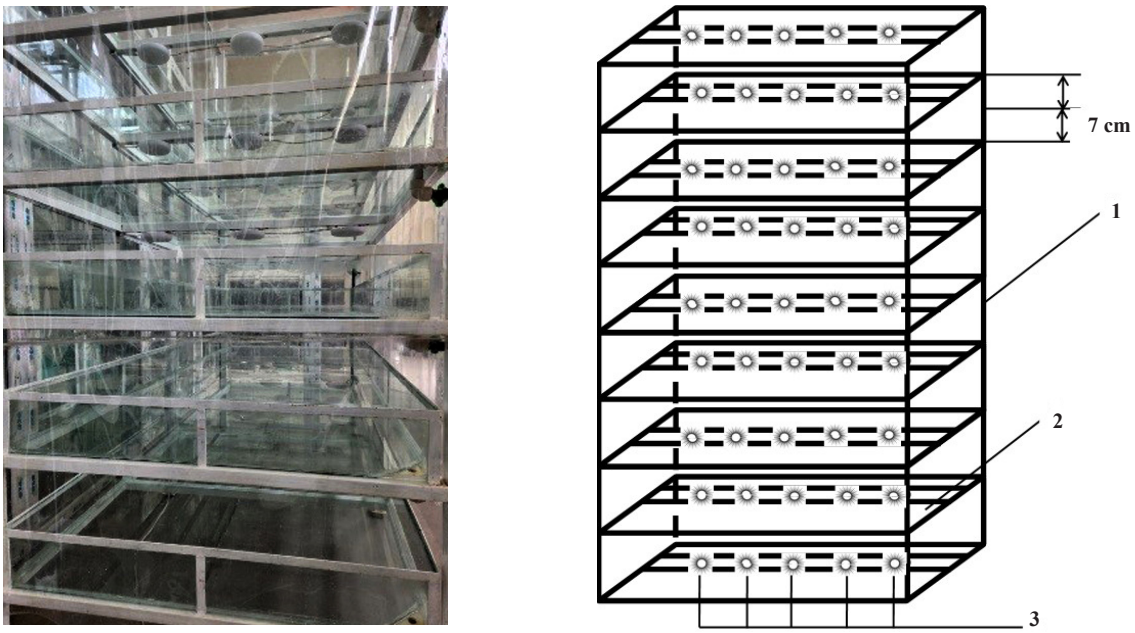
As a result of several years of studies of spirulina behavior under laboratory cultivation conditions, we have determined optimal growth conditions. Numerous experiments conducted in our modular prototype photobioreactor showed that spirulina can be cultivated efficiently indoors throughout the calendar year and cost-effectively. The designed and constructed modular photobioreactor is compact but, large.

To create a photobioreactor with compact dimensions and a large production surface, we were guided by the simple idea that the existing design should include a 10- 40 cm depth of a cell, which will be assembled as a 2 - N number of cells in a vertical arrangement one above the other, thus increasing productivity by the same number of times in the area where the cell was located previously. The next step was to use plain glass in the photobioreactor design. As a result, we got cuboid-shape cells located on top of each other, in which spirulina is cultivated. All this made it possible to radically improve the light intensity throughout the cells. This is very important for intensive spirulina cultivation.

Requirements for the photobioreactors design used for the spirulina cultivation are more stringent than for chlorella or other similar microalgae. The fact is that spirulina consists of thin spiral threads, trichomes, and contains damage that leads to microorganism death. Therefore, the next important requirement for a photobioreactor is to mix the liquid mass in such a way so as to avoid damage to the microorganism trichomes. To solve the problem, technology for microbubbles was tested and implemented in a photobioreactor. In particular, a hydraulic pump takes liquid from the photobioreactor after a 1-μm-pore filter, where a certain amount of air is simultaneously supplied. At the outlet of the pump, a soft flow of liquid rich in air microparticles is obtained. This makes it possible to periodically mix the culture liquid without damaging microorganisms.

The modular prototype of the photobioreactor (Picture) is a structure with glass shelves arranged vertically, one above the other. The height of each shelf is calculated so that the depth of the loaded suspension does not exceed 14 cm. Depending on the building height, the number of photobioreactor shelves located vertically, one above the other, can be at least 5 and not more than 30. The optimal shelf width is 1 meter.

The length is based on the building parameters and can be from 1 meter to 100 meters or more. Glass shelves are attached in a vertical position using a metal frame. The strength of the metal structure depends on the number of shelves and their dimensions. LED lamps are installed between the shelves (LEDs are a real alternative to traditional sources, including for transferring light energy to the cells of microorganisms during cultivation) to provide the necessary amount of light at night and in cloudy conditions while providing the heat necessary for optimal growth of the culture (Fontoura, et al., 2018). The glass structure of the shelves allows the lighting of the 14-cm-deep pendant layer from above and below and the lighting of the 7-cm-deep suspension layer necessary for the intensive growth of spirulina. The best range of growth for spirulina is 1500 lux to 4500 lux (Sukenik, et al., 1991). In our system, we achieve light intensity up to 4200 lux, which ensures favorable conditions for the microorganisms' intensive growth.



Picture. Cuboid cell photobioreactor (photo and general layout of structured designed and constructed in the company). *1- The metallic frame of a photobioreactor, 2- plain glass, 3- LED lighting system (composed by the authors).*

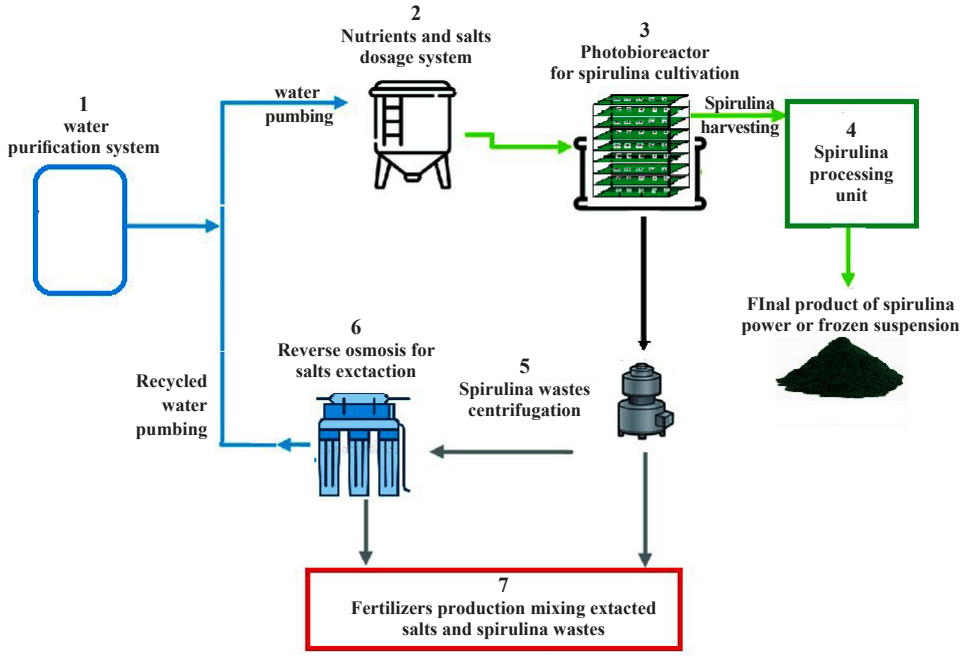


Diagram. The general layout of the full technological line for production of spirulina *(composed by the authors).*

The photobioreactor design provides easy sterilization to guarantee the high quality of the products. It is also important to ensure nutrient salt supply, periodic renewal of working suspension, periodic mixing of suspension, and the collection of ripened spirulina. All this is directly related to the photobioreactor and, in particular, to its design.

Throughout the entire period of research, the design of the photobioreactor has been constantly improved and adapted to the requirements of automatic control and adjustment of the pH of the suspension poured into the photobioreactor shelves, and optimal salt content (Belousov and Gurevich, 2005), frequency of salt mixing and working a suspension renewal, temperature and lighting. Special attention is paid to the complete automation of sterilization and harvesting ripened spirulina. The only human factor in the maintenance of the photobioreactor is that the operator monitors the technological processes of cultivating spirulina. This is done by applying special software.

Using the proposed approach, we have achieved the productivity of spirulina cultivation of 30–100 g/l of dry biomass per 1 sq. m a day. Ensuring optimal conditions such as pH >9, T-30 ^{0}C, for a 24-hour spirulina cultivation cycle, we got a minimum of 5 and a maximum of 30 g/l dried product.

Conclusion

R&D in spirulina commercial cultivation is of great practical importance for the development of the microbiological industry. The proposed technological line (Diagram) and model of the photobioreactors (representing a structure with glass shelves located vertically on top of each other), with possible further improvements, being modular can be scaled to an important industrial level. The availability of efficient equipment and analytical data based on our experience allow the development of microalgae production in a profitable and environmentally friendly way.

To sum up, the following can be stated with confidence.

- Based on modern scientific and technical achievements and more than 70 years of experience in microalgae cultivation, we have designed and constructed a photobioreactor necessary for the intensive cultivation of the spirulina microorganism with the best properties.
- Common and inexpensive materials – iron and glass – were used to manufacture the photobioreactor.
- Photobioreactors can be placed indoors with as much natural light as possible.
- Photobioreactors can be arranged from 1 to N number of parallel lines at a distance of 70 cm from each other.

- The length and number of photobioreactors depend on the length and width of the room, and the number of shelves depends on the room height.
- Photobioreactors are isolated from each other, sealed, and provide a high degree of sterilization.
- The design of photobioreactors allows the maximum use of natural light.
- The design allows automation of the entire process (from sterilization to harvesting) of spirulina cultivation.
- The design guarantees an unprecedented output of up to 100 g/l of dry biomass a day per 1 sq. m of production area in the form of the highest-quality finished product with competitive characteristics.

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Justification of Plants Water Demand in Conditions of Drip Irrigation

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ABSTRACT

In the current work, a comprehensive methodology for the soil and climatic conditions of the Republic of Armenia has been developed to justify the water requirement/demand of plants in the case of drip irrigation. The proposed methodology enables to calculate the values of the individual components of the drip irrigation regime, and in practical terms, it was used to determine the water requirement of perennial plantations in the soil and climatic conditions of the Armavir and Kotayk marzes. Based on the obtained results, the crops drip irrigation norms/rates were mapped in the GIS environment. They can serve as a background for practical planning and justification of the drip irrigation water demand for various crops and contribute to the development of introduction rates of water-saving technologies in this field.

Introduction

The lack of a drip irrigation regime for crops in the Republic of Armenia and incomplete and unsystematized research on applied irrigation technologies cause serious obstacles to the upgrading and efficient management of irrigation networks. Currently, the drip irrigation systems introduced by various programs in different irrigation zones of the republic are designed and implemented in the absence of drip irrigation regimes for crops. Moreover, the irrigation regimes of agricultural crops developed in 2007 for the irrigated lands of the republic refer exclusively to surface irrigation. As a result, the farm households and water -supplying companies appear at a dead end when WUAs should make projections for water requirements and water distribution among diverse water users in the case of drip irrigation. Multiple studies have been conducted related to the individual components of drip irrigation. To thoroughly solve the problem, it is first necessary to investigate the dynamics of crop water demand under drip irrigation conditions depending on the biological characteristics

of crops, their development stages, the depth of the root system spread, hydro-physical properties of the soil, and the complex influence of climatic and topographical conditions. The results of the conducted research will enable to evaluate the optimal water demand of crops, to develop drip irrigation regimes for crops distributed in different irrigation zones, to compare the indicators of drip irrigation with the surface irrigation regime, and to propose effective conditions for the design, construction, and operation of the drip irrigation system.

Materials and methods

One of the key issues in drip irrigation studies is the justification of crop water requirements depending on climate and soil conditions, crop type, agrotechnical, and irrigation techniques (Allen, et al., 2000; Yeghiazaryan and Miroyan, 2020).

Through the mathematical modeling of moisture movement in the unsaturated zone, the calculated formulae for the crops water demand determination depending on soil hydro-physical properties, root system depth, and planting patterns have been derived, the intensity of plants transpiration and the changing patterns of water amount in the leaves have been identified (Melikhova, 2015; Abakumova, 2006). Through the investigations, it was indicated that in conditions of drip irrigation the intensity of photosynthesis is higher than in the case of furrow irrigation. It has been found out that maximum yield can be ensured in conditions of 75…85 % field moisture capacity (Sheykin, 1980; Akopov, et al., 1985; Sahakyan, 2022). Despite the severe dry conditions in the case of drip irrigation, the plants demonstrate high resistance and even in case of 70 % of pre-watering soil moisture content they provide high yield capacity. The soil hydro-physical properties are less affected in conditions of drip irrigation in contrast to those of surface irrigation (Borodichev, et al., 2017; Davydenko, 2000; Gurenko, 2006). The constituent elements of the drip irrigation regime are the crops water consumption rate – ET_c, irrigation rate – M, watering rate – m, number – n and times – t of watering. The determination of water amount spent on the transpiration and physical evaporation in water consumption is related to certain difficulties and thus, it is very often determined as a single quantity. The total water consumption rate ET_c can be calculated through evapotranspiration ET_0. Anyhow, it is more relevant to determine the value of ET_0 with the FAO-56 method developed based on the Penman–Monteith equation, which is introduced through the following formula (Allen, 1996, Keller and Bliesner, 2000):

$$ET_0 = \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T+273} U_2 (e_S - e_a)}{\Delta + \gamma(1 + 0.34U_2)}, \quad (1)$$

where ET_0 is the estimated evapotranspiration (mm/day), R_n is the radiation reached the crop surface (MJ/m^2.day), G is the radiation reflected from the soil surface (MJ/m^2. day), T is the air temperature at 2 m high above the soil surface – ^{0}C, U_2 is the wind speed at 2 m high (km/s), e_s is the pressure of saturated evaporations (kPa), e_a is the factual pressure of evaporations (kPa), Δ is the angular coefficient, Δ is the physical constant (kPa/ ^{0}C).

In the zones with unstable and somewhat dry moisture supply, under the drip irrigation conditions, it is relevant to determine the crops water demand for the years with 50, 75, and 95 % moisture supply. In the case of such an approach, in any year, it will be possible to provide the plants with such an amount of water, so as to get a high and sustainable yield. Considering the circumstance that the crops water demand is affected by multifactorial soil and climatic phenomena, it is expedient to evaluate the years with different moisture supply percentages per the deficit of evapotranspiration:

$$def ET_0 = ET_0 - P. \quad (2)$$

The net rate of irrigation is determined by the following condition:

$$M_0 = \sum_{i=1}^{n} M_{ni}, \quad M_{nj} = ET_{cj} - 10P_j\mu_j - \Delta W_j - K_j, \quad (3)$$

where M_{nj} is the net rate of irrigation at the i-th development stage of the crop, $10P_i\mu_i$ is the active reserve of atmospheric precipitations in the soil, ΔW_i is the active reserve of moisture in the soil at the given development phase – $\Delta W_i = W_{i=0} - W_{i=k}$. Depending on the soil and climatic conditions of the specific agricultural zone and the agro-biological peculiarities of the crops, the vegetation duration can undergo some changes T(day): If we assign the number of plants development stages as n and the duration of each development phase as t_j, then it can be stated that:

$$T = \sum_{i=1}^{n} t_j. \quad (4)$$

According to the FAO-56 method, from the prospect of determining crops water demand it is reasonable to identify three development phases for the plants. The first phase is called initial – t_i, the second phase – the middle development phase t_{mid} and the third one – the final phase – t_{end}. Hence, it turns out that:

$$T = t_i + t_{mid} + t_{end}. \quad (5)$$

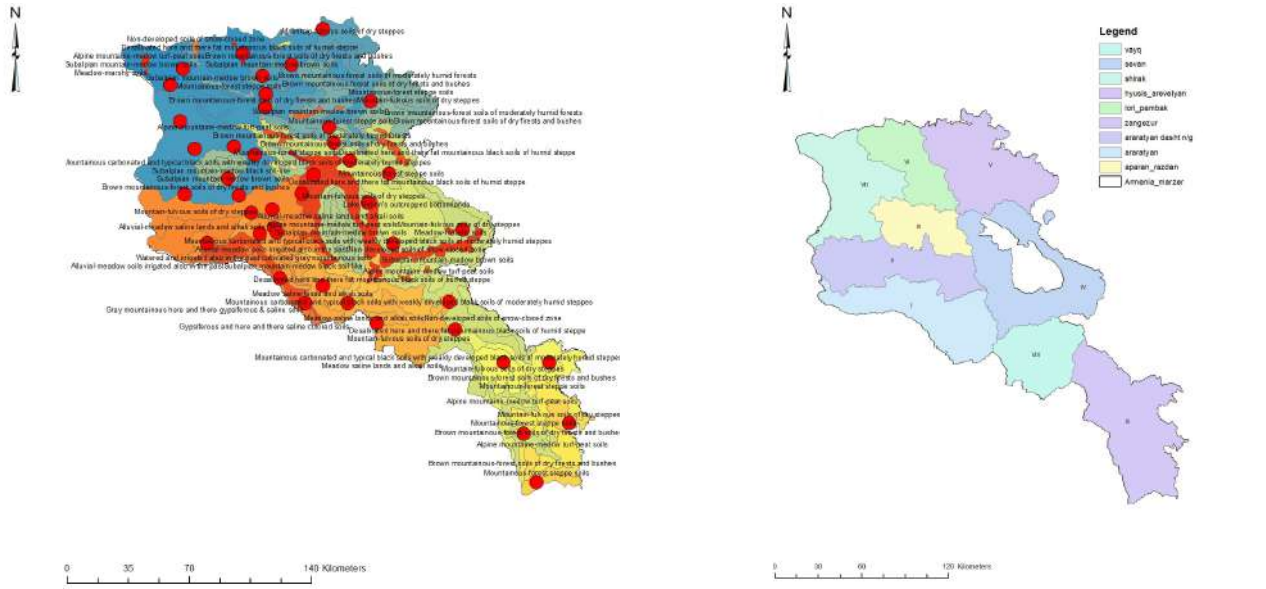

Figure 1. Digital maps of RA irrigation zones combined with the data of hydrometeorological stations and main soil types.

The value of transitional coefficient corresponding to the plant development stage is determined via the following relationship:

$$K_{cj} = \frac{ET_{cj}}{ET_{0j}}, \quad (6)$$

where ET_{cj} is the water amount consumed on the transpiration and evaporation from the soil surface per the plant development stage/phase under optimal agro-technical conditions, ET_{0j} is the evapotranspiration determined by climatic indexes. It is evident, that in certain conditions (climate, soil, crop variety, agro-technical conditions) the transitional coefficient, in its turn, is dependent on the following factors:

$$K_{cj}=K_{csoilj}*K_{cagritecj}*K_{ccropj}*K_{ctopogj}, \quad ET_c = \sum_{i=1}^{n} ET_{cj}. \quad (7)$$

The values of K_{csoilf}-coefficient are determined upon the following relationships:

$$K_{csoil} = \frac{1.5W_{wp}}{W_{FC}}. \quad (8)$$

Results and discussions

For the determination of evapotranspiration, the following parameters were obtained according to the data of meteorological stations retrieved for the period of 1992-2020: solar radiation, air temperature, relative air humidity, and wind speed. Evapotranspiration has been calculated with the support of the "CropWAT" application. Subjecting the obtained results to statistical processing, further calculations were conducted for the years with middle – 50 %, dry – 75 %, and extremely dry – 95 % moisture supply percentages. In the case of drip irrigation, it is recommended to identify the crops water demand per the irrigation zones, which correspondingly were classified according to their altitude above the sea level (Figure 1).

Table 1. K_{csoil} values depending on the soil mechanical composition, field moisture capacity (W_{FC}), permanent wilting point (W_{wp})*

Soil mechanical composition	W_{FC}	W_{wp}	K_{csoil}
Sand	12	4.5	0.56
Loamy sand	15	6.5	0.65
Sandy loam	23	11	0.72
Loam	25	12	0.72
Silt loam	29	15	0.78
Silt	32	17	0.80
Silt clay loam	33.5	20.5	0.92
Silt clay	36	23	0.96
Clay	36	25	1.04

*Composed by the authors.

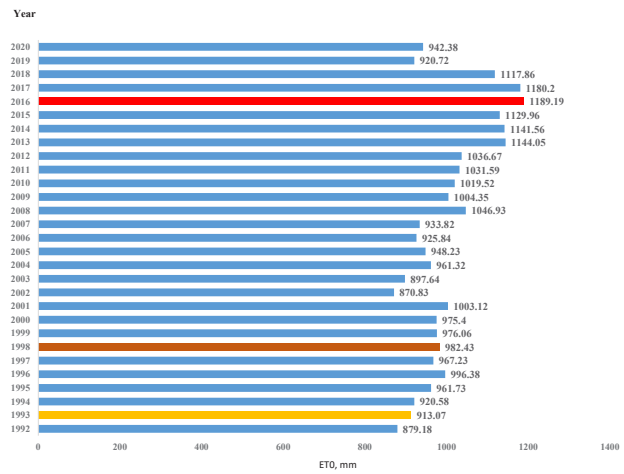

Figure 2. Dynamics of evapotranspiration during the vegetation period throughout 1992-2020 in conditions of Armavir region.

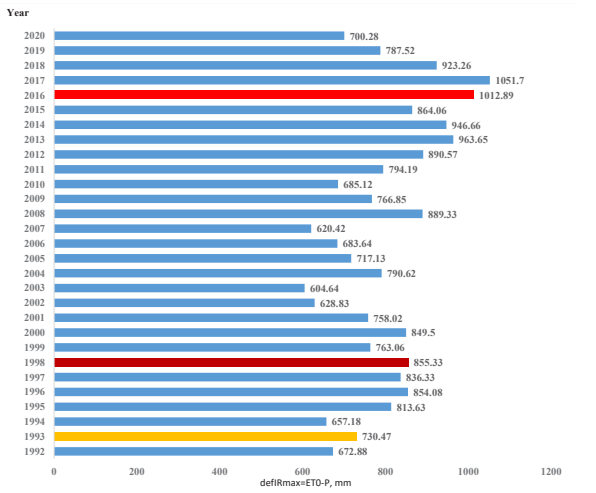

Figure 4. Dynamics of moisture deficit during the vegetation period throughout 1992-2020 in conditions of Armavir region.

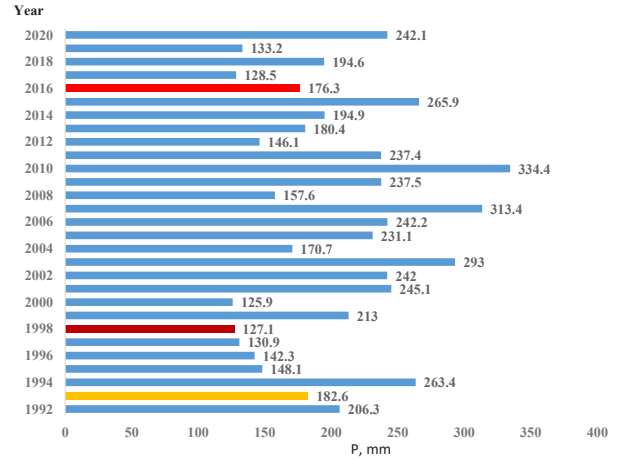

Figure 3. Dynamics of atmospheric precipitations during the vegetation period throughout 1992-2020 in conditions of Armavir region.

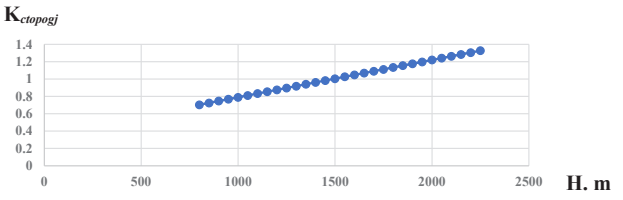

Figure 5. $K_{ctopogj}$ values per the altitudes above sea level (*composed by the authors*).

Table 2. Values of $K_{cagritecj}$ related to agro-technical conditions*

$K_{cagritecj}$ determined by agrotechnical conditions				
Grade	**Excellent**	**Good**	**Satisfactory**	**Unsatisfactory**
$K_{cagritecj}$	0.95	0.85	0.80	0.75

*Composed by the authors.

Depending on the soil mechanical composition, values have been calculated based on the quantities of Table 1.

The agrotechnical effect on the crops evapotranspiration is assessed through (Dual Crop) $K_{cagritecj} = K_{cb} + K_e$ value (Allen, 1996; Keller and Bliesner, 2000). The values of $K_{cagritecj}$ depending on agrotechnical conditions are presented in Table 2.

K_{ccropj} values are taken from Table 12 of Methodical Guideline "FAO Irrigation and Drainage Paper". $K_{ctopogj}$ values describe the effect of absolute altitude on the total evapotranspiration. Taking into account the fact that the distribution of hydrometeorological stations according to irrigation zones in the territory of the republic is discrete and limited, it is recommended to use the dependence to switch from estimated evapotranspiration to total evaporation (Figure 5).

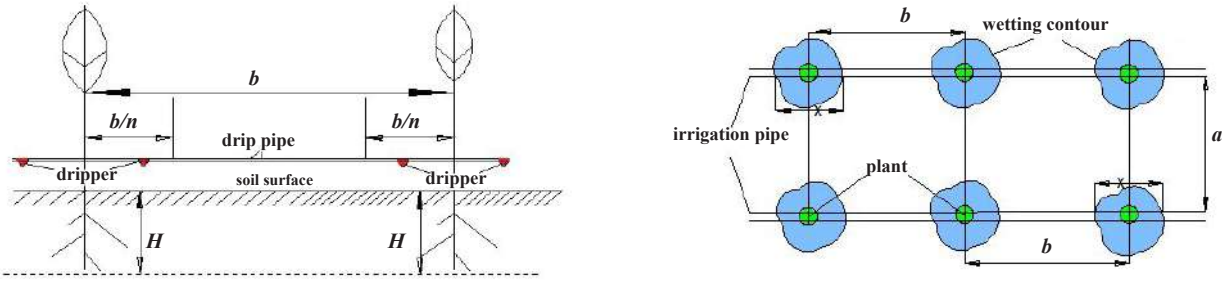

Figure 6. Linear technological diagram of drip irrigation (*composed by the authors*).

The following technological scheme is recommended to be taken as a background to identify the irrigation rate per the crops water demand (Figure 6).

The field study results of drip irrigation conducted by different authors indicate that the soil pre-watering moisture content fluctuates within 70…80 % (Akopov, et al., 1985; Davydenko, 2000; Sheynkin, 1980).

The amount of water that can evaporate from the root layer of the soil after fully moistening the soil is determined as follows:

$$TEW = 1000(W_{FC} - 0.5\,W_{WP})\,Z_e, \quad (9)$$

where W_{FC} is the field moisture capacity, W_{WP} is the permanent wilting point, Z_e is the layer of soil surface that gets dry due to evaporation: 0.10…0.15 m. The maximum water quantity provided for a plant can be calculated through the following formula:

$$IR_{pp} = 0.01\pi R^2 H \alpha (W_{FC} - \beta W_{WP}). \quad (10)$$

β values are determined:

$$\beta = \frac{\gamma W_{FC}}{W_{WP}}. \quad (11)$$

The analysis of numerical values for different soil types of the republic shows that the values fluctuate related to the soil mechanical composition: thus, in the soils with heavy mechanical composition (silt, clay) its value ranges within 1.17-1.23, whereas in the soils with light mechanical composition (sand, silt clay loam, etc.) it is within 1.56-1.73. In the soils with middle mechanical composition the latter's value makes 1.41. After summing up the above stated numbers it becomes clear that a single amount of supplied water to one plant in the soils with heavy mechanical composition is reduced to the range of 6.6-8.8 %.

The data on water amount supplied to one plant in the case of drip irrigation with account for permanent wilting point (IR_1) and 75 % pre-watering moisture conditions (IR_2) is summed up in Table 3. The digital mapping results of irrigation water demand for perennial plantations in the soil and climatic conditions of the Armavir and Kotayk regions in the ArcGIS environment are presented in Figure 7.

Table 3. Water amount supplied for one plant in perennial plantations depending on soil conditions*

Soil mechanical composition	W_{FC}	W_{WP}	R	H	α	IR_1	IR_2
Sand	12	4.5	0.5	0.7	1.1	45.33375	34.45365
Loamy sand	15	6.5	0.5	0.7	1.1	51.37825	35.66255
Sandy loam	23	11	0.5	0.7	1.1	72.534	45.9382
Loam	25	12	0.5	0.7	1.1	78.5785	49.5649
Silt loam	29	15	0.5	0.7	1.1	84.623	48.356
Silt	32	17	0.5	0.7	1.1	90.6675	49.5649
Silt clay loam	33.5	20.5	0.5	0.7	1.1	78.5785	29.0136
Silt clay	36	23	0.5	0.7	1.1	78.5785	22.9691
Clay	36	22	0.5	0.7	1.1	84.623	31.4314

*Composed by the authors.

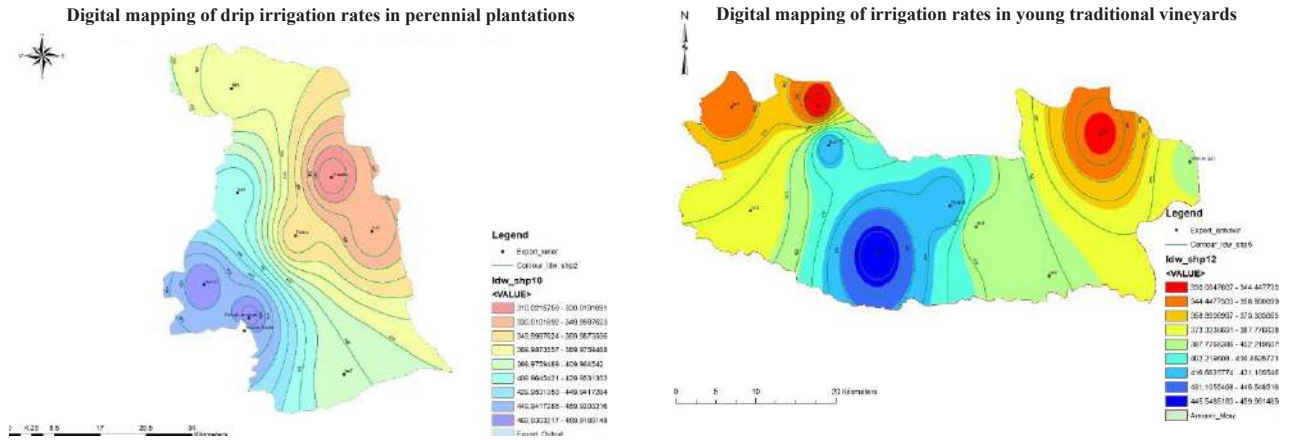

Figure 7. The digital mapping results of drip irrigation rates in conditions of Armavir and Kotayk regions.

Conclusion

An analytical model for determining the individual components of the drip irrigation regime has been developed, which takes into account soil, climatic conditions, and the phases of the biological development of the crop. The developed model has been applied in conditions of Ararat Valley and piedmont zones. Based on the obtained results the digital maps of crops water demand for vineyards and orchards in GIS medium have been compiled. The acquired results enable to plan and justify the crops water demand in case of drip irrigation in practice and to contribute to the development of introduction rates of water-saving technologies in this sector.

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The Analysis of State Agrarian Policy, Support Programs, Laws and Legislative Acts Regulating Viticulture in RA: Existing Problems and Their Solutions

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ABSTRACT

This article presents a comprehensive analysis of the state agrarian policy, laws, and legislative acts regulating the viticulture sector of RA. An analysis of the strategic documents, legislative documents regulating the sector, laws, normative legal acts, and other documents related to state policy was conducted. Obtaining results led to the conclusion that the legislative acts regulating the sector need to be updated and, in some cases, amended. In addition, the control mechanisms of the sector have gaps in law enforcement. There is no strategic document aimed at viticulture development, and existing policy measures and programs are not properly implemented and are ineffective. The article concludes with recommendations aimed at alleviating the issues revealed by this research.

Introduction

State policy and regulation are very significant in ensuring the regular operation and development of any sector of the economy. Under the influence of their peculiarities, priorities, characteristics, and external and internal factors, countries adopt certain financial policies. This becomes the main environment in which various economic activities operate. The state forms a certain legislative framework regulating economic sectors, defines taxation systems, and implements support measures that directly and indirectly affect economic sectors' development. The viticulture complex is one of the most important and strategic sectors of the RA economy. Alcoholic beverages obtained from grape processing were in second place among the products with the highest customs value exported from RA in 2021. These are products with comparative advantages in international trade, and viticulture provides a high level of employment in rural areas (Asatryan, 2022). The main goal of the research is to analyze the state policy in the field of viticulture of RA. This will allow us to assess the existing opportunities, and threats, as well as highlight the strengths and weaknesses of the state policy.

There is a rich literature about EU and US viticulture complex regulations. In particular, the researchers note that in the EU the sector is subject to very strict regulation, while in the USA this regulation is carried out in the scope of individual states (Bradley, et al., 2018). According to the EU wine policy, the EU has the strongest regulated wine market in the world (Deconinck and Swinnen, 2015). Today's European wine

policy is centered on a system of appellations, implemented as geographical indications (GIs), that entail significant technological regulations restricting the varieties grown while imposing maximum yields per hectare and other rules regarding grape production and winemaking practice (Alston and Gaeta, 2021).

Interestingly, even due to such strict regulations, EU wine policy has not always had a positive effect. Researchers point out that some components of EU wine policy have caused certain distortions instead of solving them (Meloni and Swinnen, 2012). Restrictions on planting rights are among such controversial regulations, which were introduced in 2007. After causing large controversies the restrictions were lifted in 2018 (Deconinck and Swinnen, 2014). This indicates that even in developed countries with a long history of state policy development, problems may arise regarding state policy's effectiveness. Regardless of the country's level of development, geographical location, and demographic characteristics, the state policy should be subject to constant monitoring and respond flexibly to changing economic conditions and sectoral priorities.

Materials and methods

In this research, all state policy documents related to viticulture were comprehensively studied. Specifically, "The Strategy of the Main Directions Ensuring Economic Development in the Agricultural Sector of the Republic of Armenia for 2020-2030", and "Action plan 2020-2022 for the implementation of the strategy of the main directions ensuring economic development in the Agricultural sector of the RA for 2020-2030", the reports of that action plan and state support programs were all analyzed. In the context of legislative regulation, the laws and legislative acts that regulate the viticulture complex were analyzed. All the necessary data were obtained from the official websites and reports of the RA Ministry of Economy, the Legal information system of Armenia, and other related departments.

Results and discussions

According to the law of RA about alcoholic beverages based on grape raw materials, the grape-growing regions in Armenia are Ararat, Armavir, Aragatsotn, Kotayk, Lori, Tavush, Vayots Dzor, Syunik, and Yerevan wine-growing regions. The main part of vineyards – 73 % – is located in the Armavir and Ararat regions, where technical brandy varieties of grapes are cultivated. According to the data of the Statistical Committee of the RA, there are 66.591 grape-producing farms in Armenia, of which 46.321 are farms with a size of up to 0.1 ha (69.6 % of the total). Table shows the dynamics of the main viticulture indicators of Armenia from 2017 to 2021. Gross grape harvest and average yield increased over the years, and that is conditioned by the proper cultivation of grapes by farmers and favorable climatic conditions. The vineyard areas, though, started to decrease. Studies show that farmers demolish their vineyards, and it is mainly conditioned by the very low selling prices of grapes (Asatryan, 2022).

Table. The main viticulture indicators in RA, 2017-2021*

2017		2018		2019		2020		2021	
	%		%		%		%		%
Vineyard areas. ha									
15814	100	16099	100	16497	100	16681	100	16524	100
Gross Grape Harvest. thousand tons									
210.0	100	179.7	100	217.5	100	283.2		237.1	100
Average Yield. tons/ha									
14.14		12.01		14.61		18.93		15.79	

*Composed by the authors (www.armstat.am).

In comparison wine and brandy production volumes as well as export volumes are increasing. Figure shows the dynamics of production volumes for wine and brandy products. Both have a steady growth trend except in 2020 when the global pandemic halted most industries' production and trade. Overall the analysis of the grape, wine, and brandy sectors of the viticulture complex reveals that the development of the complex is described by a growth trend. This was a summary of the current state of viticulture. Now let's discuss the main topic of this research – state policy and legislative framework regarding viticulture. According to the Ministry of Economy of RA, one of the landmark documents of RA agrarian policy is "The Strategy of the Main Directions for Ensuring Economic Development in the Agricultural Sector of the Republic of Armenia for 2020-2030".

The strategy's vision for the next ten years is to have sustainable, innovative, high value-added agriculture in harmony with the environment, ensuring the care of natural living in the village. The Strategy outlines the key priorities of the RA agricultural policy, defines the scope of priority issues, as well as the Action Plan for the implementation of the Strategy for 2020-2022. Within the framework of the mentioned policy document, among the programs to be implemented in RA in the medium-term period, measures for viticulture development are included. Part 29.1 of the strategy is directly related to viticulture. The "D" subsection of point 3 aims to introduce an updated pricing system for milk, grapes, and other products, which will be based on quality standards. In essence, the proposal is very relevant, because of the complicated and controversial situation related to grape pricing, which has been discussed many times (Linda Bitsch, et al., 2022). The part of the strategy, called "Form 2", also presents the target indicators of agriculture, which will be achieved by the implementation of the strategy. In particular, the average yield of grapes is planned to increase from 12.0 tons/ha to 15.1 tons/ha and the target figure of 273 thousand tons is set for the gross harvest of grapes. The next document analyzed within the framework of the research is "Action plan 2020-2022 for the implementation of the strategy of the main directions ensuring economic development in the agricultural sector of the RA for 2020-2030".

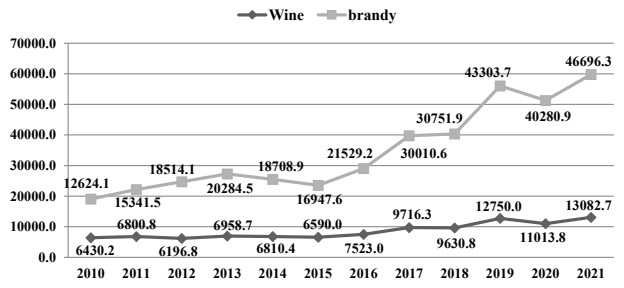

Figure. The dynamics of wine and brandy production volumes (thousand liters), 2010-2021(www.armstat.am).

The program M15 is titled "Introduce a new pricing system for milk, grape, etc. based on the quality standards". This program expects to achieve the inclusion of relevant regulatory provisions in the Draft Law of the RA on Agriculture Activity:

• Draft Decree of the RA Government

• Definition of a mandatory requirements for the pricing calculation method

• Definition of a pricing method.

According to subsection M15-5 of this program, the established pricing system should be enforced in 2021. In 2022, the financing of the monitoring of the implementation of the established pricing system should be done. On the official website of the Ministry of Economy, two reports were published about the implementation of strategic measures. The 2020 (IV quarter) report on measures implementation states: The Ministry of Economy applied to the South Korean Development Fund for the development and implementation of the pricing system for milk, grapes, etc… Within this program, experts visited Armenia in February 2020. In November of the same year, a joint research program was developed, and the Powerpoint version was presented. The first trimester of 2021 was considered the expected start date for the project. The results report states: "During the mission, assessment of needs, transfer of information, and development of a new pricing system mechanisms were carried out".

The next report chronicles the state of strategy implementation in 2021. This report mentions the following progress in implementation: "To introduce a new pricing system for milk, grapes, etc., within the KAPEX program of the South Korean Development Fund, the technical tasks of the joint research were developed and mutually agreed upon, and then the relevant documents were signed. The implementation of the process started in April 2021." For 2022 there are no reports. This program was promising in its nature, and if applied, it could introduce regulation in grape pricing in RA. However, in 2022, the grape procurement process was the same as in previous years. In terms of priorities in the strategy, some points will also, even if indirectly, impact the development of the viticultural complex. For example, the priorities "Export Diversification" and "Commercialization and value-add activities" directly relate to the sale of brandy, wine, and fresh grapes. In particular, the following objectives and measures are distinguished in the scope of these priorities:

• Supporting market opening, investment attraction, and export promotion

• Developing tools to increase agricultural investment opportunity

• Developing and implementing the "Adding value to the products" program

• Promoting well-organized agro-wholesale, retail, and farmer's markets

• Fostering cooperation, aggregation, and value chain integration.

The achievement of the listed objectives will have a positive impact on the development of the entire viticulture complex. The implementation of the above-mentioned measures in all links of the grape-wine-brandy chain will lead to the solution of sales problems and the stabilization of the sector's development dynamics.

In the context of state policy, the next link is the "State support programs in agriculture" implemented in RA, which refers to the viticulture complex. As of June 2023, 13 state support programs are operated. The first of the programs related to the viticulture complex is the "State assistance with leasing for financial lending of agri-food equipment in the RA", which can be used by grape processing companies, wine producers, brandy producers, etc… The next one is "Subsidizing interest rates on loans provided to the agricultural processing sector for the purchase (acquisition) of agricultural raw materials". The latter was very helpful in 2020 when the sector was paralyzed by an unprecedented grape harvest. The implementation of this program partially alleviated the situation, but grape procurement problems for the following years 2021 and 2022 were not resolved, and this program mitigated the situation for the time being but did not completely resolve it. The last of state support programs refers to assistance with vineyards establishment with modern technologies. In the past, there was also a program of subsidizing loan interest rates, when loans given for grape cultivation were subsidized, or loans were given on preferential terms. According to the report published by the Ministry of Economy, in 2018-2021, 539 hectares of intensive vineyards were established within the framework of this state support program. The state assistance programs for the implementation of insurance systems in the agricultural sector, co-financing, the introduction of modern irrigation systems, and hail protection nets in the agricultural sector of the RA are partially related to grape production and their efficient implementation could positively affect viticulture development. As a result of surveys conducted among grape growers in the Armavir region, the following feedback was received regarding these programs (surveys were carried out by the author):

• When asked whether they use crop insurance or not, only 20.3 % of grape growers answered yes. Some indicated that they would not use it again in the future. According to the Ministry of Economy of RA in 2022 the overall insured vineyard area was 340 hectares, which is only 2 % of total

vineyards (Source: "State support programs in agriculture, 2022 annual report", page 19).

• When asked if they are aware that there is a state support program for vineyard establishment and have used or will use this program or not, 46.3 % of grape growers stated that they are aware that such a program exists. Only 1.6 % of respondents have ever benefited from this program.

• Only 1.9 % of respondents use modern irrigation systems and only 2.5 % use hail protection nets.

In our assessment, state support programs regarding viticulture need revision and readdressing. These programs should be problem-oriented and solve specific problems of viticulture. For example, the loan subsidy program for grape purchases contributes to easing processing companies' financial difficulties, but it is still not sufficient for that company to increase its procurement volumes, or set such prices that will benefit grape producers. In the same way, within the vineyard establishment program, loans are provided to establish 0.5-10 ha vineyards. However, what are the justifications for these vineyard sizes? It has been repeatedly discussed that one of the weaknesses and threats of the RA agrarian sector is the small farms. Therefore, optimal vineyard sizes for grape producers should be determined, in which case maximum efficiency is ensured. This should be derived from the enlargement of RA lands and the effective use of land resources. If one of the priorities of the strategy of the RA agrarian sector is the optimization of farm sizes and their more practical use as a result of land enlargement, then the scientifically based definition of their sizes in support programs will be the first step towards increasing land resource efficiency. In other words, these programs should be aimed at solving concrete, relevant and current problems, rather than merely serving senseless economic growth in the agricultural sector. This is not combined with economic development.

The main laws and legislative acts regulating the viticultural complex in RA are:

1. Law of RA about alcoholic beverages based on grape raw materials
2. Establishing a quarantine to prevent the phylloxera penetration in regions free of grape phylloxera infection
3. Defining the borders of phylloxera-free and phylloxera-infected regions of grape cultivation and the list of phylloxera-resistant grape varieties
4. Defining grape varieties intended for the production of Armenian cognac, brandy, and grape vodkas and the minimum amounts of natural alcohol in the wines
5. Defining the aging procedure for Armenian cognac, brandy spirit, and grape vodka distillate
6. Deciding to set the terms and procedure for the distillation of Armenian cognac and brandy wine material, raw alcohol, spirit water, aromatic water, and grape vodka
7. Recognizing a state-authorized body to ensure the enforcement of the RA Law about alcoholic beverages based on grape raw materials
8. Defining the procedure for declaring wine product residues, grapes used to produce wine products with geographical indication, and quantities of wine products with geographical indication
9. Defining the technical requirements for the distillation of Armenian cognac and brandy wine material, raw alcohol, spirit water, aromatic water, and grape vodka
10. The decision of the RA Prime Minister on measures of the implementation of the RA Law about alcoholic beverages based on grape raw materials
11. The order of the Minister of Agriculture of the RA on the approval of the amount of actual (natural) losses arising during the storage and transportation of grapes and fruit juices, wine materials, and wines, to be deducted from the gross income for taxation purposes
12. Law of the RA on State Duties
13. Law of the RA on Activity Implementation Notification.

The main legislative act regulating the sector is the Law of RA about alcoholic beverages based on grape raw materials. The other listed legal acts mostly derive from this law and ensure its more detailed and thorough regulation. The law regulates different relations that arise during the cultivation of grapes for winemaking, sale and production, processing, labeling, and marketing of alcoholic beverages made from grapes. The law distinguishes:

• State regulation of the sector

• Basic concepts related to the sector

• Viticulture grape-producing and winemaking are defined

• Classification of wines, attenuating methods, processing, technical requirements, and geographical indications

• Definitions of Armenian cognac, brandy, and grape vodka

• Issues of labeling and marketing of alcoholic beverages based on grape raw materials

• Requirements for the production and purchase of wine products with a geographical indication

• Certification of compliance and state control in the field, etc.

In our opinion, the specification of the requirements for the production of wines with geographical indication for Armenian cognac and brandy in the law is remarkable. These are the provisions of the law, in respect of which manufacturers often commit violations. Proper control of that by authorized bodies is very important for the sustainable development of the sector. The problem is that the law clearly defines that wines with a geographical indication must be made from grape varieties produced in a specific region and even defines the maximum output of wine and the yield of grapes per 1 ha. In many cases, producers violate the established rules and release wines with a geographical indication, which, however, does not meet the specified requirements. Control over the observance of the rules will allow preventing illegal actions by processing companies, which are related to the exaggeration of procurement volumes, the unnecessarily low setting of the price of the procured grapes, etc. In addition, article 49 of the law stipulates that grapes for the production of wine products with a geographical indication must be purchased under a contract between the grape producer and the wine product manufacturer before the start of the cultivation season. Ensuring the implementation of this part of the law is very

important in solving the procurement problems of grapes. In the same way, the law stipulates that Armenian Cognac and Cognac spirit must be produced only from grapes produced in the territory of the RA. In other words, if the product shipped by the distributor bears the name of Armenian Cognac, Cognac, or is sold as Cognac spirit, then that product cannot contain raw wine produced and imported outside the territory of the RA. If imported raw materials are used in the content of a product, then the product must be called brandy. The strict control and observance of these rules will allow preventing possible violations by brandy-making organizations.

There are many cases when brandy producers complain that they lack the production capacity and financial means to procure all the produced grapes. Instead, they import raw grape spirits produced abroad at low prices and sell the product obtained from them under the name of Armenian Cognac. State-authorized bodies should strictly supervise these provisions since they directly affect grape procurement and market competition. State control over labeling is a top priority too. Regarding the marking of wines and brandy products, it is clearly defined that such information which can create a false impression and mislead consumers should not be used during labeling. It should be noted that companies in the sector often violate this provision, and the Commission recorded many such cases.

According to the RA Government's decision N 1058 of September 17, 2009, the Ministry of Agriculture was recognized as the state-authorized body for the implementation of some provisions of the Law of RA about alcoholic beverages based on grape raw materials. Currently, RA does not have a Ministry of Agriculture. It has been merged with the Ministry of Economy and it is unclear if those liabilities have also been transferred to the Ministry of Economy or not. Therefore, the Law has some gaps regarding the definition of an authorized body. Regarding specific articles and provisions, it is not defined which state institution can act as a state-authorized body.

According to the RA Government's decision N 646 of June 23, 2016, the Vine and Wine Foundation of Armenia (VWFA) was established. The VWFA's purpose is the efficient and coordinated development of the sector, as well as the introduction of new strategies for state policy and developmental programs (www.vwfa.am). In countries with well-developed wine industries, various state bodies operate that deal with the regulation of the viticulture sector, in parallel with the Ministry of Economy or Ministry of Agriculture. For example, at the federal level in the USA, that body is the Alcohol and Tobacco Tax and Trade Bureau ("TTB"). TTB collects taxes on alcohol, tobacco, firearms, and ammunition, protects the consumer by ensuring the integrity of alcohol products, ensures only qualified businesses enter the alcohol and tobacco industries and prevents unfair and unlawful market activity for alcohol and tobacco products. In the case of the EU, there is a whole list of national and regional bodies that ensure compliance of laws and rules in the field of viticulture in the EU member states with the laws established by the Union. In Argentina, since 2000, large winemaking organizations, together with the Government of Mendoza, the National University of Chuyo, and the National Institute of Agricultural Technology, began working on the development of a strategic plan for the wine industry and, as a result of a heated compromise process, adopted "National Law 25849", which created the Argentine Wine Corporation (COVIAR). The latter is a public, non-governmental organization, whose central mission is the management and coordination of the Strategic Plan of the Wine Industry 2020. According to the Law of the RA on Activity Implementation Notification, engaging in the production of grape wine and brandy is considered a type of activity subject to notification. A state fee is charged for engaging in these types of activities, which is 25,000 AMD for grape wine (www.arlis.am). As for cognac/brandy, the situation is a little different. In 2018, after the relevant amendments and additions to the Law of the RA on State Duties, the number of state duties for the production, sale, and import of brandy products up to 100 thousand liters (calculated with 100 percent alcohol) was set at 10 million AMD. Those changes were a significant step in the background of the previous regulations. This is because in the past the amount of duty was 15 million AMD, and there was no state duty for brandy imports. These new changes contributed to creating more favorable conditions for domestic producers. However, in our opinion, the current 10 million state duty should be reduced. This issue was also addressed by the Commission. Its sectoral study emphasizes the review of the state duty amount for the right to produce and import cognac/brandy. In our opinion, a review should be carried out only regarding the duty of acquiring the right to produce cognac/brandy. There is no need to change the state duty on imports. In this case, only local production will be promoted, and the risk of importing cheaper foreign brandy and cognac (which could harm local producers) will be limited. As a result of the revision of state duties it was stated that:

• Equal competitive conditions will be created for companies engaged in the production of brandy/cognac and brandy spirit. Other small companies will start producing brandy/cognac, which will decrease concentration levels on the market. This will reduce the risk of a dominant position by any market participant

• The emergence of new companies in the industry will increase grape demand, which will solve the grape procurement problem. In particular, it will contribute to the increase in the prices of grape procurement, the increase in the level of marketability of grapes, and the timely execution of payments for procurement.

In the "Results of the study conducted in the field of cognac turnover" (carried out by the Commission), the following problems were pointed out (www.competition.am).

• Some companies in the brandy industry produce so-called "Armenian special drink", "Armenian original drink", "Armenian strong drink" and other products with similar names, which consumers identify with cognac and brandy.

• The negative impact of the requirements established by the RA State Duties Law on the competitive environment of the brandy industry.

• Cases of the possible use of non-Armenian spirits for the production of Armenian cognac by some companies in the

brandy industry were registered.

• Lack of mechanisms for proper control over the implementation of the Law of RA about alcoholic beverages based on grape raw materials.

Conclusion

Taking into account the results of analyses carried out in this research we concluded that there is no single, wholesome state policy regulating the sector. The state policy is expressed in the strategy of the agrarian sector and the measures and state support programs are included in it. There are several issues in terms of strategy, measures, and support programs. State implementation of strategic measures is not adequate and support programs are not effective enough. Regarding the legislative regulation in the viticulture, there is an imperative to add and change the laws. The current law comprehensively addresses various issues of sector regulation, but there is a need for some clarifications regarding the state authorities, as well as the prices of grape procurement and the organization of procurement processes. A serious problem is the lack of proper control over the implementation of the viticulture legal framework. On the one hand, low loopholes do not clearly define the levers of control over enforcement. On the other hand, this control is not ensured. To alleviate existing issues and contribute to viticulture development in RA, the following recommendations were made:

• Reviewing the state fee for granting the right to produce brandy

• Adding new components to state support programs aimed at promoting cognac and wine production, so new producers enter the wine market

• Amending the Tax Code to establish a preferential tax regime for small businesses engaged in wine and brandy production

• For the improvement of the control mechanisms of the state regulation we propose to establish a state inspection body, which will perform the following functions:

- Supervision of procurement activities to prevent violations of laws by processing companies
- Strict control of technological processes of production of wines with GI, Armenian brandy, brandy spirit
- Continuous monitoring of the whole viticulture sector (including grape production, wine, and brandy industries) to reveal existing problems and develop measures aimed at solving them
- Cooperate with the Commission and the Food Safety Inspection Body of the RA within its activities.

The scientific novelty of this research article lies in the fact that by comprehensively studying state policy, state support programs, and the legislative framework regulating the RA viticulture complex, the existing issues have been highlighted and practical recommendations for their improvement were provided.

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The Creation of a Gene Library of Valuable Plant Species in Armenia

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ABSTRACT

Using DNA banks or gene libraries is the most efficient method of protecting and managing valuable, endangered, and rare gene pools. Genomic libraries are collections of bacteria colonies with restriction sites on their genomic DNA that contain desired genes from a specific organism. The "Scientific Center of Agro-Biotechnology" branch of ANAU has created gene libraries of 34 plant species, which are preserved in the National Genebank of Crops and their Wild Relatives, which forms a basis for genomic selection. This allows genetic identification, barcoding, DNA formula decoding, and, most importantly, is the basis for genome identification. Based on the results, plasmids can incorporate restriction segments of genomic DNA and be efficiently transformed. The high level of correlation between the number of necessary clones and the efficiency of transformation of all studied plant species proves the completeness of the gene pool.

Introduction

In every country, genetic resources are a source of wealth. Due to their natural evolution and human activity, they play an essential role in food production and environmental balance. Breeding cultivated plants and animals contributes to economic growth, national autonomy, and food security (Eastwood, et al., 2022). For preventing genetic resources erosion, utilizing them responsibly, conserving resources, and developing the economy sustainably, the preservation and diversity of the gene pool are essential, being closely related to science, technology, economics, law, ethics, and international politics (Altukhova, 2004; Chandra and Idrisova, 2011).

It is a necessity to create and cultivate varieties of crops that can meet population and production needs within a short period. This is at the current stage of agricultural development. Genomic and classical selection is necessary to improve existing ones. As a result, DNA banks or gene libraries are the most practical way to preserve valuable gene pools, and catalog, locate, and obtain genes.The forced construction of a genomic library can be divided into two types based on the DNA source used: (a) Nuclear Genomic Library: this is the genomic library that contains the entire nucleus' DNA. This type of library is made by extracting nuclear DNA and using to create it. An organelle genome library excludes nuclear DNA and targets the DNA of either mitochondria, chloroplasts, or both (www.biotechnologynotes.com).

An organism's genomic DNA can be inserted into a gene pool by inserting restriction sites into a vector. Colonies like these are considered sources of desired genes. As a result, DNA segments from valuable species are stored in bacterial cells (Goncharenko, 2005). Each bacterial cell contains genomic DNA. Clones are created when a bacterial cell multiplies, receiving hybrid DNA. DNA banks consist of bacterial cells that contain all fragmented DNA fragments from a particular organism (David and Clark, 2019). This study aimed to create gene libraries of 34 plant species preserved in the "Scientific Center of Agro-Biotechnology" branch of the Armenian National Agrarian University (ANAU). By doing this, we established a basis for genomic selection. The mentioned works were performed for the first time in the Republic of Armenia.

Materials and methods

The research was carried out in the Biological Research Laboratory of the "Scientific Center of Agro-Biotechnology" branch of the ANAU, during 2021-2023. Plant material was collected from the ex-situ seed collection of the National Genebank of crops and their wild relatives. The SDS method was used to obtain genomic DNA from crops and wild species (Padutov, et al., 2007). The NanoDrop One spectrophotometer (Thermo Scientific, USA) was used to determine denatured DNA concentration. To obtain colicinogenic plasmids against ampicillin (as a selection marker), ampicillin resistance was identified in *E. coli* according to the following scheme (Gvozdeva, et al., 2012). At 37 ^{0}C for 18 hours, *E. coli* colonies were grown in 100 ml (LB) nutrient medium containing 50 μg/ml ampicillin. Fresh (LB) nutrient medium containing ampicillin was added to a portion of the overnight culture to ensure a 100-fold dilution. After 16-18 hours of growth, the optical density (2-3∗10^8 cells/ml) was reached. The basic degradation method was used to isolate plasmid DNA from bacterial cells (Padutov, et al., 2007; Kutlunina and Ermoshin, 2017).

Genomic DNA and vector were restrictedly digested using *EcoRI* restrictive enzyme (Kayumov, 2016), restriction mixture containing: water – 15 μl, 10x buffer – 2 μl, DNA –2 μl (2 μg/ml), restriction enzyme – 1 μl (5 units/ μl). At 37 ^{0}C, the mixture was incubated for 16 hours. A restriction method was used to determine DNA incorporation into transformed cells (Padutov, et al., 2007). In the case of a restrictive enzyme, to avoid cleaving of the split ends of the vector, dephosphorylation of plasmid DNA was carried out immediately after restriction according to the following scheme: adding 5 μl of 10x buffer (5 μg DNA, 50 μl deionized water, 2 μl alkaline phosphatase) to a 50 μl reaction mixture to avoid all this. At 37 ^{0}C, the mixture was incubated for one hour. A phenol-chloroform method was used to purify DNA, and 96 % ethanol was used to remove phosphatase.

Ligation was used to seal the insert into the vector, which is done by using T4 DNA ligase, according to the following scheme: A 10x buffer was used, a plasmid DNA sample (0.1 μg/ml) of 1.5 μl, restriction fragments of genomic DNA sample of 3 μl (0.1 mg/ml), a T4 DNA ligase sample of 1 μl, and water were used and incubated at 16 ^{0}C, for 2 hours (Kayumov, 2016; Zhuravleva, 2022).

According to (Yprintsev, et al., 2008), complementary cells were obtained by treating them with 0.1 M calcium chloride:

1. *E. coli* colonies from the Petri dish were transferred to a tube containing 2 ml of LB nutrient medium at 37 ^{0}C and were incubated overnight for 16-18 hours.
2. 200 μl of the overnight culture was transferred to a tube containing 10 ml of LB and was grown for 2.5 hours at 37 ^{0}C.
3. 1.5 mL of cell culture was centrifuged at 13.000 rpm for 1 minute.
4. Cells were suspended in 200 μl cold and sterile solution of 0.1 M $CaCl_2$, then transferred to ice under 4 ^{0}C conditions for 2 hours.

In this case, heat shock was used to carry out the genetic transformation (Koltovaya, 2010).

Clark's formula was used to determine the amount of recombinant DNA (clone) needed (Yeprintsev, et al., 2008):

$$N = Ln(1-p)/Ln(1-f),$$

where N = number of recombinants, p = probability, and f = fraction of the genome contained in a single average insert. According to (Zhuravleva, 2022), transformation efficiency was calculated by the formula:

$$T=M/A,$$

where T = transformation efficiency, M = the total number of transformed colonies per cluster, and A = the amount of incorporated DNA (μg).

Results and discussions

In creating gene libraries, inserting as many restriction segments of genomic DNA of a specific organism into the vector as possible and undergoing genetic transformation is extremely significant. Vectors are DNA molecules that self-replicate within cells of different organisms, transferring genetic information to the recipient. Genomic DNA restriction insert size guides vector selection. As a result of digestion with restriction enzymes, these inserts are generated.

The current study used the EcoRI restriction enzyme, which has the following nucleotide sequence at its site:

5′-G-A-A-T-T-C-3′
3′-C-T-T-A-A-G-5′

EcoRI recognizes six bases, so they cut every 46 kb or 4096 bases. According to this fact, plasmids were chosen as vectors. It is possible to clone DNA inserts up to 15 kb on most plasmids (Goncharenko, 2005).

DNA bank formation is directly influenced by the number and efficiency of clones carrying restriction inserts. Our study found that breadfruit had the largest genome size among the plant species we studied (table). The range was from $1.68*10^6$ kb to $17*10^6$ kb. Spinach (*Spinacia tetrandra Steven*.) has a genome size of $989*10^3$ kb, while Physalis (*Physalis alkekengi L.*) has a genome size of $157*10^3$ kb.

Depending on the number of clones, restriction inserts of genomic DNA are more likely to be inserted into the genome (90 %).

The number of clones needed to capture the genomic DNA of the studied plant species, the number of clones for DNA extraction is presented in the same table as well. Based on the numerical values, it becomes evident that the number of clones needed depends on the genomic DNA size. Hence, clones are needed more when the genome is large. The largest number of clones was 1920-19131 kb, and in other cases, it ranged from 174.4 kb to 1292 kb (table).

Table. Analysis of the transformation efficiency of the studied plant species*

№	Plant species/varieties	Genome size (kb)	The number of clones (N)	Transformation efficiency (T)
1	*Triticum urartu Tumanian ex Gandilyan*	$5*10^6$	5625	$2.4*10^8$
2	*Triticum araraticum Jakubz*	$5*10^6$	5625	$2.2*10^8$
3	*Triticum boeoticum Boiss.*	$5*10^6$	5625	$2*10^8$
4	*Triticum aestivum L.. variety "Alti Aghaj"*	$17*10^6$	19131	$3*10^8$
5	*Triticum aestivum L.. variety "Voskehask"*	$17*10^6$	19131	$3.8*10^8$
6	*Triticum aestivum L.. variety "Gyulgiani"*	$17*10^6$	19131	$2.8*10^8$
7	*Triticum aestivum L.. variety "Garaseferyani"*	$17*10^6$	19131	$3.6*10^8$
8	*Triticum aestivum L.. variety "Qrik"*	$17*10^6$	19131	$2.4*10^8$
9	*Triticum aestivum L.. variety "Galgalos"*	$17*10^6$	19131	$2*10^8$
10	*Hordeum bulbosum L.*	$5.3*10^6$	5987	$3.2*10^8$
11	*Hordeum vulgare L.. variety "Hayk 1"*	$5.3*10^6$	5987	$3.7*10^8$
12	*Hordeum vulgare L.. variety "Hayk 2"*	$5.3*10^6$	5987	$3.1*10^8$
13	*Hordeum vulgare L.. variety "Marina"*	$5.3*10^6$	5987	$3*10^8$
14	*Aegilops tauschii Cosson.*	$4.2*10^6$	4704	$2.8*10^8$
15	*Aegilops umbellulata Zhuk.*	$4.2*10^6$	4704	$2.5*10^8$
16	*Lactuca serriola L.*	$2.5*10^6$	2881	$3.6*10^8$
17	*Spinacia tetrandra Steven.*	$989*10^3$	1098	$3.8*10^8$
18	*Daucus carotaL.*	$473*10^3$	530.1	$4.2*10^8$
19	*Beta macrorhiza Steven.*	$758*10^3$	850.8	$3.8*10^8$
20	*Beta lomatagona F. et M.*	$758*10^3$	850.8	$2.1*10^8$
21	*Beta corolliflora Zoss.et Butler*	$758*10^3$	850.8	$2.8*10^8$
22	*Beta vulgaris L. variety "Bordeaux 237"*	$758*10^3$	850.8	$2.7*10^8$
23	*Physalis alkekengi L.*	$157*10^3$	174.4	$3.7*10^8$
24	*Coriandrum sativum L.*	$213*10^4$	2395	$2.4*10^8$
25	*Phaseolus vulgaris L.* "Buzhakan local" variety-population	$587*10^3$	658.4	$3.2*10^8$
26	*Phaseolus vulgaris L.* "Goris local" variety-population	$587*10^3$	658.4	2.2*108
27	*Phaseolus vulgaris L.* "Armenian red" variety-population	$587*10^3$	658.4	2.8*108
28	*Phaseolus vulgaris L..* "Kotayk local" variety-population	$587*10^3$	658.4	$4.2*10^8$
29	*Cicer arietinum L.* variety" Leninakan 313"	$740*10^3$	830.6	$3.6*10^8$
30	*Cicer arietinum L.* variety" Karin"	$740*10^3$	830.6	$2.4*10^8$
31	*Glycine max Merr.* variety "Milena"	$115*10^3$	1292	$2.8*10^8$
32	*Vitis vinifera ssp. sylvestris*	$500*10^3$	560.4	$4.4*10^8$
33	*Rubus L.*	$240*10^3$	267.8	$3.3*10^8$
34	*Pistacia mutica Fisch et C.A.Mey.*	$600*10^3$	673	$3.2*10^8$

*Composed by the author.

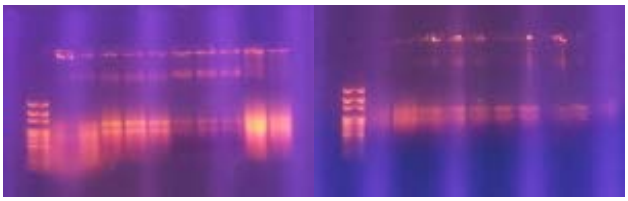

Figure. The results of DNA recombination in transformed cells.

Transformations in the gene pool indicate outcomes. The highest transformation results were recorded in wild grape ($4.4*10^8$), local Kotayk bean variety ($4.2*10^8$), and wild carrot ($4.2*10^8$) species. While the lowest was observed in the Galgalos wheat variety, wild Triticum boeoticum, Triticum araraticum species, the wild Beta corolliflora species of the beet, and the local variety-population of bean from Goris, which equaled $2*10^8$, $2*10^8$; $2.2*10^8$, $2.8*10^8$ and $2.2*10^8$ respectively. There were also cases where it ranged between $2.4*10^8$ – $3.8*10^8$. According to the study, genomic DNA is fully incorporated into the genome of the studied plant species. Agarose gel electrophoresis was used to determine DNA incorporation into transformed cells (Figure).

As can be seen from the agarose gel, recombinant cells of the tested plant species contain a vector carrying recombinant DNA. As a result, the genome is complete. After growing transformed colonies in LB medium for 8 hours and preserving them in 50 % glycerol solution, a 3:1 ratio formed the DNA bank. To preserve transformed cells for a long period, they were stored at -70 ^{0}C.

Conclusion

Species from the Poaceae family had the largest genome size among the studied plant species, whereas *Physalis alkekengi L.* had the smallest. Due to their 15 kb size, plasmids can efficiently transform genomic DNA while containing restriction segments. Gene pool completeness is demonstrated by the high correlation between the number of necessary clones and transformation efficiency for all species studied.

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The Efficiency of Mineral and Water-Soluble Complex Fertilizers in Potato Fields

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yield capacity

ABSTRACT

Application of fertilizers is among the prior agriculture measures to achieve sustainable potato yield in conditions of leached soils of Lori region, poor in essential plant nutrients. The field experiments established that to obtain maximum potato yield in addition to the introduction of the main mineral fertilizer (*NPK*) it is necessary to apply complex water-soluble fertilizer (CWF) via foliar feeding thrice during the vegetation period with 10-12 days of interval. In this case the yield amount was 286 c/ha, 40.0 % of which was large and 35.0 % were medium size. The tuber's chemical composition was also improved. In the case of using $N_{120}P_{120}K_{120}$ the yield amount was 261.0 c/ha, out of which 30.2 % were large and 40.5 % were medium size, while in the control variant 207.5 c/ha yield was received, out of which only 15.1 % were large and 35.9 % – medium in size.

Introduction

Potato is a leading crop in the Lori region, where both large and small-scale farms are engaged in potato cultivation. Anyhow, according to statistical data, its average yield capacity and profitability are still low. According to the observations and studies conducted in different communities of the mentioned region, one of the main reasons for low potato yield capacity is the use of an unjustified amount of fertilizers in conditions of soils with poor fertility, which results in the destruction of plant nutrition, slow-down of its growth, reduction of yield capacity and deterioration of its quality (Atlas of soils of RA, 1990; Yurevna, 2020; Yakushev, et al., 2008). Another reason for low yield production is the damage caused by diseases and pests which are not controlled efficiently. Therefore the vegetation period of plant terminates much earlier (Avagyan, et al., 2017; Yeritsyan, et al., 2017).

It is known that potato's nutrient demand is rather high. For example, to produce a 300-350 c/ha yield the plant takes up 180-210 kg nitrogen (*N*), 60-70 kg phosphorus (P_2O_5) and 270-315 kg potassium (K_2O) from the soil. Whereas, in the carbonate and leached black soils of the RA, including in those of the Lori region, such amounts of available nutrients are missing, which is confirmed upon the results of agrochemical analyses and field experiments (Atlas of soils of RA, 1990; Edilyan, 1976). So, in the soils of Lori region there is a lack of plant-available potassium, then phosphorus and nitrogen.

The soils of the mentioned region are also very poor in micronutrients, which have a multifunctional role in the improvement of plants nutrition process. They promote the yield increase, yield quality and tubers' retention capacity by improving the nutrition with nitrogen, phosphorus and potassium (Atlas of soils of RA, 1990; Chernavina, 1970).

To regulate the nutrition process of the plants, including potatoes, in fast manners, such fertilizers are used in recent years, which are rapidly imbibed in case of spraying them on the leaves and thus, exert a quick effect on the plant nutrition process, growth, yield capacity and tuber's quality. Considering the paramount importance/urgency of applying such fertilizers, currently numerous fertilizers with the mentioned characteristics (*N:P:K=20:20:20*; *N:P:K=0:52:34*) are being imported into the republic (Gasparyan, et al., 2020; Sahakyan, et al., 2020).

Taking into account all aforestated circumstances, an objective was set up to develop an efficient fertilization system for the increase of potato yield capacity, which would also partially mitigate the development of diseases and pests.To solve the mentioned problems, our research group has studied the effect of different combinations of mineral and water-soluble complex fertilizers on the growth, yield capacity and tuber's chemical composition of the potato variety "Marfona" in the leached black soils of the Lori region.

Materials and methods

The field experiments have been conducted in the Aygehat community of the Lori region in 4 replications per the following experimental patterns:

1. Without fertilization (control)
2. N_{120}
3. $N_{120}P_{120}$
4. $N_{120}P_{120}K_{120}$
5. $N_{120}P_{120}K_{120}+CWF$ by soaking the tubers, then conducting foliar feeding during the vegetation period thrice with 10-15-day intervals.

The laboratory studies were conducted at the ANAU branch of H. Petrosyan Scientific Center of Soil Science, Agrochemistry and Melioration, in 2020-2021. The size of the experimental bed was 98 m^2 (4.9 m x 20 m), the feeding area per plant was 0.21 m^2 (0.7m x 0.3 m), and the potato variety was Marfona (www.potato.professor home.ru). It is a mid-early-ripening variety with 80-90 vegetation period; anyhow, in conditions of relatively temperate climate conditions of the Lori region the tubers maturation and the natural dying of vines are somehow delayed. The studied variety has a more than average yielding capacity, the marketable yield of the tubers amounts to 90-95 %. The resistance towards foliar blight, leaf curl and tuber scab is rather high (www.kartofan.org). In the experiments the mineral fertilizers – double superphosphate and potassium chloride – were applied during the pre-treatment of the soil, while the ammonium saltpeter (30 %) was applied via sowing and the remaining amount was used during the weeding-hilling period. The water-soluble complex fertilizer was used before planting via tubers spraying and then it was applied when the plant height increased up to 10-15 cm. The process was repeated twice more with 10-15-day interval. The potato tops had been reaped 12 days before harvesting process. During the vegetation period phenological observations, measurements and calculations were carried out. The yield accounting has been conducted after harvesting the entire yield from the experimental bed via weighing method. The tubers have been grouped into large, average, and small sizes. The experimental error has been estimated (Ex%) and the least significant difference ($LSD_{0.95}$) between the variants according to Dospekhov's method (Dospekhov, 1965). The soil, plant and tubers analyses have been conducted per the methods accepted in the RA (Viktorov, 1969; Yagodin, 1987).

Results and discussions

The experiments of field fertilization were conducted in the mid-power leached black soils, which are characterized by the following chemical properties: the mechanical components are clay and sandy, heavy; when drying out they get partially compacted and encrusted, the humus content in the arable layer (0-30 cm) makes 4.41 %, and in the sub-surface layer it is only 1.25 %. They are free from carbonates, the soil reaction (*pH*) is weakly alkaline and makes 7.8. In the soil water extract the ratio of *Ca-Mg* makes 60:40 %. The content of mobile nitrogen in the soil arable layer makes 4.35 mg per the Turin-Kononova method, as to the Machigin's method the phosphorus content makes 1.08 mg and the potassium content in the extract of Machigin's solution makes 14.58 mg in 100 g soil. According to the data on the limited numbers of the nutrient provision of soil accepted in the RA, the mentioned soils are considered to be poorly provided with essential nutrients (*NPK*).

It should be added that the lack of nitrogen and phosphorus is peculiar to the soils of the RA, while the shortage of mobile potassium is recorded in numerous land areas of the Lori province. Thus, the application of potash fertilizers in this zone is ultimately important (Atlas of soils of the RA, 1990; Edilyan, 1976). The observations, measurements and calculations conducted throughout the plant vegetation period have testified that the application of fertilizers has exerted a noticeably positive effects on the transitional phases in the plant development period, their growing process, as well as on the tubers' yield capacity and qualitative indices.

So, let's consider the impact of fertilizers on the transitional phases of the plant development process: the mineral fertilizers showed zero effect on the tuber's sprouting time, whereas by the use of CWF the sprouting process went ahead by one day. Therefore, it can be concluded that during the sprouting period the applied CWF affected the biological processes taking place in the tuber (Ivanyushkin, et al., 2018; Khoroshkin, 2015).

A similar effect was also recorded in the fields of cereal crops, e.g., winter wheat and spring barley. Moreover, the Complex Water-Soluble Fertilizer has demonstrated favorable effect on the vigorousness and sprouting capacity of the seeds in these and other crops (Gasparyan, et al., 2020; Abitov, 2014; Yeritsyan, et al., 2017). So, the following picture is depicted when studying the effect of fertilizers on the times of plant budding, blossoming and natural dying of potato tops. In the control variant the budding, as well as the blossoming period was sped up. The natural dying period of the potato tops was also accelerated. This indicates that the life span of the plants was reduced, which also results in the reduction of plants yield capacity and in the decline of marketable tuber yield. Whereas in the variants where fertilizers were applied, particularly in the case of applying CWF on the background of mineral fertilizers, the budding and further phases were delayed, which is more obvious in the case of applying water-soluble fertilizers on the background of mineral fertilizers. This testifies that the transitional phases in the plants development period, particularly the period of natural death were prolonged upon the use of CWF. That is, the complex water-soluble fertilizers have promoted the sustenance of plants bio-activity, as a result of which the yield is still increasing. Regarding the mentioned facts, it is known that the crops with long vegetation, for instance, late-ripening potatoes, are endowed with higher yielding capacity (Butov, et al., 2013; Vlasenko, 1987; Zamotaeva, 1987). Considering that the potato yield capacity is also related to the intensity of the aboveground mass growth and that a correlative relationship is established between the plant growth and yield capacity within a certain limit, the effect of fertilizers on the plant growing peculiarities has been determined (Table 2).

Table 1. The effect of mineral and water-soluble complex fertilizers on the times of potato developmental stages (average for 2020-2021)*

N	Variants	Times of developmental stages, day			
		sprouting	budding	blossoming	from sprouting to the natural dying of tops
1	Without fertilization (control)	18	39	61	91
2	N_{120}	18	45	68	99
3	$N_{120}P_{120}$	18	45	65	98
4	$N_{120}P_{120}K_{120}$	18	45	62	98
5	$N_{120}P_{120}K_{120}$ +CWF	17	47	70	114

Table 2. The effect of mineral and water-soluble complex fertilizer on the growing peculiarities of potato plant (average for 2020-2021)*

N	Variants	Plants height, cm	Number of stems, n	Branching degree, n	The weight of aboveground mass after blossoming, g
1	Without fertilization (control)	34	4.3	3.5	341
2	N_{120}	40	4.7	4.0	392
3	$N_{120}P_{120}$	42	5.1	4.4	409
4	$N_{120}P_{120}K_{120}$	42	5.6	4.6	429
5	$N_{120}P_{120}K_{120}$ +CWF	45	5.6	5.1	474

Table 3. The effect of mineral and water-soluble complex fertilizer on the potato yield structure (average for 2020-2021)*

N	Variants	The weight of the tubers mass per a bush, g			
		total	including		
			large	average	small
1	Without fertilization (control)	442.9	66.8	159.1	217.0
2	N_{120}	502.1	125.5	180.7	195.9
3	$N_{120}P_{120}$	524.4	146.8	204.0	173.6
4	$N_{120}P_{120}K_{120}$	553.5	167.2	224.2	162.1
5	$N_{120}P_{120}K_{120}$ +CWF	604.9	242.0	211.5	151.4

*Composed by the authors.

According to the data in table 2, the indices of plant height, stem number per a bush, branching degree and the weight of aboveground mass are lower in the unfertilized variant. Thus, the plants height makes 34 cm, the number of stems per a bush amounts to 4.3 and after blossoming the weight of a bush aboveground mass makes 341 g. In the fertilized variants, the mentioned indicators are much higher but the highest result was recorded in the variant where CWF on the background of mineral fertilizers was applied. In the latter variant the plant height was 45 cm, the number of stems per a bush was 5.6 and the weight of a bush aboveground mass amounted to 474 g. Whereas, in the variant where only nitrogen was applied (N_{120}) the plant height made 40 cm, the number of stems was 4.7 n and the weight of a bush aboveground mass amounted to 392 g. The mentioned data are much higher than those of the control variant but they considerably yield to those where $N_{120}P_{120}$ or $N_{120}P_{120}K_{120}$ have been used, while are well behind the variant where $N_{120}P_{120}K_{120}$ +*CWF* has been applied.

It is evident that when studying the efficiency of any agro-technical measure in the potato sowings, it is essential to reveal the effect of the implemented measures on the yield structure, which accounts for the share of commercial products (Table 3).

According to the data in the Table 3 the weight of tubers per bush and the share of different fractions therein depend on the fertilization variant. So, in the control variant the total tubers mass per bush makes 442.9 g, from which the share of the large tubers makes only 66.8 g (15.1 %), while the share of the small and medium tubers makes 217.0 g (49.0 %) and 159.1 g (35.9 %) respectively. Whereas, in the variants where fertilizers have been used, the share of large and then small-sized tubers sharply increases amounting to 25.0-40.0 % and 36.0-40.5 %, respectively, besides, higher yield (large and mid-sized) was received in the variants where also CWF was applied in the $N_{120}P_{120}K_{120}$ fertilization system. In this variant the tubers mass per a bush exceeds that of the control variant by 36.6 %, while against the variant of $N_{120}P_{120}K_{120}$ the surplus makes 9.3 %, while the mass of large tubers makes 242.0 g (40.0 %) and that of the medium-sized tubers makes 211.5 g (35.0 %). In addition, the tubers' marketable appearance in the $N_{120}P_{120}K_{120}$+*CWF* variant has improved and the damage caused by the disease (potato scab) has been reduced.

It is clear that by contributing to the growth of aboveground mass of the plant, the increase of leaves chlorophyll content, as well as to the considerable prolongation of the plants bioactivity and the period of natural dying of the potato tops/haulms, the fertilizers promoted the increase of the tubers yield capacity, the intensity of which is again related to the fertilization system (Table 4).

Thus, per the two-year average data, the tuber yield in the control variant makes 207.5 c/ha, while in the fertilized variants it amounts to 235.5-286.0 c/ha, which exceeds the same index of the control variant by 28.0-78.5 c/ha (13.5-37.8 %). According to the data of Table 4, there is a significant difference between the fertilized variants as well. The yield index is considerably lower in the variant where only nitrogen (N_{120}) with the dosage of 235.5 c/ha has been used. In the case of applying phosphorus along with nitrogen, the yield capacity increased up to 246.5 c/ha, while per the use of $N_{120}P_{120}K_{120}$ it amounted to 261.0 c/ha. It should be noted that the efficiency of phosphoric and potash fertilizer is related to the low content of phosphorus and potassium in the experimental plot, which is characteristic of the land areas of the Lori region.

Table 4. The effect of mineral and water-soluble complex fertilizers on the yield capacity and qualitative composition of the potato tuber*

Variants	2020	2021			The content in the tubers					
	yield, c/ha	yield, c/ha	Average yield, c/ha	Surplus, c/ha	dry matters, %	starch, %	NO_3 mg in 1 kg fresh mass, mg	Nitrogen (*N*), %	Phosphorus (P_2O_5), %	Potassium (K_2O), %
1	225	190	207.5	-	18.6	13.2	39.6	0.18	0.16	0.31
2	258	213	235.5	28.0	17.8	13.0	64.2	0.45	0.11	0.25
3	270	223	246.5	39.0	18.7	14.0	48.5	0.38	0.23	0.24
4	283	239	261.0	53.5	19.0	14.3	31.6	0.27	0.25	0.51
5	312	260	286.0	78.5	19.5	15.1	20.3	0.25	0.32	0.63

Note. *Variants: 1. Without fertilization (control), 2. N_{120}, 3. $N_{120}P_{120}$, 4. $N_{120}P_{120}K_{120}$ 5. $N_{120}P_{120}K_{120}$+CWF*

*Composed by the authors.

The higher yield was recorded in the variant where on the background of $N_{120}P_{120}K_{120}$ CWF was applied by soaking the seeds with the solution of the mentioned fertilizer and then implementing foliar feeding twice during the vegetation period. In the mentioned variant the average tuber yield has amounted to 286.0 c/ha, which exceeds the analogous index of the control variant by 78.5 c/ha (37.8 %), and that of the background variant – by 25.0 c/ha (9. 6 %).

Conclusion

The amount of humus substances in the leached and regular carbonate black soils of the Lori region is lower than those peculiar to the soils of the same type. The mentioned soils are also poor in the plant-available basic nutrients, such as nitrogen, phosphorus and potassium. They are also endowed with unfavorable agro-physical properties. In such soil conditions the potato plant provides only 190-225 c/ha yield. In order to improve the fertility of the mentioned soils and to increase potato yield capacity, it is important to apply an efficient fertilization system.

Among the studied fertilization systems (N_{120}; $N_{120}P_{120}$; $N_{120}P_{120}K_{120}$ and $N_{120}P_{120}K_{120}$+CWF); $N_{120}P_{120}K_{120}$+CWF system is the most efficient one, which has considerably improved the plant growth and yield capacity against the variant of $N_{120}P_{12}K_{120}$. The yield surplus against the control variant has made 78.5 c/ha (37.8 %), while against the variant of $N_{120}P_{120}K_{120}$ it amounts to 25.0 c/ha (9.6 %).

In all fertilization variants the natural dying times of the haulms were prolonged by 8-23 days; the longest period was recorded in the variant of $N_{120}P_{120}K_{120}$+CWF, which lasted 23 days. Besides, when applying the mentioned fertilization system, the tuber qualitative properties were improved, the content of dry matters in the tuber increased by 0.9 %, that of starch – by 1.9 %, and phosphorus and potassium contents – by 0.16 % and 0.32 %, respectively, as compared to those of the control variant.

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Agrochemical and Ecological Conditions of Brown Forest Soils Determined by Degradation Factors by the Example of Bazum Community of Lori Region

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ABSTRACT

The article presents the direction of degradation processes in eroded forest brown soils based on the example of the Bazum community of the Lori region. It also shows the qualitative characteristics of these soils, the thickness, the degree of humus content, and the availability of nutrients. New technologies and methods of resource-saving tillage and plant care are proposed to mitigate erosion processes, degradation, and desertification of soils based on the research conducted.

Introduction

One of the global problems facing human society today is land degradation and desertification. The land is very precious and essential to everything in this world. The Sustainable Development Goals (SDGs) are one of the ways proposed by the United Nations in 2015 to achieve a better and more sustainable future for all. An increase in pressure on land is very likely to happen in order to achieve the SDGs related to food, health, water and climate (www.sdgs.un.org).

Soil erosion is a growing problem that threatens soil quality and soil's ability to provide environmental services. Soil detachment, deposition, and transport processes occur simultaneously during erosive rainfall. As a result, nutrients, soil organic carbon, and valuable soil biota are transported. At the same time, the species diversity of plants, animals, and microbes is significantly reduced (www.fao.org). The off-site impacts of eroded soil and runoff, primarily eutrophication of water bodies, sedimentation of gravel-bedded rivers, loss of reservoir capacity, and flooding of roads and communities, are increasingly recognized and the costs estimated (Ascough and Flanagan, 2011). Against the background of climate change and accelerated human activities, changes in natural rainfall regimes have taken place and erosion processes will be expected to become more pronounced in future decades (United Nations, 2000; www.fao.org). Long-term shifts may challenge cultivation systems worldwide and eventually alter land use and topography spatiotemporal patterns. All these changes will increase pressure on soil erosion processes, making accurate erosion prediction and control harder. Thus, improved knowledge and understanding of the soil-erosion process will be essential for dealing with forthcoming challenges regarding soil-conservation practices (Flanagan, 2002).

Being located in the dry and terrestrial part of the subtropical climate zone the Republic of Armenia, represents a drought risk zone (Kroyan, et al., 2022). Desertification processes, which became more active, especially during the socio-economic crisis, with their special manifestations, currently cover most of the republic's territory (Markosyan, 2007). Although desertification phenomena are very diverse and multifaceted, one of the main factors contributing to land degradation and desertification processes in RA is soil erosion (Kroyan, et al., 2022).

Materials and methods

The object of research was the brown forest soils of the Lori region. Comparative studies were conducted on non-eroded, slightly, and moderately eroded arable and uncultivated soils of the Bazum community. To assess the agrochemical and ecological conditions of the mentioned soils, soil sampling was done according to soil layers (0-10; 10-20; 20-40; 40-60 cm). The studies were carried out through field experiments and laboratory research.

Field investigations were conducted on brown forest soils. All soil samples were taken using the Burkle Soil Sampling Kit. After being transported to the laboratory, the stones and plant remains were removed from the samples, then dried under room conditions (20-22 ^{0}C). After drying samples were ground and passed through a 2 mm sieve (soil was not crushed only for humus determination). Soil agrochemical and physicochemical indicators (*pH*, humus content, carbonates, mobile nutrients, water-resistant aggregates, absorbed cations, concentration of the mobile form microelements, and mechanical composition) have been determined. The *pH* of the soil was estimated by dipping the pH electrode meter in the saturation paste (ISO 10390:2005 Soil quality – Determination of pH). Total carbonate content was quantified by acid dissolution and subsequent release of titrimetric CO_2 (ISO 10693:1995 Soil quality – Determination of carbonate content). The sample content of humus substances was determined by Turin's method for the determination of organic carbon (using titration with phenyl anthranilic acid). The mechanical composition was determined by the classical pipette method and evaluated according to the Kachinsky classification scale. The easily hydrolyzable nitrogen in the samples was extracted and determined using the method of Tiurin and Kanonova, which is considered to be an indicator of mobile nitrogen compounds, mobile phosphorus was determined using Machigin's method, which is based on the principle of removing them by a 1 % $(NH_4)_2SO_4$ solution, while the exchangeable potassium was quantified using Maslova's method (USSR scientists), the quantity of exchangeable calcium (Ca^{2+}) and magnesium (Mg^{2+}) according to Arinushkina method (1962). The mobile or leachable microelements fraction was extracted using an acetic acid–ammonium hydroxide as a buffer solution adjusted for *pH* 4.8. The samples were left at room temperature in the buffer solution for 24/h soaking and shaken 5-7 times during the soaking period.

After 24/h, the solution was shaken again and filtered. The mobile or leachable forms of microelements (*Cu, Pb, Cd, Zn, Mo*) in the filtrate were quantified using an atomic absorption spectrophotometer (AAS-1) (He, et al., 1979; Taylor, 2001).

Results and discussions

Field experiments were conducted in 2021–2022 on the brown forest soils of the Lori region at Bazum community, which is 1615 m high above sea level. Preparatory activities were implemented per trial patterns presented in appropriate tables and figures. Forest brown soils are of medium strength and have a medium and heavy loamy-sandy mechanical composition. The structure is grainy-lumpy, porous, and carbonate-free.

The results of field and laboratory research have indicated that along with the depth of the soil layer, a certain weighting of mechanical components is observed. The content of physical clay in the 40-60 cm layer reaches 55.1 % (Table 1). The soils are saturated with alkaline-earth metals, where Ca^{2+} predominates. Its amount in the upper layer reaches 41.27 mg/eq per 100 g soil. The Mg^{2+} content is 6.22 mg/eq per 100 g soil. Table (2) shows that in uncultivated, non-eroding soils, the amount of water-proof aggregates is quite high, which ensures favorable hydrophysical properties of these soils. Particles with diameters of 3-5 mm and 1-3 mm prevail in water-proof aggregates. In the soils upper horizons, the water-resistant aggregates content is 64.58-71.3 %, and in the lower horizons, a noticeable decrease in their content is observed (Table 2).

The slightly and moderately eroded uncultivated areas are weakly, moderately and strongly rocky at the surface. The lower horizons appear rocky. Humus content is quite high, 7.4 % in the 0-10 cm layer. Humus-accumulating horizons in eroded plots range from 20-40 cm. The humus content in the upper horizons of slightly eroded uncultivated soils is 2.6-6.0 %. In the same horizons of moderately eroded soils, this indicator is 2.1-4.2 % (Table 2). The composition of alkaline earth metals is dominated by Ca^{2+}, whose content in the upper layer of slightly disturbed soils is 29.08 mg/eq per 100 g soil, and 19.07 mg/eq per 100 g soil in medium/moderately disturbed soils (Table 1).

Table 1. Physico-Chemical Characteristics of Test Site Soils (Lori Region. Bazum Community)*

The State of the soil. Degree of Erosion	Sampling depth. cm	Absorbed cations. mg eq in 100 g soil			< 0.01 mm – the sum of particles (phys. clay). %	$CaCO_3$ according to CO_2. %
		Ca^{2+}	Mg^{2+}	Total		
Uncultivated. Non-Eroded	0-10	41.27	6.22	47.49	43.6	-
	10.0-20	14.19	2.11	16.3	48.3	-
	20-40	15.38	0.83	16.21	42	-
	40-60	21.72	2.05	23.77	50	-
Uncultivated. Slightly Eroded	0-10	29.08	6.01	35.09	42	5.38
	10.0-20	26.1	6.11	32.21	44.1	5.53
	20-40	25.35	6.27	31.62	36.8	7.04
	40-60	-	-	-	47.9	10.8
Uncultivated. Moderately Eroded	0-10	19.07	10.1	29.17	40.7	0.32
	10.0-20	18.2	12.2	30.22	46	0.33
	20-40	17.2	10.43	27.63	51	0.33
	40-60	-	-	-	55.1	0.83
Arable land. Slightly Eroded	0-10	23.01	2.08	25.09	54	6.44
	10.0-20	24.2	2.79	26.99	40.5	6.03
	20-40	34.36	2.19	36.55	36	6.49
	40-60	-	-	-	51	6.79
Arable land. Moderately Eroded	0-10	22.29	3.1	25.39	32	-
	10.0-20	23.28	4.01	27.29	34.5	-
	20-40	23.07	4.05	27.12	44	-
	40-60	-	-	-	46.3	-

*Composed by the authors.

Table 2. Agrochemical Characteristics of Test Site Soils (Lori Region, Bazum Community)*

The state of the soil, degree of erosion	Sampling Depth, cm	*pH*	Humus, %	Water-resistant aggregates, %
Uncultivated, Non-Eroded	0-10	6.8	7.4	-
	10.0-20	7.1	5	64.58
	20-40	7.2	2.4	71.3
	40-60	7.6	1.6	50
Uncultivated, Slightly Eroded	0-10	7.3	6	-
	10.0-20	7.9	3.8	57
	20-40	7.8	2.6	65.4
	40-60	7.9	1.3	74.6
Uncultivated, Moderately Eroded	0-10	7.2	4.2	-
	10.0-20	7.2	3.5	67.1
	20-40	7.6	2.1	69.9
	40-60	7.6	0.89	71.52
Arable land, Slightly Eroded	0-10	7.9	4.1	-
	10.0-20	8.4	2.8	54.92
	20-40	8.6	2	52
	40-60	8.7	1.1	52
Arable land, Moderately Eroded	0-10	7.2	3.9	-
	10.0-20	7.6	3.2	38.32
	20-40	7.6	2.6	40.2
	40-60	8	1.8	32.4

Research results show that there is a significant difference between uncultivated, non-eroded, slightly, and moderately eroded soils and cultivated variants. That difference is particularly evident in the hummus content. If it is 7.4 % in the upper horizon of uncultivated soils, it decreases to 6.0 % in weakly eroded soils, and 4.2 % in moderately eroded versions (Table 2). Significant differences are also felt in absorbed Ca^{2+} and Mg^{2+}. The content of Ca^{2+} in uncultivated non-eroded plots of land is higher (41.27 mg/eq per 100 g soil) than in arable soils subjected to different degrees of erosion, where it is 29.08 mg/eq per 100 grams of slightly eroded soils and 19.07 mg/eq in per 100 grams of moderately eroded soils. In contrast to Ca^{2+}, *Mg* content in soils exposed to different degrees of erosion significantly increases (Table 1). From the analysis of the water-resistant aggregate data, it becomes clear again that there is a big difference between the uncultivated non-eroded versions and the cultivated eroded plots. Thus, if the indicated index in the upper layer of uncultivated soils is 64.58 %, then in the arable layer of slightly eroded soils it is 54.92 %, and in moderately eroded versions it is 38.32 % (Table 2). According to Tables 1 and 2, the humus content in the upper layers of the soil (0-10 cm) varied from 3.9-7.4 % depending on the degree of erosion, the reaction of the soil solution (*pH*-6.8-7.9) varies between neutral and alkaline, they have a loamy-sandy medium and heavy mechanical composition.

A similar pattern is also observed from the point of view of the content of mobile nutrients-nitrogen (*N*), phosphorus (P_2O_5), and potassium (K_2O) in the soil. Thus, the highest amounts of these elements were recorded in the A horizon of the untreated, non-eroded version: *N*-7.0, P_2O_5-4.7, and K_2O-35.6 mg/100 g of soil, decreasing with depth (Figure 1).

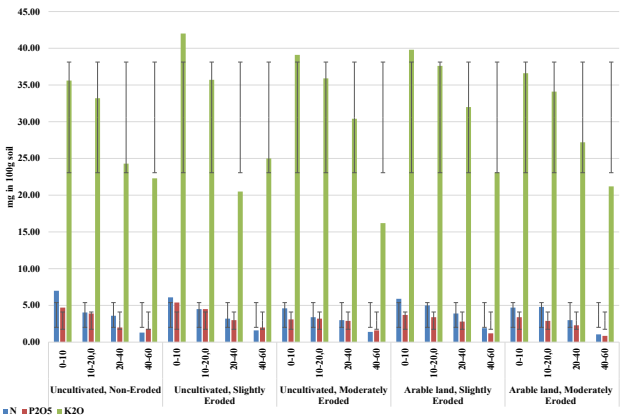

Figure 1. Mobile nutrient content of test site soils *(composed by the authors)*.

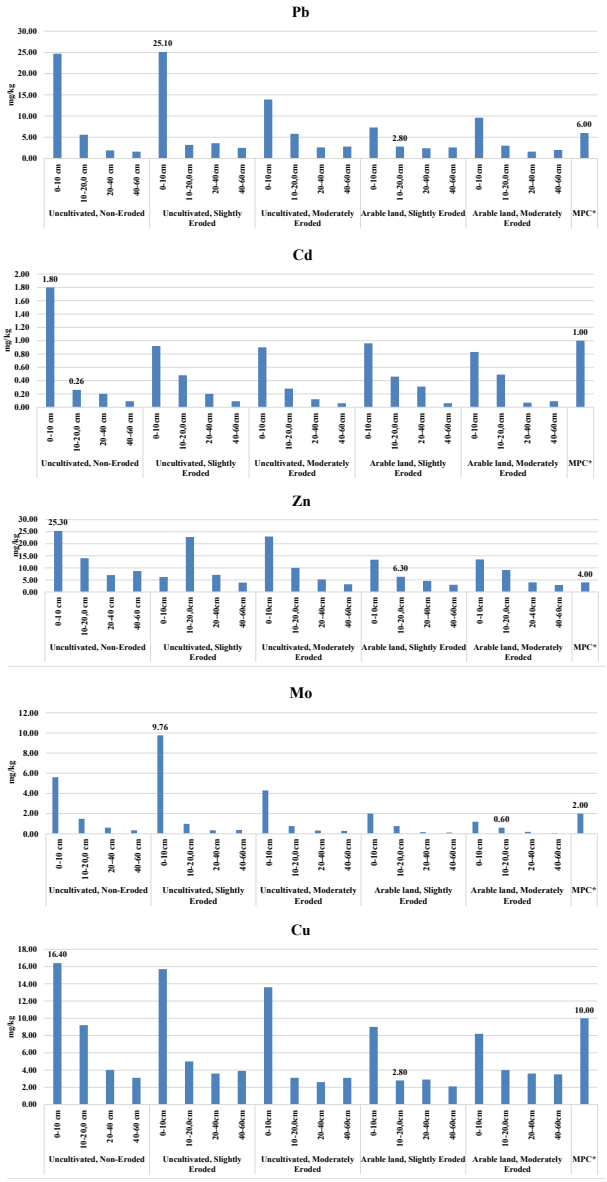

Figure 2. The concentration of the mobile form microelements (*Pb, Cd, Zn, Mo, Cu*) in different degrees of forest brown soils (Lory Region, Bazum Community) *(composed by the authors)*.

The mobile content of microelements, depending on the degree of soil erosion, the amount of humus, mechanical composition, and other factors, varies in the upper 0-20 cm layer: *Cu*-2.8-16.4, *Pb*-2.8-25.10, *Cd*-0.26-1.80, *Zn*-6.3-25.3, *Mo*-0.60-5.6 mg/kg (Figure 2). In Figure 2, microelements are mostly found in the humus horizon (0-10), which proves the correlation between humus and microelements, and the content decreases with depth. To compare the content of microelements, the MPC* indicators (maximum permissible concentration) were used (Chernykh and Sidorenko, 2003).

Conclusion

Based on the studies and analysis, we can say that, along with the increase in the degree of erosion of brown forest soils, the capacity of soil shear, the amount of humus and essential nutrients, the amount of water – resistant aggregates of high value from an agronomic point of view (>0.25 mm) decreases, the specific and volumetric weights increase, the overall porosity decreases, the background contents of microelements decrease along with the depth, which ultimately leads to a decrease in the efficiency of agricultural land types.

It is recommended:

• To fight against erosion phenomena and the degradation of these lands, it is necessary to practice farming agriculture according to the outline-landscape principle. i.e., to carry out all the agricultural works (tilling, sowing, fertilization, etc.) according to the contours of the slope, to perform fertilization at high rates (organic fertilizer: 30-35 t/ha, the purpose of fertilization with such a dose is to bind microelements in the soil and is to make them available for further use by plants).

• To place crops according to soil protection properties, and apply crop rotations. In heavily eroded and uncultivated arable lands it is necessary to cultivate butterfly-flowered and perennial cereal grass mixtures, which will significantly reduce erosion processes, restore the degraded soil structure, increase the reserves of organic matter, improve the agrophysical properties of the soil, making it possible to return it to agricultural circulation.

• To increase the efficiency and productivity of grasslands and pastures (especially near the community), it is necessary to implement radical (fertilization NPK 60:60:60 and sowing with grass mixtures) and surface improvement measures and apply effective pasture rotation/ circulation schemes.

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Studying the Adaptive Capabilities of Imported Apple-Tree Cultivars in Lowland Conditions of the Republic of Armenia

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ABSTRACT

To study the adaptive and acclimatization capabilities of imported apple cultivars to expand the geographical boundaries of their use in the specific lowland conditions of the Ararat Valley as well as to understand their genetic resources in new growing conditions, production experiments were set up. The following encouraging data were obtained Based on a two-year study of five new novel cultivars of apple trees in three different areas.

Five apple tree cultivars were investigated: Granny Smith, Jonagold Decosta, Fuji, Mutsu, and Gala. During observation and study, they showed a high yield productivity. As a result, all five imported cultivars of apple trees are recommended for growing in intensive orchards and under similar agro-climatic conditions.

Introduction

The objective was to study five imported varieties of apple (Ibanez and Dandekar, 2007) trees in the three communities (Garni, Akunk, and Karbi), at the Kotayk and Aragatsotn marzes of Armenia. All communities were located in the Ararat Valley's foothill zone (at an altitude of 1000-1800 m above sea level), under relatively similar natural (soil, climate) and agro-technical conditions. It was also intended to identify the selected varieties' production and economic features compared with the control (C) variety – Golden Delicious accepted as a standard assortment in the Republic of Armenia since 1981. In 1930-1940, the different varieties of apple trees, namely, local (Margaryan.1960), imported (Zakarian et al., 1989), and selective (Karanyan and Agulyan,1981) were studied in the Armenian SSR. As a result, a list of standard apple varieties (Winter Banana, Bellefleur Jaune, Bellefleur Kitaika, Winter Golden Parmain, Pepin Saffron, Renet Simirenko, Virginia Pink), was compiled in 1959, which has practically remained unchanged until now. Out of 46 local apple varieties examined by Margaryan (1960), 12 varieties were recommended for State variety testing (1970) and identified as potential donors for breeding. The same happened with the variety Melba (1976) and

5 selective varieties examined by P.G. Karanyan and S.L. Agulyan (1981). Later, some new apple (Shaomin Chen et al., 2022) varieties were added to the list (Aurora Krimskaya, Cooper Zangi 3012 and 3006 (1989), Sima (2015) (2020). The current research aims to prove the efficiency of intensive gardening and explore the possibilities for its further development in the foothill zones of the Ararat Valley, as well as to identify possible distribution routes, and reveal the favorable properties of the new varieties.

Materials and methods

The imported apple tree varieties, namely Granny Smith, Jonagold Decosta, Fuji, Mutsu, and Gala, grown in Armenia, have been investigated. A preliminary analysis of soil composition was conducted. The studied orchards were cultivated using intensive horticultural methods: double-grafted saplings, crown creation according to the Spindelbusch system, drip irrigation, fertigation, and hail protection/anti-hail system The research was carried out by the methodological programs of the Lobanov,1973; Dospekhov, 1985; Popov, 200; Khachatryan 2002; Sahakyan, 1989. Three replications were conducted with five trees each. Field experiments were carried out in 2017-2018 in the Garni community (Kotayk Marz) on the Mutsu and control (C) varieties, which were grafted onto the rootstock MM106, with a feeding area of 4x3m^2 in the Akunk community (Kotayk marz).

During 2019-2020, field experiments were conducted on Granny Smith, Fuji, Jonagold Dekosta, and control varieties on rootstock M9, a feeding area of 4.0x1.20 m^2. Field experiments in Karbi (Aragatsotn marz) were carried out in 2020-2021 on the Gala, Granny Smith, and control (C) varieties on the M9 rootstock. The feeding area was 3.80x0.85 m^2. All trees were 3-5 years old in the observation period when fruiting started.

Results and discussions

Akunk community is situated at 1360 m above sea level. The garden exposure is northeast. Garni village is located 1000-1400 meters above sea level. In most of the district, the slopes do not exceed 8°, so they are relatively easy to exploit economically. The village of Garni is located in the Kotayk marz of Armenia, 63 km south of the marz capital (Ashtarak). The height of the Karbi community of Aragatsotn marz above sea level is on average 1700 meters. Orchards are located in the northeast.

The saplings were purchased from Holland. They were grafted on M9 and MM106 rootstocks; Paradise 9 or M9 is a dwarf rootstock, 2 m in height, drought-resistant, and frost-resistant. MM106 – issue of the Dusen subspecies is a medium-sized dwarf rootstock. The bushes are wide-spreading.

Trees were planted in Akunk and Karbi in 2014. They were planted in Garni in 2011. Production started in Akunk and Karbi in 2017 and Garni in 2014. In Garni, the orchard was established with 4 varieties, which pollinate each other. The varieties were planted in the orchard in a ratio of 3/1, with each variety covering 25 % of land area. In Karbi and Akunk, the ratio of the pollinating varieties in the park was 3/1. Every four tree was a seed pollinator.

The imported apple tree varieties – Granny Smith, Jonagold Decosta, Fuji, Mutsu, and Gala -growing in Armenia have been used for our research. Before the research, an analysis was carried out. The studied orchards were cultivated using intensive horticultural practices: double-grafted saplings, crown creation according to the Spindelbusch system, drip irrigation, fertigation, and hail protection systems. The study guides issued under the editorship of the following authors have been used (Lobanov, 1973; Dospekhov, 1985; Popov, 2000; Khachatryan, 2002; Sahakyan 1989).

Table 1 presents the results of the soil sampling analysis in the Aragatsotn marz, and Table 2 introduces the results of the soil sampling in the Kotayk marz.

Soil analysis shows that the soil is clay and heavy. Analysis of the content of soil nutrients in the field enables to determine the number of fertilizers and the timing of their introduction into the soil.

It is known that 4-5 hours after grafting, the scion's inoculation with the rootstock begins. This lasts for several years until complete adhesion. In our case, the varieties showed a high grafting assemblage of 85-90 %, and out of them, a growth of 90 % was recorded in the Gala variety.

Our study on the transition of phenological stages showed that vegetation duration was observed within 233-300 days, starting with swelling of the buds in the period of 02.03-07.04 in different years and different varieties, and ending with the end of leaf fall in the period of 15.11-18.12.

Based on the data obtained, it can be stated that vegetative buds developed in April-June. The formation of green cones and shoots lasted from 7 days (Mutsu) to 21 days (Golden Delicious) and went on until 15.04-20.05. The period of intensive bud growth lasted from 10 (Granny Smith) to 15 days in most varieties during the period 08.05-10.06. The period of apical bud formation lasted from 2 (Mutsu) to 7 days (Gala) in the period of 04.05-20.06.

Table 1. Experimental results of agrochemical analyses of soil samples in Akunk*

Soil sampling depth (cm)	Mechanical composition of soil	*pH*	$CaCO_3$	Composition of water-soluble salts	Water extract in 100 g soil, mg\ eq		The content of absorbed nutrients in the soil, mg\ 100 g		
			%		Ca^{2+}	Mg^{2+}	*N*	P_2O_5	K_2O
0-30	Clay, sandy, and heavy	7.8	n/a	0.056	0.6	1.0	3.00	26.77	111.34
30-60				0.037	0.5	1.0	2.01	13.00	92.87

Table 2. Experimental results of agrochemical analyses of soil samples in Karbi*

ha/soil	Humus (%)	Content of water-soluble salts (%)	*pH*	In water extract (mg/100 g)		The content of absorbed nutrients in the soil in mg\100 g				
				Ca^{+2}	MG^{+2}	$N\text{-}NH_4$	$N\text{-}NO_3$	$P\text{-}P_2O_5$	K_2O	*Fe*
1 (1.7 ha) 0-30 (cm)	0.8	0.2	7.6	2.0	1.6	0.3	3.4	19.2	3.24	0.2
2 (1.7 ha) 30-60 (cm)	1.2	0.8	7.9	1.4	1.1	3.4	0.3	6.0	59.6	0.3

* Composed by the authors.

It should be mentioned that these factors are important for the application of agro-technical measures in these conditions.

Observations and records show that various agricultural activities (including the repeated application of fertilizers) have exerted a significant effect on the development of the assimilation surfaces of tree leaves, as well as on the growing intensity of annual shoots in an apple tree. Observations showed that in comparison with the Golden Delicious variety, only the Mutsu variety surpassed the number of shoots on one of its trees in terms of length and leaf surface area (129.57 cm, 87975 cm^2).

Besides, the Mutsu variety, which has the largest leaf area (87975 cm^2), also has the highest yield capacity per tree (41.0 kg). In general, the problem comes down to the number of leaf-bearing branches and the size of the total leaf area on one tree capable of assimilating solar energy.

As a result, the indicator of total leaf surface becomes of paramount importance, since it directly correlates with the main economic indicator – productivity. Among the three varieties of leaves studied in Akunk, the total foliage indicators were quite similar to those found in control samples.

Picture 1. A design system applied in an intensive apple orchard (Akunk 2019-2020).

Table 3. Survival rate of grafted trees in Karbi, during 2020-2021*

Varieties	Budding/ grafting	Survived graft, adhesion	Survival rate
	(n)		%
Gala	1130	1017	90
Granny Smith	935	795	85
Golden Delicious	1031	896	87

Picture 2. Harvesting Golden Delicious and Galla apple varieties in the garden (Karbi 2020-2021).

Table 4. Duration of vegetation stages in Akunk, Karbi, and Garni*

Cultivar	Years	Bud swelling and scaling	Defoliation			Vegetation duration (days)
			beginning	mass	end	
Granny Smith	2017-2018	04.04-10.03	25.10-05.11	30.11-03.12	10.12-18.12	245-275
Fuji	2017-2018	25.03-02.03	23.10-27.10	27.11-30.11	07.12-10.12	247-275
Jonagold Decosta	2017-2018	04.04-05.03	20.10-25.10	25.11-30.11	05.12-10.12	240-273
Golden Delicious	2017-2018	02.04-02.03	20.10-25.10	25.11-30.11	05.12-10.12	242-275
Mutsu	2018-2019	25.03-03.04	23.10-27.10	27.11-30.11	07.12-10.12	240-248
Golden Delicious	2018-2019	27.03- 07.04	01.11- 05.11	15.11-03.12	30.11-18.12	233-248
Gala	2020-2021	25.03-27.03	15.10-25.11	22.10-25.10	18.11-10.12	239-248
Granny Smith	2020-2021	23.03-25.03	26.10-03.11	15.11-17.11	25.11-27.11	248-252
Golden Delicious	2020-2021	20.03-25.03	07.10-25.10	01.11-15.11	15.11-27.11	250-300

*Composed by the authors.

In Garni, the Mutsu variety slightly surpassed the control. In Karbi, the indicators of the Granny Smith variety exceeded the control in 1.6 times and 3.5 times the Gala variety. As we know, it is generative buds that provide fruit formation and yield. Therefore, timely and normal implementation of vegetative buds developmental phases ensures proper yield. The dynamics of generative buds' development and quantitative data of branches and leaf surfaces (Tables 3-8) indicate the normal adaptation and development of imported varieties in the lowland zones, timely and regular implementation of functions in their organs, which plays a favorable role in the normal formation and maturation

of their crops. On the other hand, wide opportunities are created to stimulate the further quality and quantity of yield of these varieties through agro-technical measures. Observations show that the feeding area indicator is directly proportional to the yield index.

In Akunk, generative buds swelled on March 23-30, they burst out on April 7-15, a single flowering took place on April 17-24, mass blossom – on April 28-03, and the end was recorded on May 6-14, fruit formation – on May 13-20, fruit ripening – on September 12-16, and the end was on 03-15.10.

In Garni swelling occurred on 25-29.03, bursting – on 07-13.04, single flowering –on 17-23.04, mass – on 25.04-01.05, end – 03-12.05, formation of fruits – on 11-16.05, beginning – 25-28.08, end – 01-07.10.

Table 5. Changes in vegetative buds (Akunk, Karbi, and Garni)*

Varieties	Years	Formation of green cones and shoots (1-5 cm)	Period of intensive bud development	Period of apical buds' formation
Granny Smith	2017-2018	05.05-20.05 15	10.05-25.05 15	06.05-10.05 4
Fuji	2017-2018	03.05-18.05 15	08.05-23.05 15	04.05-08.05 4
Jonagold Decosta	2017-2018	04.05-19.05 15	09.05-24.05 15	05.05-09.05 4
Golden Delicious	2017-2018	03.05-18.05 15	08.05-23.05 15	04.05-08.05 4
Mutsu	2018-2019	04.05-11.05 7	09.05-17.05 15	07.05-09.05 2
Golden Delicious	2018-2019	05.05-15.05 10	12.05-23.05 11	04.05-09.05 5
Gala	2020-2021	17.04-05.05 18	23.05-07.06 14	10.06-17.06 7
Granny Smith	2020-2021	15.04-02.05 17	25.05-05.06 10	10.06-15.06 5
Golden Delicious	2020-2021	19.04-10.05 21	27.05-10.06 13	15.06-20.06 5

Table 6. Growth indices of branches and leaf surfaces (Akunk 2019-2020, Karbi 2020-2021, Garni 2017-2018)*

Varieties	1 tree					
	Shoot length (10 n average per cm)	Number of branches	Number of scaffold branches	Number of leaves on a tree	Leaf surface	The surface of all leaves on a tree
		(n)			(cm^2)	
Granny Smith	54	16	13	290	71	20590
Fuji	50	13	10	300	68	20400
Jonagold Decosta	30	9	9	260	75	19500
Golden Delicious	87	15	16	320	78	24960
Mutsu	129	57	16	1173	75	87975
Golden Delicious	95	51	16	1126	75	84450
Gala	25	20	6	200	35	7000
Granny Smith	40	35	12	400	60	24000
Golden Delicious	35	30	10	300	50	15000

* Composed by the authors.

Table 7. Development of generative buds in Akunk, Karbi, and Garni*

Varieties	Years	Buds		Blossom			Fruits		
		swelling	bursting	beginning	mass	end	formation	ripening	
								beginning	full
Granny Smith	2017-2018	25.03-30.03	09.04-15.04	19.04-25.04	30.04-03.05	08.05-14.05	15.05-20.05	14.09-16.09	08.10-15.10
Fuji	2017-2018	23.03-28.03	07.04-13.04	17.04-23.04	28.04-01.05	06.05-12.05	13.05-18.05	12.09-14.09	05.10-10.10
Jonagold Decosta	2017-2018	24.03-29.03	08.04-14.04	18.04-24.04	28.04-02.05	07.05-13.05	14.05-19.05	13.09-15.09	03.10-05.10
Golden Delicious	2017-2018	23.03-28.03	07.04-13.04	17.04-23.04	28.04-01.05	06.05-12.05	13.05-18.05	12.09-14.09	03.10-05.10
Mutsu	2018-2019	25.03-26.03	09.04-11.04	18.04-20.04	25.04-27.04	03.05-05.05	11.05-12.05	26.08-28.08	01.10-06.10
Golden Delicious	2018-2019	28.03-29.03	07.04-13.04	17.04-23.04	25.04-01.05	06.05-12.05	13.05-18.05	25.08-27.08	01.10-07.10
Gala	2020-2021	20.03-25.03	01.04-05.04	21.04-25.04	01.05-03.05	12.05-15.05	20.05-22.05	01.09-05.09	15.09
Granny Smith	2020-2021	20.03-23.03	03.04-05.04	23.04-25.04	01.05-05.05	10.05-15.05	19.05-23.05	09.09-13.09	22.09-24.09
Golden Delicious	2020-2021	25.03-27.03	03.04-07.04	25.04-27.04	01.05-04.05	10.05-13.05	22.05-26.05	09.09-15.09	25.09-27.09

Table 8. Yield capacity of the cultivar in Akunk, Karbi and Garni*

Varieties	Years	Yield harvesting period	Harvested yield per tree (kg)		Average fruit weight (g)	Weight of the biggest fruits (g)	Regularly colored fruits	
			yield on a tree	fallen yield			Weight (kg)	%
Granny Smith	2017-2018	08.10-15.10	19.5-20	1-1	285-290	294-300	18.5-19	94.8-95
Fuji	2017-2018	05.10-10.10	14.5-15	1-2	160-165	160-170	12.5-13	86.2-86.6
Jonagold Decosta	2017-2018	03.10-05.10	18.5-20	2-2	250-260	260-270	17.5-19	94.5-95
Golden Delicious	2017-2018	03.10-05.10	21-22	3-3	299-300	299-310	20.5-21.5	97.6-97.7
Mutsu	2018-2019	05.10-07.10	33-41	2.5-4	280-300	350-370	31-37	90.2-93.9
Golden Delicious	2018-2019	02.10-06.10	37-40	3-3	220-250	299-310	33-36	89.1-90
Gala	2020-2021	24.09-30.09	6-6	1-1	150-145	200-200	4.30-5	75-75
Granny Smith	2020-2021	24.09-07.10	13-14	1-1	250-250	300-300	12-14	95-95
Golden Delicious	2020-2021	17.09-30.09	11-12	1-1	200-200	280-300	11-12	95-95

* Composed by the authors.

Picture 3. Growth intensity of of vegetative organs of apple (Garni 2017-2018).

In Karbi swelling was recorded on 23-27.03, bursting – on 01-07.04, single flowering – on 21-27.04, mass on – 01-05.05, end – 12-15.05, fruit formation – on 19-26.05, beginning – on 01-15.09, full – 15-27.09.

LSD_{05} = 3.51 kg /tree, $E\chi_0$%=8.5 % –Akunk,
LSD_{05} = 2.3 kg /tree, $E\chi_0$%=3.2 % – Garni,
LSD_{05} = 14.4 kg /tree. $E\chi_0$%=2.9% – Karbi.

The results of conducted statistical calculations for the yield data on the three studied plots of apple tree plantations using the Student's formula show that their LSD (Least Significant Difference) is at the 5 % significance level (which corresponds to a 95 % probability level) and the data from Karbi and Garni plots do not go beyond ± LSD_{05}, while the data from the Akunk plot have a positive deviation from the average yields of the standard (control) option. In Akunk, the harvest was collected on 03-15.10. The maximum yield was Golden Delicious (25.0 kg/day); the commercial yield was 21.5 kg/day. The minimal yield was in the Fuji variety – 15.0 kg/tree and 11.0 kg/day, while for varieties of Granny Smith and Jonagold Decosta, the yield amount was 21.5 kg/tree and 19.0 kg/tree, respectively. Harvest in Garni took place on 02-07.10.

The maximum yield capacity was recorded in the variety Mutsu – 41.0 kg/tree and 37.0 kg/tree. The minimum index was observed in the variety of Golden Delicious – 40.0 kg/tree and 36.0 kg/tree. In Karbi, the yield was harvested on 17.09-07.10. The maximum yield amount was obtained in the variety of Granny Smith – 14.0 kg/tree and 12.0 kg\tree. Minimum yield was recorded in the Gala variety – 6.0 kg\tree and 4.3 kg\tree. In the variety Golden Delicious, the yield was 11.0 – 12.0 kg\tree. Such a yield amount is accounted for by the fact that the plants haven't yet entered the period of full fruiting. Concerning the key economic indicators, the yield capacity of the control variant of the Golden Delicious variety demonstrated high results in all three communities. In the first community – Akunk, the indices of Granny Smith and Jonagold Decosta varieties approached the control variety data. In the second community – Garni the best indicators were found in the Mutsu variety, and in the third community – Karbi. The variety of Granny Smith even exceeded the control.

Conclusion

Soil analysis indicates that it possible to cultivate apple trees on such soils. The study of apple tree varieties shows a high survival rate, with the Gala variety having the highest survival rate of 90 %. The vegetation period lasts between 233-300 days, with swelling of buds occurring between 02.03-07.04 and leaf fall ending between 15.11-18.12. The developmental stages of vegetative buds occur between April-June, with the formation of green cones and shoot lasting from 7 days (Mutsu) to 21 days (Golden Delicious).

Observations showed that in comparison with the Golden Delicious variety, only the Mutsu variety surpassed the number of shoots on one of its trees and in their length. The Mutsu variety, which has the largest leaf area, has also the highest yield amount per tree.

In Akunk, the maximum yield was in the control option of Golden Delicious amounting to 25.0 kg/day, commercial yield was 21.5 kg/day. The minimal yield was in the Fuji variety – 15.0 kg/tree and 11.0 kg/day, while for the varieties of Granny Smith and Jonagold Dacosta, the yield amount was 21.5 kg/tree and 19.0 kg/tree, respectively. In Garni, the maximum yield capacity was recorded in the variety Mutsu – 41.0 kg/tree and 37.0 kg/tree. The minimum index was observed in the variety of Golden Delicious – 40.0 kg/tree and 36.0 kg/tree. In Karbi, the maximum yield amount was recorded in the variety of Granny Smith – 14.0 kg/tree and 12.0 kg/tree. Minimum yield was recorded in Gala variety – 6.0 kg/tree and 4.3 kg/tree. In the variety of Golden Delicious, the yield amount was 11.0 – 12.0 kg/tree. Such a yield amount is accounted for by the fact that the plants haven't yet entered the period of full fruiting. Concerning the key economic indicators, the yield capacity of the control variant of the Golden Delicious variety has demonstrated high results in all three communities. In the first community – Akunk, the

indices of Granny Smith and Jonagold Decosta varieties approached the data of the control variety. In the second community – Garni – the best indicators were recorded in the variety of Mutsu, and in the third community – Karbi, the variety of Granny Smith even slightly surpassed the control.

We recommend that Granny Smith, Mutsu, Jonagold Decosta, and Fuji varieties can be used for a wide production expertise and in establishing intensive orchards under similar conditions. According to the important data of economic index, i.e. yield capacity, the varieties Granny Smith and Jonagold Decosta have good prospects for further wide industrial testing and distribution.

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Evaluation of Grain Yield and Drought Tolerance Indices in Armenian and Iranian Wheat Varieties Under Irrigated and Non-Irrigated Conditions

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ABSTRACT

Drought is one of the main abiotic stresses that depends on soil type and rainfall patterns on agrarian land. However, it is mainly responsible for major yield losses in crops. Climate change further compounds the challenges to advanced crop yields. Failure stress reduces crop yield. This research was conducted on grain yield in fifteen Armenian and Iranian wheat varieties to evaluate grain yield and drought tolerance indices. The study was performed with three replications under normal and drought-stress conditions for two years. According to grain yield, eight drought tolerance indices are estimated, including stress tolerance (STI), tolerance index (TOL), stress susceptibility index (SSI), mean productivity (MP), harmonic mean (HM), geometric mean (GMP), yield index (YI), and drought index (DI) for all varieties. The combination of yield analysis, eight drought tolerance indices, and correlation revealed that Navid, Voskehask, Sabalan, and Zare varieties were desirable for drought tolerance. Other varieties were identified as semi-tolerant and sensitive to drought stress.

Introduction

Wheat ranks second after rice in terms of dietary intake volume, with 68 % of the wheat produced used for food, approximately 19 % for feed, and the rest for other purposes, including industrial biofuels. Wheat imports from developing countries, including the tropics where wheat is not grown, are increasing. Droughts cause reduced crop and pasture yields, crop failures, and scarcity of water supplies, even with advanced technologies and management in recent years (www.climatedata.ca; Qian, et al., 2018). Grain yield is the basis for drought tolerance selection. However, grain yield is affected by many factors aside from drought (Khadka, et al., 2020). Measuring yield by the attribution of physio-morphological traits independent of grain yield improves selection effectiveness by reducing reliance on final grain yield. Stable yield performance of varieties under both normal and failure stress conditions is vital for factory breeders to identify failure-tolerant varieties (Pierivatolum, et al., 2010). Multiple indicators have been proposed to evaluate failure-resistant

characteristics in colorful crops based on fine connections between stressed-out and unstressed-out conditions. These indicators include the stress vulnerability indicator (SSI) (Fischer and Maurer, 1978); relative failure indicator (RDI) (Fischer and Wood, 1979); mean productivity (MP) (Rosielle and Hamblin, 1981), stress forbearance indicator (STI) and geometric mean productivity (GMP) (Fernandez, 1992). More recently, indicators of failure forbearance have included the abiotic forbearance indicator (ATI), stress vulnerability chance indicator (SSPI), and a relative drop in yield (RDY) (Farshadfar and Elyasi, 2012). The present study compares and estimates different yield-grounded failure-forbearance selection indicators for Armenian and Iranian wheat varieties.

Materials and methods

A pot experiment was conducted to determine the effects of water stress on grain yield. The experimental material consisted of fifteen Armenian and Iranian wheat varieties (Sardari, Navid, Alvand, Mihan, Azar2, Sabalan, Zare, Pishgam, D92, G31, Sateni22, Akhtamar, Armianka60, Voskehask, and Nairi68). Wheat varieties were collected from the East - Azerbaijan Agricultural and Natural Resources Research and Education Centre and the Department of the Scientific Center for Agronomy and Plant Protection of the Republic of Armenia.

The study was conducted at the "Arman Naghsh-Sabz Aras Co." greenhouse site of Ara's free zone in Iran during the 2018–19 and 2019–20 growing seasons (October to July). Jolfa is located northwest of East Azerbaijan Province. In terms of longitude, it lies between 45°17' and 46°31', and in terms of northern latitude, it lies between 38°39' and 39°2'. It is a narrow strip on the province's northern border. AFZ is located in Iran's semi-dry and semi-cold North-West. Annual rainfall is about 225 to 400 milliliters per year, and the average temperature is about 15 degrees Celsius. About 50 days per year are cold. The AFZ is near high mountain ranges, with a chilly climate (www.en.wikipedia.org).

Three replications in two years were conducted with six seeds in every pot. Grain yield was measured at physiological maturity per pot. Eight drought indices including SSI, STI, TOL, GMP, MP, HM, YI, and DI were calculated based on grain yield under normal and drought stress conditions according to the following equations:

stress susceptibility index = SSI= (1-(Ys/Yp))/SI
(Fernandez, 1992).

Stress tolerance index = STI= (Yp*Ys)/ $\bar{Y}p2$
(Fernandez, 1992).

Tolerance = TOL=Yp-Ys
(Hossain, et al., 1990).

Geometric mean productivity= GMP = $\sqrt{(Y_p * Y_S)}$
(Fernandez, 1992).

Mean Productivity =MP=(Yp+Ys)/2
(Rosielle and Hambline, 1981).

Harmonic Mean = HM=2*(Yp*Ys)/(Yp+Ys)
(Chakherchaman, et al., 2009).

Yield index = YI = Ys/ $\bar{Y}s$
(Chakherchaman, et al., 2009).

Drought resistance index = DI = Ys x(Ys/Yp)/ $\bar{Y}s$
(Lan, 1998),

where Yp is the yield under non-stress conditions, Ys is the yield under stress conditions; $\bar{Y}p$ is the mean yield of all verities; $\bar{Y}s$ is the mean yield of all varieties; and SI = 1-($\bar{Y}s$/$\bar{Y}p$) (Khosravi, et al, 2020). After calculating different indices, the correlation between grain yield under normal and stress conditions (Yp, Ys) and stress tolerance indices was calculated and the best index was determined. So, indices with a significant correlation with grain yield under both conditions were introduced as the most reliable indicators.

Results and discussions

A total of eight indices of drought tolerance were calculated for each variety (Figure 1 and Table 1). The use of these indices is considered most suitable for selecting drought-tolerant varieties by many researchers. The grain yields of non-irrigated and irrigated fields were significantly different. Grain yield decreased by 50 % when not irrigated. The stress intensity index (SI) ranges from 0 to 1. More significant values of stress intensity indicate more severe stress conditions (Raman, et al., 2012). The SI value in this study was 0.45. Drought-tolerant varieties have high values of GMP, MP, HM, and DI and small values of SSI and TOL (Gitore, et al., 2021). Navid, Voskehask, Sabalan, and Zare are classified as tolerated varieties according to the TOL index. In the case of the yield index, tolerance is defined as a value that is greater than one. In contrast, susceptibility is defined as a verity with a value less than one. SSI and YI indicate that Navid, Voskehask, Sabalan, and Zare are drought-tolerant as are Azar2, Sateni22, and Akhtamar. Under stress conditions, verities with high-value MP, GMP, and HM indices are more admirable

(Gitore, et al., 2021). Varieties Navid, Voskehask, Sabalan, Zare, and Azar2 were detected as tolerant based on these three indices. Gitore et al. (2021) define tolerance as a verity greater than one, while susceptibility as a verity less than one. DI is another index of drought resistance, which was commonly accepted to identify varieties producing high yield under both stress and no-stress conditions by Lan (1998). In this study, the DI index selected only Navid and Voskehask varieties as drought tolerant.

STI-high varieties usually have a significant difference in yield under two different humidity conditions (Lan, 1998). The DI and STI consider not only the ability of varieties to grow well under stressed environments but also superior performance in non-stressed environments (Bahrami, et al., 2020; Sabaghnia and Janmohammadi, 2014). High STI values indicate tolerance to moisture stress in Navid, Voskehask, and Sabalan. Low values indicate low tolerance to moisture stress, such as Mihan, G32, and D31.

Other researchers have also used different indices for selecting resistant varieties of various crops. For instance, STI and GMP in maize (Khallili, et al., 2004), and safflower (Majidi, et al., 2011; Bahrami, et al., 2014). According to grain yield and drought indices, varieties Navid, Voskehask, Sabalan, and Zare were detected as drought tolerant. Azar2, Sateni22, and Akhtamar were classified as those that were not too tolerant but showed a high grain yield value. On the other hand, Alvand, Pishgam, following Sardari, Nairi68, and Armianka60 were identified as semi-sensitive varieties, and Mihan, G32, and D92 were listed as sensitive to drought stress. In our previous study, we used reverse transcription PCR to evaluate the relative water content (RWC) and expression level of Wdhn13 and WCS120 DHN genes. According to the results, the Navid, Sabala, Zare, and Voskehask varieties were highly resistant. In the second place were Azar 2, Sateni 22, and Akhtamar. Sardari, Alvand, Pishgam, Nairi 68, and Armyanka 60 were in the third place. Mihan, D92, and G31 were evaluated as sensitive varieties because the expression of their genes began when the percentage of water content decreased (Vahramians and Melikyan, 2022).

Various strategies for improving plant yield in stressful environments have been proposed to increase plant breeding efficiency. Given the fact that successful breeding programs can be measured against a range of indices, an ideal tolerance index should have high discriminative power to identify superior varieties with long-term yield stability (Mevlut and Sait, 2011).

As shown in Table 2, grain yield under irrigated and non-irrigated conditions is correlated with drought-tolerant indices. The correlation between YP and YS was strong and significant for all indices. A strong correlation of grain yield with other indices for selecting tolerant varieties is essential (Farshadfar, et al., 2012). The correlation between grain yield, SSI, and TOL was negative, and significant under two conditions. These results agree with those reported by (Anwar, et al., 2020; Khosravi, et al., 2020) in wheat.

The drought-tolerant indices showed a strong positive correlation under both non-stress and stress conditions, indicating that these indices were comparably effective for selecting and predicting better grain-yielding varieties under both moisture regimes, corroborating previous reports (Sardouie-Nasab, et al., 2015; Darzi-Ramandi, et al., 2016).

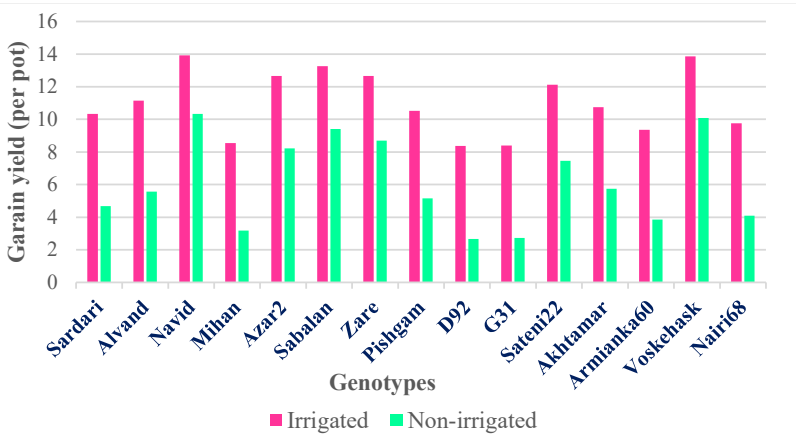

Figure 1. Grain Yield of 15 wheat verities under irrigated and non-irrigated conditions *(composed by the author)*.

Table 1. Yield potential (YP), stress yield (YS), and eight indices of drought tolerance for 15 wheat verities*

	TOL	SSI	STI	MP	GMP	HM	YI	DI
Sardari	5.66	1.22	0.45	7.51	6.96	6.44	0.76	0.35
Alvand	5.58	1.11	0.58	8.35	7.87	7.42	0.91	0.45
Navid	3.58	0.57	1.35	12.13	12.00	11.87	1.69	1.26
Mihan	5.36	1.39	0.26	5.87	5.22	4.65	0.52	0.19
Azar2	4.43	0.78	0.97	10.44	10.20	9.96	1.34	0.87
Sabalan	3.85	0.65	1.17	11.34	11.17	11.01	1.54	1.09
Zare	3.96	0.70	1.03	10.67	10.48	10.30	1.42	0.98
Pishgam	5.36	1.13	0.51	7.83	7.36	6.91	0.84	0.41
D92	5.70	1.52	0.21	5.51	4.72	4.04	0.43	0.14
G31	5.67	1.50	0.21	5.57	4.79	4.12	0.45	0.14
Sateni22	4.68	0.86	0.85	9.79	9.51	9.23	1.22	0.75
Akhtamar	4.99	1.03	0.58	8.24	7.85	7.48	0.94	0.50
Armianka60	5.52	1.31	0.34	6.60	6.00	5.45	0.63	0.26
Voskehask	3.79	0.61	1.31	11.97	11.81	11.66	1.65	1.20
Nairi68	5.69	1.29	0.37	6.93	6.31	5.76	0.67	0.28

*Composed by the author.

TOL and SSI showed a significantly negative correlation with all selection indices, which agrees with the observations. STI and GMP indices were the more objective criteria used to select heat-tolerant and high-yielding varieties. These correlations indicate that higher MP and GMP varieties are superior under stress conditions. These results agree with those reported by some scientists (Khosravi, et al., 2020).

There was a significant positive correlation between STI, MP, GMP, HM YI, and DI. Since GMP is calculated based on MP, so high MP values distinguish high-yielding drought-tolerant wheat varieties (Anwar, et al., 2020). The TOL index correlated negatively with all traits except the SSI, which showed a positive correlation. The results were consistent with those reported by Golabadi, et al., (2006) in durum wheat and Khalili, et al., (2012) in canola.

Table 2. Correlation coefficients between drought tolerance indices and seed yield in normal conditions*

	TOL	*SSI*	*STI*	*MP*	*GMP*	*HM*	*YI*	*DI*
TOL	1							
SSI	0.94	1						
STI	-0.96	-0.99	1					
MP	-0.94	-0.99	0.97	1				
GMP	-0.94	-0.98	0.97	0.99	1			
HM	-0.94	-0.98	0.97	0.99	0.99	1		
YI	-0.95	-0.99	0.97	0.99	0.99	0.99	1	
DI	-0.97	-0.98	0.97	0.97	0.99	0.97	0.97	1

(Yp) grain yield under irrigated conditions; (Ys) grain yield under non-irrigated conditions; (SSI) Stress susceptibility index; (STI) Stress tolerance index; (TOL) tolerance; (MP) mean productivity; (GMP)Geometric mean productivity; (HM) Harmonic mean; (YI) Yield index; (DI) Drought resistance index

*Composed by the author.

Conclusion

Among the varieties identified, Navid, Voskehask, Sabalan, and Zare were recognized as drought-resistant and high-yielding varieties. Regardless of whether they are irrigated or not, they can perform well. This potential can be used in future breeding programs or genetic engineering programs for drought-stress abilities. Also, the results of an evaluation of the effect of failure stress on grain yield using stress forbearance indices suggested that breeders should choose the indices based on stress inflexibility in the target terrain.

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Brucellosis Prevalence in Livestock of Tavush and Kotayk Marzes of Armenia and Assessment of Diagnostic Algorithm

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ABSTRACT

Brucellosis is a common bacterial zoonosis caused by *Brucella spp.*, which are facultative intracellular gram-negative cocco-bacilli, causing disease in cattle, small ruminants, and humans. The occurrence of brucellosis is widespread across a wide geographical area as well as endemic throughout the Caucasus region. The Ministry of Agriculture of Armenia has adopted a diagnostic algorithm consisting of screening samples with the Rose Bengal Test (RBT) followed by confirmation with the complement fixation test (CFT). This study determined the feasibility of the algorithm and diagnostics in Armenia. Sampling and testing were conducted in two marzes of Armenia with disparate brucellosis prevalence. The screening was performed in 2020 at regional laboratories, followed by retesting at the reference laboratory with RBT and confirmation by CFT. Results indicated that RBT testing was reproducible between laboratories, although positive rates in CFT testing were slightly higher. Brucellosis incidence in cattle was not geographically dependent, while sheep incidence was affected by location. The addition of CFT to the diagnostic algorithm improves the results.

Introduction

Brucellosis is a common bacterial zoonosis caused by *Brucella spp.*, which are facultative intracellular gram-negative cocco-bacilli. It is most commonly a disease of cattle, small ruminants, and humans, with animal cases typically recognized by abortion (Acha, 2003; Głowacka, et al., 2018). The main species are *B. abortus, B. suis, B. melitensis, B. neotame, B. ovis*, and *B. canis* (Theron, 2014). Brucellosis is transmitted to humans through contact with infected animals and animal products, especially milk products (Khurana et al., 2021). The Food and Agriculture Organization (FAO), World Health Organization (WHO), and World Organization for Animal Health (WOAH) consider brucellosis one of the most pervasive zoonoses in the world (Corbel, 2005). Brucellosis also poses a hazard to laboratory staff handling specimens containing Brucella species, as the pathogen is

readily aerosolized and has a low infective dose. Therefore, brucellosis is becoming one of the most prevalent laboratory infections (Yagupsky and Baron, 2005; Sayin-Kutlu, et al., 2012). The most effective prevention strategy is infection elimination in animals (Corbel, et al., 2006). Many countries at risk of brucellosis lack diagnostic capacity to identify disease cases, which threatens affected countries and their neighbors (Kisman, 2010).

Brucellosis is widespread geographically, with cases reported in North Africa, the Mediterranean, the Middle East, and Central Asia. The disease is endemic in the Caucasus region (Porphyre, et al., 2010; Yumuk and O'Callaghan, 2012; Akhvlediani, et al., 2017). In Armenia, brucellosis is one of the most widespread zoonotic diseases, with more than 300 new cases annually (2019). Disease incidence is depends on geographical and demographic factors. Geographically, the Kotayk marz is dominated by pastures located centrally in the country, where it is a crossroads for seasonal animal movement. Farmers from multiple regions use pastures. The Tavush marz is dominated by forest zones. A primary demographic factor is the presence of a national minority group, the Yezidis in the Kotayk marz. This group maintains small ruminants in large numbers. In Armenia, previous risk mapping indicated a significant prevalence of 29 % and 21 % for cattle and small ruminants, respectively, in 858 communities surveyed. However, the distribution was uneven, which prevents accurate disease predictions risk, and suggests a possible problem with testing methods (Porphyre, et al., 2010).

Diagnosis of brucellosis is primarily accomplished through serological methods, but bacterial culture methods can also be used, although, culturing requires additional safety precautions (Dal, et al., 2019). Primary binding assays, such as enzyme-linked immunosorbent assay (ELISA), in serological diagnosis, determine the interaction of antibodies and antigens. Conventional tests measure secondary phenomena such as agglutination in the Rose Bengal Test (RBT), or the activation of complement in the complement fixation test (CFT). Each diagnostic has performance and cost considerations, such as low-cost screening tests versus high-cost and highly specific binding assays (Gall and Nielsen, 2004). One potentially confounding factor in brucellosis testing programs is vaccinating animals with strains such as S19 in cattle and Rev-1 in sheep and goats. These vaccine strains cause false positives in RBT. This issue is not important for Armenia. A State vaccination program has not been initiated to date. Importation and use of vaccines are restricted by the Armenia government and private individuals can not access them.

In 2019, the Ministry of Agriculture of Armenia made changes to its state brucellosis testing algorithm due to past limitations on the information supplied by the brucellosis testing regime in Armenia. Currently, in addition to screening livestock within each marz by RBT, positive samples must be confirmed by the Armenia Reference Laboratory for Especially Dangerous Pathogens (RLEDP) followed by an additional confirmatory test by complement fixation test (CFT). In 2020 following the implementation of the CFT, a pilot study was conducted in two marzes of Armenia. This study determined the feasibility of the algorithm and diagnostic tests in two marzes of Armenia with disparate brucellosis prevalence.

Materials and methods

The investigation was performed in 2020. The tests were conducted in the Reference Laboratory for Especially Dangerous Pathogens of the "Republican Veterinary-Sanitary and Phytosanitary Center of Laboratory Services" SNCO, under the Food Safety Inspection Body of the Republic of Armenia.

Sample collection

The two marzes selected for this study were Tavush and Kotayk, with a historically low and high prevalence of brucellosis, respectively. All regions of the Tavush marz were sampled, while two of three regions in Kotayk were sampled due to geographical barriers of the mountainous region. A total of 1.298 samples were collected with 535 blood samples from Tavush marz (cattle=257, sheep=278) and 763 samples from Kotayk marzes (cattle=215, sheep=548). From each animal, 5-10 ml of blood was collected in vacutainer tubes and transported to a regional laboratory. Blood was processed by centrifugation and serum was removed and stored at +4 ^{0}C until initial analysis.

Diagnostics

The diagnostic algorithm used for the processing of samples through the final result was as follows: Screening was conducted by RBT at the regional laboratory level. All sera were then transferred on ice to the RLEDP for retesting by RBT, followed by confirmation with CFT. Final results were determined at RLEDP and reported through government-access.

Screening for antibodies against Brucella was first conducted by the RBT (ANTIGEN, LTD) at regional laboratories. Then samples were transferred to RLEDP where RBT was repeated in duplicate. Positive samples were confirmed using CFT. In the case of split results between RBT and CFT, CFT was used as the final diagnosis. Briefly, serum samples and controls were aliquoted in 96 well plates and diluted 1:4 (25 µl) in veronal buffer. Equal volumes of diluted antigen (IDEXX) and complement (Rockland, USA) were added and incubated for 16-20 hours at +2-8 ^{0}C. Plates were incubated for 10 minutes at +37 ^{0}C followed by the addition of 50 µl of hemolysis serum consisting of

equal volumes of 2.5 % sheep red blood cells and optimized hemolysin (Rockland, USA). Plates were incubated at +37 ^{0}C for 30 minutes followed by 60 minutes at +2-8 ^{0}C. The percentage of hemolysis was determined by comparison to prepared standards, consisting of a series of dilutions of complement control from 0-100 % hemolysis. Samples with less than 50 % hemolysis were considered positive.

Statistical analysis

The relation of marz to disease status in cattle and sheep was calculated by the Pearson chi-square test for independence, computation of odds ratio, and likelihood test ratio. Analysis was performed utilizing SAS software.

Results and discussions

Among tested cattle from Tavush, 11.3 % tested positive (29/257) for brucellosis as indicated by the confirmatory CFT results. Incidence among Kotayk cattle was higher, with 18.6 % positive (40/215). Analysis indicates that there was a significant interaction between marzes (environment) and disease status in cattle, with Kotayk cattle 1.8 times more likely to be positive. An even stronger association between marz and disease status was indicated in sheep, with Kotayk sheep 2.4 times more likely to be positive for brucellosis. Disease incidence was higher in sheep than in the cattle in both marzes. This was 18.3 % sheep positive (51/278) in Tavush marz and 35.8 % positive (196/548) in Kotayk marz. The higher density of sheep kept in the Kotayk marz coupled with shared-farming husbandry practices should account for the higher prevalence.

When the results were examined by region within each marz (Table 1), there was no significant difference in the incidence of brucellosis among cattle. However, in sheep, the incidence of disease was significantly dependent on the region (Table 2). In the Kotayk marz, the odds of having a brucellosis-positive sheep are 2.0 times greater in the Nairi region than in the Abovyan region. The Tavush marz also revealed significant differences in brucellosis incidence by region. The odds of detecting a positive sheep in the Dilijan region were 3.5x higher than in Ijevan, 5.1x higher than in Noyemberyan, and 11.0x higher than in the Berd regions. The difference in odds of positive sheep was 3.1x higher in the Ijevan region than in Tavush. Brucellosis testing results by marz followed the historical trend of higher incidence in Kotayk marz than in Tavush. The current diagnostic algorithm for brucellosis was an initial screening test by RBT in each region's laboratory. This was followed by confirmatory testing at RLEDP by RBT and CFT. For this pilot study, all samples were tested by RBT and CFT for comparison purposes. Under normal testing parameters, only RBT positives and 10 % of negative samples would be forwarded to RLEDP for confirmation.

Test results were nearly identical between regional laboratory RBT results (59/472 positive cattle, 231/826 positive sheep) and Reference Laboratory results (63/472 cattle, 238/826 sheep). There were no statistical differences in the odds of testing positive by RBT the regional versus reference in laboratories. CFT produced slightly more brucellosis positives than RBT, especially in Tavush marz cattle (29 by CFT versus 18 by RBT), where additional positives were detected in samples that tested negative by RBT in both laboratories. Differences by test and region may be due to the skill level of technical staff or the condition of testing reagents. It has been shown that RBT antigens can deteriorate when used frequently due to repeated cold-to-room temperature cycles.

Table 1. Brucellosis incidence in cattle by marzes and region communities*

Marz	Region	Brucellosis positive animals quantity/ animals total quantity	Positive frequency within the region, %
Tavush	Ijevan	11/69	15.9
	Dilijan	5/55	9.1
	Noyemberyan	4/57	7.0
	Berd	9/76	11.8
Kotayk	Abovyan	40/206	19.4
	Nairi	0/9	0.0

Table 2. Brucellosis incidence in sheep by marzes and region*

Marz	Region	Brucellosis positive animals quantity/ animals total quantity	Positive frequency within the region, %
Tavush	Ijevan	15/64	23.4
	Dilijan	12/27	44.4
	Noyemberyan	15/86	17.4
	Berd	9/101	8.9
Kotayk	Abovyan	154/463	33.26
	Nairi	42/85	49.4

*Composed by the authors.

Conclusion

A summary of the pilot study results indicates that adding a confirmatory test (CFT) to the state brucellosis testing algorithm is an important step to the improvement of the

results before reporting. Additionally, baseline data on the prevalence of brucellosis in Tavush and Kotayk marzes has increased since 2010. These results also indicate that while geographic region does not impact the incidence of disease in cattle, there is a significant impact of location on the incidence of brucellosis in sheep. This pilot study should be expanded in the future to all marzes of Armenia. Further expansion of the data available would better inform public health policy as well as allow the development of improved testing and slaughter management plans. This would reduce the incidence of brucellosis in Armenia.

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Declarations of interest

The authors declare no conflict of interest concerning the research, authorship, and/or publication of this article.

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Evaluation of Service, Perinatal Period and Milk Productivity of Fleckvieh Breed Cows in the Vamaks LLC Farm of the RA Syunik Region

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ABSTRACT

The data of the study indicate that the service period of the cows of the 4th-5th lactation is 5-58 days higher than the norm in the "Vamaks" LLC cattle farm of the Syunik region of the RA, and the coefficient of reproductive ability varies in the range of 86.3-98.6 %. Due to sterility, the loss of lactations in the 5th lactation is 16 and the loss of milk quantity is 126 tons.

We propose to identify and inseminate (mate) animals with sexual desire in a timely manner, as well as to establish strict veterinary control to monitor and regulate the service period of 4-5 calving cows.

Introduction

"Vamaks" LLC cattle farm in the Syunik region of the RA was built by the Federal Republic of Germany for breeding German breed Fleckvieh animals. It is equipped with modern facilities and a software control system. The departments are provided with the ideal conditions of livestock: hygiene and microclimate, year-round management conditions, and whole animal feeding (Berry, 2014). Farm animals' reproduction is guided by a complex hormone system. Knowledge of the animals age, sexual cycle, the timing of insemination, and the physiological processes of the organism allow to properly organize reproduction and increase economic efficiency (Anisimova, 2008; Milovanov, 1962; Koeck, et al., 2010).

The most complex and labor-intensive process in animal husbandry is herd reproduction, which accounts for animal breeding, food production, intensive use, longevity, and economic profitability (Bocharov, 1990; Dedov, et al.,

1988).

Materials and methods

The studies were carried out in 2020-2021 upon the data of the completed lactation of the 3rd, 4th, and 5th calving of Fleckvieh (Simmental) cows bred in the cattle farm of "Vamaks" LLC, Syunik region/Marz, RA. According to the summary data of the complex assessment (bonitation) of the farm's livestock, 279 cows were assessed, including 73 heads of 1st-calf generation, 103 heads of 2nd generation, 35 heads of 3rd generation, 53 heads of 4th-5th generations, and 15 heads of 6-7 generations. The average age of 1st generation cows was 789 days.

21 cows of the 3rd generation and 19 cows of the 4th-5th generation were included in the study scope. Cows were selected according to calving duration (3, 4, and 5) and the service period (30-70 days, 71-100 days, and 101-150 days). Cow mating season, length of service (from birth to insemination), sexual lust (flow), and perinatal periods were investigated (Crowe, 2018; Anokhin, et al., 2019).

The coefficient of reproductive ability (CRA) of cows was determined through the following formula:

$$CRA = \frac{365}{PP} \times 100 \ \%,$$

where 365 is the number of days in a calendar year, and *PP* is the perinatal period in days.

If the cow's coefficient is above 100 %, the reproductive ability is excellent, 95-100 % – good, 90-95 % – satisfactory, 85-90 % – poor, and below 85 % very bad (Marmaryan, et al., 2001).

Conditional loss of calves due to sterility per 100 cows was determined by the following formula:

$$CRA = \frac{(ASP - 80) \times 100}{365} \ \text{head},$$

where ASP is the actual service period in days, 80 is the guaranteed service period in days, and 365 is the guaranteed perinatal period in days.

The 305-day milk yield data taken from the complex evaluation (bonitation) book of cows were processed by the biometric method (Merkuryeva, 1970; Karymsakov, 2020).

Results and discussions

According to the electrical control data, during the examination of cows in the insemination room, the beginning and end of the breeding period were noted through daily observations. At the same time, the animals in lust are identified per the behavior mode, the genitals of cows swell, turn red and secrete mucus, the cow behaves restlessly, bells, appetite worsens, the milk gets reduced, and the animal becomes unable to protect himself from other cows jumping on it (Pantelic, 2011; Koriakina, 2019).

It is clear from the study that the average duration of autumn-winter lactation was 27.2 hours (variation of 24-32 hours), and the spring-summer calving cycle was 24.2 hours (variation of 20-30 hours). After insemination, sperm cells live in the cow's genital tract for 12-24 hours. 8-12 hours after the sexual desire occurs, ovulation happens. The ovum fertilizes within 6-10 hours after ovulation. After the cow's insemination, the sperm cells reach the upper third of the fallopian tube within 6-8 hours. To conceive, it is advisable to inseminate (mate) cows 10-12 hours after sexual desire (Ulimbashev, 2015; Schmeling, 2022). After parturition, cow conception occurs after the uterine has regrown. In most cows, uterine retrogression occurs within 28 days. Some authors estimate it at 42-50 days. The duration after birth to conception (service period) should not exceed 3 months on average, in which case the perinatal period will be 365 days, including service, lactation, pregnancy, and dry period (Troitskiy, 1961; Kozlo, 1984; Trimberger and Davis, 1943).

From the analysis of data in (Tables 1 and 2), it was found that the service period of the 3rd calving cows in case of 30-70 days duration was 63 days on average, the perinatal period was 349 days, the milk of 305 days was 6665 kg, the correlation of milk amount and service period indicators is -0.12, and regression coefficients are -12.0 and -0.001. The variability coefficients of milk quantity, milk fat, milk protein, milk fat+ milk protein, and service period in 305 days are consistent and range from 16.4 to 17.5, the CRA was 104.6 %, which is excellent, whereas due to sterility in 3rd generation cows, no calving loss was observed.

The service period of the 4th calving cows in the range of 71-100 days was 85 days, and the other indices were 370 days, 7495 kg, -0.19, -26.0, and -0.001, respectively. The coefficients of variation of 305-day milk quantity, milk fat, milk protein, milk fat + milk protein, and service period were normal and ranged from 9.6 to 15.9, and the CRA was 98.6 %, which is acceptable; due to infertility in 4th generation cows, the loss of calving makes 1 head.

The service period indices of the 5th calving cows with a duration of 101-150 days are 138 days, 423 days, and the other indices are 7877 kg, -0.21, -31.15, and -0.001, 305 days, respectively.

Table 1. Biometric processing of milk yield and reproductive ability indicators of Fleckvieh cows*

Indicators	Lim	M±m	σ	Cv
Service period in the 3rd calving animals, 30-70 days (n=21)				
Milk in 305 days, kg	4054…8543	6665±238.5	1093.0	16.4
Fat content in milk, %	3.8…4.1	3.9±0.02	0.09	2.3
Protein in milk, %	3.3…3.4	3.4±0.01	0.05	1.5
Milk fat, kg	154…325	258±9.46	43.3	16.8
Milk protein, kg	138…290	224±8.03	36.8	16.4
Milk fat + milk protein, kg	292…615	482±17.41	79.8	16.6
Perinatal period, day	322…357	349±2.45	11.23	3.2
Service period, day	35…70	63±2.4	11.0	17.5
Service period in the 4th calving animals, 71-100 days (n=19)				
Milk in 305 days, kg	6164…10831	7495±258	1125	15.0
Fat content in milk, %	3.7…4.1	3.9±0.02	0.09	2.3
Protein in milk, %	3.0…3.4	3.4±0.02	0.1	2.9
Milk fat, kg	246…444	290±10.6	46.2	15.9
Milk protein, kg	208…368	251±9.1	39.5	15.7
Milk fat + milk protein, kg	455…812	541±19.6	85.2	15.7
Perinatal period, day	358…385	370±1.9	8.2	2.2
Service period, day	73…100	85±1.9	8.2	9.6
Service period in the 5th calving animals, 101-150 days (n=19)				
Milk in 305 days, kg	5552…9836	7877±240.7	1049	13.3
Fat content in milk, %	3.7…3.9	3.8±0.02	0.08	2.1
Protein in milk, %	3.1…3.4	3.3±0.02	0.08	2.4
Milk fat, kg	217…374	300.2±8.5	36.9	12.3
Milk protein, kg	183…325	261±8.1	35.4	13.6
Milk fat + milk protein, kg	400…698	561±16.5	71.9	12.8
Perinatal period, day	412…436	423±1.5	6.7	1.6
Service period, day	127…150	138±1.6	7.0	5.1

Table 2. The correlative relationships between the milk yield and service period indicator, regression coefficients*

Indicators	Coefficients of correlative relationships (r) and regression (R)		
	r	$R_{1/2}$	$R_{2/1}$
Service period 30-70 day	-0.12	-12.0	-0.001
Service period 71-100 day	-0.19	-26.0	-0.001
Service period 101-150 day	-0.21	-31.15	-0.001

*Composed by the authors.

The coefficients of variability of milk quantity, milk fat, milk protein, milk fat + milk protein amounts, and service period are normal and range from 12.3 to 13.6, the CRA was 86.3 %, which is poor, the loss in calving due to sterility is 16 heads, and the loss of milk quantity is 126 tons.

The coefficients of variability in the amount of milk, milk fat, milk fat + milk protein, and the maintenance period are regular and range from 9.6-15.9, the coefficient of reproductive ability (CRA) was 98.6 %, which is good; in cows of the 4th calving, the calf loss makes 1 head due to sterility.

Conclusion

The data of the study prove that the service period of the cows of the 4th-5th calving is 5-58 days higher than the norm in the "Vamaks" LLC cattle farm of the RA Syunik region, while the coefficient of reproductive ability varies between 86.3-98.6 %. Due to sterility, the loss in lactations in the case of the 5th calving is 16, and the loss of milk quantity is 126 tons. It is recommended to identify and inseminate animals with sexual desire and to establish strict veterinary control to track and regulate the maintenance period of the 4th-5th lactation cows.

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Prevalence of Demodicosis of Dogs in Some Districts of Yerevan

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ABSTRACT

In recent years, the successful development of dog breeding in Armenia has been hindered by various diseases, the most common of which is demodicosis. The spread of the demodicosis invasion in Yerevan is mainly due to the uncontrolled breeding of dogs, violation of sanitary and hygiene rules for dog rearing, and unauthorized sale and purchase of dogs. Lack of information about the disease among the dog owner and breeders is another important reason for the spread of the disease.

Demodicosis in dogs proceeds with certain variations. The extent of invasion in autumn was 51.7 %, in spring – 34 %, in winter – (32.2 %), and in summer it was 18.5 %. The relatively low temperature of the external environment and the high relative humidity of the air are probably favorable conditions for the reproduction, growth, and development of causative agents of demodicosis in dogs.

Studies have shown that the highest degree of demodicosis was observed among maternal livestock, and animals older than three months. The lowest degree of disease damage was observed in puppies up to three months old.

Introduction

Dog invasive diseases of, both worldwide and in the Republic of Armenia, demodicosis is characterized by high prevalence. The first information about pathogenic mites – the causative agents of demodicosis, is found in Homer works (800 BC). Drawings with images of mites were found on the walls of Egyptian tombs. This indicates that a person has been familiar with pathogenic animal mites and the peculiarities of their structure since ancient times. In his book “The History of Animals” Aristotle described the mites that lived on dogs, suggesting using sulfur to destroy the latter (Naghashyan, 2003).

According to Dabry and Wolff (1899), mites of the genus Acarus were known to the Chinese 400 years ago. In the 20th century, comparative and evolutionary arachnology was studied by Balashov, Lange, and many other scientists (Naghashyan, 2003).

The causative agents of demodicosis of dogs and humans belong to the order Acariformes, which, in turn, is divided into subtypes Sarcoptiformes, Trombidiformes, and Oribatida. The Trombidiformes subtype includes many free-living and parasitic mites and the causative agent of demodicosis in dogs Demodex canis (Naghashyan, 2003).

In the city of Tyumen of the Russian Federation, according to the data of surveys conducted at different times of the year, demodicosis of dogs was recorded in the following percentage: in winter – 16.2 %, in spring – 23.7 %, in summer – 38.6 %, and in autumn it was 21.5 %. Infected dogs mainly have lesions of the skin of the eyelids, cheeks, lips, lower jaw, head, neck, chest, front, and hind limbs (Korotaeva, 2005). The results of epizootological studies of demodicosis of dogs conducted in 2011 in Moscow indicate a high degree of infection – 37.1 %. (Yarovaya, 2011). The high degree of infection in March-April and October-November is a consequence of a decrease in the general nonspecific resistance of animals in transitional seasons (Kataeva and Kostyleva, 2008). Outside the host body at a temperature of 18-20 ºC and relative humidity of 90 %, the mites remain viable for 9 days (Krasev, et al., 2004).

Animals infected with demodicosis have itching, which is why they rub the affected parts of the body against various objects, scratch themselves with their limbs, and bite the affected areas of the body, thereby contributing to the appearance of wounds seeded with a second pathogenic microflora. Studies have shown that milk production decreases by 12-20 % in cows affected with demodicosis. In heifers and bulls affected with demodecosis, there is poor growth, weight loss, and even death (Akbaev, et al., 2016). Also, in the blood picture of sick animals, there is a decrease in the hemoglobin content and the number of red blood cells (Romensky, 2001).

The causative agent Demodex canis occurs mainly in dogs, but in some cases, it is transmitted to humans through contact with a sick animal. In puppies infected by mothers during feeding, the mouth area, eyes, and forelimbs are affected (Romanina, 2014).

For the diagnosis of demodectic disease, deep skin scrapings are necessary, and in rare cases, a skin biopsy is performed. Immune suppression caused by endoparasitism or malnutrition in young dogs and endocrine diseases, neoplasms, and chemotherapy in older dogs are considered predisposing factors and should be diagnosed and treated to optimize therapeutic outcomes (Mueller, et al., 2012).

Based on various studies it can be inferred that demodicosis is widespread all over the world but data on scabies is still scanty and more detailed studies are required. The work aims to find out the prevalence of dog demodicosis in some communities of Yerevan, taking into account the seasonality, age group of animals, conditions of care, and behavior.

Materials and methods

The study was conducted in 2021-2022 at the Veterinary Medicine and Veterinary Sanitary Examination Research Center laboratory of the Armenian National Agrarian University.

The diagnosis of demodicosis in dogs was made based on epizootological data, clinical signs, and results of generally accepted methods of laboratory examination of scrapings taken from the affected areas of the skin which are used in the diagnosis of demodicosis in dogs (Naghashyan, et al., 2016). The prevalence of demodicosis of dogs was studied within the city of Yerevan, taking into account factors such as animal habitats, time of year, and age of dogs.

The clinical expression of canine demodicosis was studied in dogs belonging to different age groups infected with demodectic ticks. A total of 161 clinical experiments were performed on domestic, yard, and stray dogs. The study of clinical signs of demodicosis was carried out by observation and palpation methods of skin and hair. During the experiment, attention was paid to clinical signs, such as the absence of hair, tousled hair, lack of hair luster, lack of skin elasticity, flaking of the skin, thickness, and presence of lumps and bumps.

The diagnosis of demodicosis was confirmed when eggs, larvae, pupae, as well as mature mites or their body parts, were detected by microscopy of preparations prepared from skin scrapings taken from the affected areas of the body. However, the absence of mites in the preparations in the presence of specific clinical signs of the disease does not exclude the possibility of their presence in the animal's skin. Therefore, repeated sampling of clinically ill animals under control was carried out after 5-10 or 15-20 days (Naghashyan, et al., 2016).

Results and discussions

The result shows that in Malatia-Sebastia, Shengavit, and Ajapnyak communities, the infection rate in autumn was 51.75 %, in spring – 34 %, in winter – 32.2 %, and in summer it was 18.5 %. It can be concluded that demodicosis in dogs showed certain fluctuations. Demodex canis penetrates hair follicles of the skin, and the ducts of the sebaceous and sweat glands, multiplies rapidly, and stay protected from the influence of environmental factors.

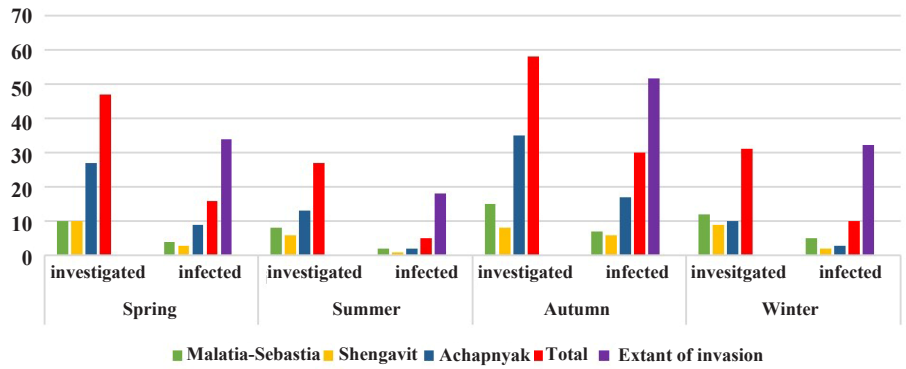

Figure. Summarized data on the prevalence of demodicosis of dogs according to the time of the year in different administrative districts of Yerevan *(composed by the authors)*.

Table. The prevalence of demodicosis of dogs in some administrative districts of Yerevan, according to the age of the animals*

Communities	Maternal livestock		Up to three months		Older than three months	
	Investigated (head)	Infected (head)	Investigated (head)	Infected (head)	Investigated (head)	Infected (head)
Malatia-Sebastia	15	7	10	2	20	8
Shengavit	10	4	7	1	14	3
Ajapnyak	30	14	12	3	43	15
Total	55	25	29	6	77	26
The extent of the invasion (%)	-	45.5	-	20.7	-	33.8

*Composed by the authors.

Picture. Dog with demodicosis.

The data on the extensiveness of dogs' infection level with demodicosis in some communities of Yerevan, according to the age of the animals are shown in Table.

The degree of demodicosis manifestation in dogs of different age groups had the following percentage ratio: in the communities of Malatia-Sebastia, Shengavit, and Ajapnyak, the extensiveness of the disease in maternal livestock was 45.5 %, in under three months old puppies 20.7 %, and in older than three months old puppies it was 33.8 %. The intensity of demodectic infestation was highest in the maternal population, lowest in pups under three months old, and relatively lower in animals over three months old. This prevalence of demodicosis, depending on the age of infected animals, is obviously due to the

frequent movement of maternal livestock, males, and dogs older than three months, contributing to numerous contacts between healthy and sick animals.

Puppies up to three months old are infected by their mothers during the period of milk nutrition by contact with the mother's breast. For this reason, puppies of this age initially have lesions in the mouth area then spread to other parts of the body.

Conclusion

1. Demodicosis in dogs proceeds with certain variations. The invasion intensity was in autumn – 51.7 %, in spring – 34 %, in winter – 32.2 %, and in summer – 18.5 %. The relatively low temperature of the external environment and the high relative humidity of the air are probably favorable conditions for the reproduction, growth, and development of causative agents of demodicosis in dogs.
2. In Yerevan, demodicosis of dogs occurs regardless of the time of year and hygienic conditions of keeping animals. The manifestation of demodicosis according to age groups had the following picture: in Malatia-Sebastia, Shengavit, and Ajapnyak communities, the prevalence of infection in mothers was 45.5 %, in puppies up to three months old – 20.7 %, and in dogs over three months old it was 33.8 %.

Studies have shown that the highest degree of demodicosis was observed among maternal livestock, and animals older than three months. The lowest degree of disease effect was observed in up to three months old puppies.

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Research of Colibacteriosis in Honey Bees at Aragatsotn Region and Susceptibility of the Detected Pathogen to Antibiotics

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ABSTRACT

The research was carried out in the Ashtarak and Avan communities of the Aragatsotn region of the Republic of Armenia. During the research enterobacteria were found in the samples brought from the Ashtarak community. *Salmonella, Escherichia coli*, or *Proteus* diseases were suspected. API 20 E microbial identification test-system was used for identification. As a result of research carried out in 2022, using the above method, *Escherichia coli* was confirmed in bees in the Ashtarak community of the Aragatsotn region. The susceptibility of the pathogen to antibiotics was also checked and it was found out that the most effective antibiotics are *Gentamicyn, Levofloxacin, Streptomycin*, and *Amoxicillin*.

Introduction

Beekeeping is one of the most important branches of agriculture. On the one hand, it stands out for its high profitability, and on the other hand, the bee is the main pollinator of a number of agricultural crops, due to which the seeding and yield of vegetables, fruit trees, and herbs increases (Mkrtchyan and Pepelyan, 2015). Besides, natural honey is known for its high caloric content, nutritional value, and curative-preventive properties (Abrahamyan et al., 2008).

Beekeeping existed in prehistoric times. It developed in several stages, from wild beekeeping to framed beekeeping (Ayvazyan, 2015). Armenians have been beekeepers for centuries. This is documented in "Anabasis" by Xenophon (431-354 BC), "Bibliotheca Historica" by Diodorus Siculus, "History of Armenians" by Moveses Khorenatsi, and "Bee" by V.Yu. Nekrasov, and also "History of Armenians" by Hovhannes Draskhanakerttsi. A part of the honey produced by Armenia in ancient times was exported to Persia and Byzantium. Even in the "Judgment Book" of Mkhitar Gosh and Smbat Sparapet, there is a special law regarding the purchase and sale of beehives (Kotoghyan, et al., 1961).

It is clear that the number of beehives in the world has increased between 2010 and 2021. In 2010, there were 80.65 million beehives in the world, while in 2021 there was 101.62 beehives as per statistician (www.statista.com).

In parallel with the increase in beehives, assessment and application of appropriate medical and pre-treatment measures is necessary.

All living organisms can be attacked by their natural enemies and bees are no exception. Parasites and predatory insects (www.fao.org), as well as diseases, can harm bee colonies. The symptoms observed in bee colonies differ depending on the type of pathogen causing the disease, so the most effective way to confirm the presence of the pathogen is an appropriate laboratory diagnosis (The National Bee Unit, 2017).

Bees`diseases are infectious and non-infectious. Non-infectious diseases are not transmitted from sick bees to healthy bees, as they lack pathogens. Colibacteriosis is an infectious, bacterial disease caused by Escherichia coli. As a rule, weak bees get sick and die. The occurrence of the disease is facilitated by the decline in bee resistance, insufficient feed, temperature, and high humidity (Hakobyan, et al., 2014).

Bee gut microbiota is simpler than that of mammals (Kwong and Moran, 2016). The gut microbiota of worker bees is dominated by 9 bacterial phylotypes, 5 of which are the main ones: *Gilliamella, Snodgrassella, Lactobacillus Firm-4, Lactobacillus Firm-5, Bifidobacterium*, and a number of secondary ones: *Frischella, Bartonella, Commensalibacter or Bombella* (Kešnerová, et al., 2020). Recent studies have shown that the gut microbiome of bees is essential for metabolism, immune function, growth, development, and defense against pathogens (Raymann and Moran, 2018), while disruption of the microbiome can lead to reduced bee adaptability (Zheng, et al., 2018).

Materials and methods

The research was carried out in the Ashtarak and Avan communities of the Aragatsotn region of the Republic of Armenia. It was cloned first in the field, then in the laboratory.

According to the beekeepers of the Ashtarak community, the disease started in April and after about 10 days, the bee's decline was observed. The preliminary examination of newly fallen bees was done by eye observation.

In order to conduct laboratory research, the declined bees were transferred from the communities in a thermal bag to the Microbiology Department of the "Scientific Center for Risks Assessment and Analysis in the Food Safety Area" CJCS of the Republic of Armenia.

For the purpose of the research, newly declined bees, microbial identification test system were used: API 20E, Vortex Mixer, dyes, and Italian-made antibiotics such as *Streptomycin* 10 mg, *Levomycetin (Chloramphenicol)* 30 mg, *Levofloxacin* 5 mg, *Amoxicillin* 10 mg, *Tetracycline* 30 mg, *Gentamicin* 10 mg, *Penicillin-G (Benzylpenicillin)* 10 mg, *Erithromycin* 15 mg, *Vancomycin* 5 mg, *Cephalotin* 30 mg, mortar, saline, ethyl alcohol 96 %, meat peptone agar, bismuth-sulfite- agar, Muller-Hinton agar, etc.

The Research was carried out in the following methods: observation (eye observation) and microbiological (Gram staining, microscopic observation by immersion method, identification test system and disco-diffusion using discs containing antibiotics in agar medium, the study of the formation of the zone of suspension of bacterial growth caused by the phenomenon of diffusion).

In the laboratory, suspensions were prepared from declined bees taken from the Avan and Ashtarak communities. After 15 minutes of extraction, primary injections were performed on meat peptone agar in test tubes and on bismuth-sulfite agar in Petri dishes in 1 ml portion sizes. Test tubes with meat peptone agar were placed in a thermostat at 37 ^{0}C for 48 hours and Petri dishes with bismuth-sulfite agar for 72 hours. After removing the test tubes and Petri dishes from the thermostat they were inspected with the eye.

In order to continue the research, smears were prepared from bacteria taken from test tubes and Petri dishes. They were stained by Gram's method, and observed under a microscope with a 100- magnification lens using the immersion method.

Reseeding was carried out to identify the bacillus found in the smears prepared from samples of bees brought from the Ashtarak community. It was placed in a thermostat at 37 ^{0}C for 24 h, after which 5 ml of physiological solution was added to the test tube to which bacteria from a single colony grown from seeding were added, and shaken using Vortex a Mixer. Then it was adjusted to the McFarland 0.5 standard. According to the accompanying methodology, a diagnosis was made using the API 20E test system for microbe identification.

The susceptibility of the detected pathogen to antibiotics was determined using the disco-diffusion method according to the instructions (Determination of the susceptibility of microorganisms to antibacterial medicines, 2004): Muller-Hinton's agar medium and 10 Italian-made antibiotic discs were used to set up the reaction. A 1 ml portion size of bacterial suspension corresponding to the above-mentioned standard was added to the Muller-Hinton agar medium in Petri dishes. The reaction results were read

after 24 hours. The zone formed around the antibiotic disc was measured with a Circinus. The size of the zone of microbial growth suspension was determined in mm by placing it on the ruler.

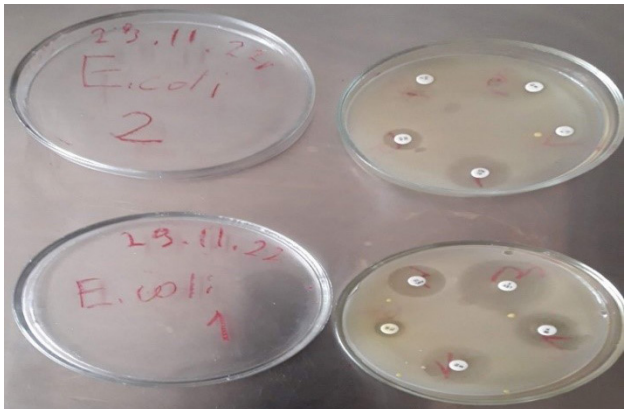
Results and discussions

During field observations, traces of diarrhea were found on honey bread and beehive walls.

During the laboratory research, after removing the thermostat, the test tubes and Petri dishes were examined with the eye. As a result, it was found out that there was expressed white growth in the test tubes of bee samples brought from the Avan community. No bacterial growth was observed in the Petri dishes. In the test tubes of the bee samples brought from the Ashtarak community, expressed colony growth with a light yellowish coloring was observed, and in the Petri dishes, well-established, medium, and large-sized dark brown colonies were noticed, whereupon enterobacteria were suspected (Picure 1).

Picture 1. Growth of brown colonies on bismuth-sulfite agar.

In smears prepared from samples brought from the Avan community, bacteria were absent on bismuth-sulfite agar, and gram-positive stained cocci were found on meat peptone agar. Observations obtained from meat peptone and bismuth-sulfite agars of samples collected from the Ashtarak community revealed 2-3 µm long, gram-negative, non-spore-producing bacteria with concave edges. Microscopic examination showed that these bacteria belong to the Enterobacteria group (*Escherichia coli, Salmonella, Proteus*).



Picture 2. Susceptibility of *Escherichia coli* pathogen to antibiotics.

An identification diagnosis was made using the API 20 E test system for microbe identification, as a result of which *Escherichia coli* 1 was confirmed out of 3 suspected pathogens through the API Web computer program: 99.6 % (very reliable identification).

Bee colibacteriosis was diagnosed through laboratory research. As a result of the microbiological experiments, it was found out that *Escherichia coli* was isolated only from samples brought from the Ashtarak community.

The susceptibility of the *Escherichia coli* pathogen was tested against 10 antibiotics, and the following results were obtained (Picture 2 and Figure).

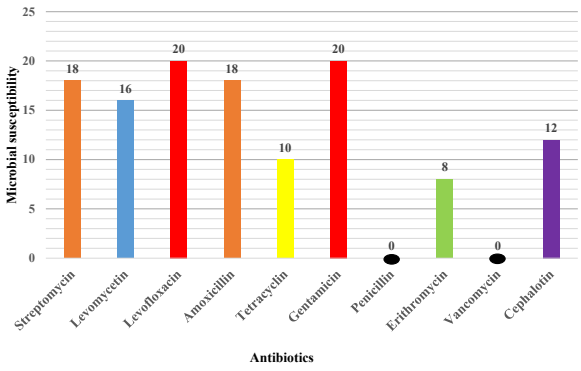


Figure. Susceptibility of the pathogen to antibiotics (*composed by the authors*).

The diagram shows that the following results were obtained when using different antibiotics: *Levofloxacin* and *Gentamicin* with 20 mm inhibition zone, *Streptomycin*, and *Amoxicillin* with 18 mm inhibition zone, *Levomycetin*: 16 mm, *Cephalotin*: 12 mm, *Tetracyclin*: 10 mm, *Erythromycin*: 8 mm and *Penicillin-G* while *Vancomycin* antibiotics did not give results.

As a result of laboratory research on samples from the Avan community of the Aragatsotn region, Escherichia coli, the pathogen of colibacteriosis, was not found. However, gram-positive stained monococci and tetracocci were found in the microscope field of view. But the research is still incomplete.

Conclusion

According to the research results, colibacteriosis (the pathogen: Escherichia coli) disease was detected in the bees brought from the Ashtarak community, while it was not recorded in the bees brought from the Avan community. The decline of bees in the Avan community was not related to colibacteriosis disease and clarification of the reasons needs further research.

Based on the obtained results, it can be concluded that the pathogen is susceptibile to *Gentamicin, Levofloxacin*, *Streptomycin*, and *Amoxicillin* antibiotics. In addition the pathogen shows intermediate resistance to the *Levomycetin* antibiotic and resistance to the remaining antibiotics.

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Development of the Second Ingredient of the New Product "Yogrik"

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ABSTRACT

Research in this area aims to develop a milk-protein product similar to ricotta cheese. This product will be the second component of the newly developed two-component Yogrik product, a mixture of yogurt and ricotta. Combination of the biological value of yogurt and ricotta will create a balanced amino acid food.

Cheeses obtained by acid and thermo-acid coagulation are usually fresh soft cheese varieties made by the coagulation of milk, cream, whey, or their mixtures, by direct chemical acidification, bacterial cultures coagulation, or by a combination of chemical acidification and high-temperature processing.

In appearance and production technology, ricotta is between cheese and cottage cheese. But in terms of the softness of the clot and versatility, it is an outstanding product. Ricotta is rich in proteins including albumin and contains about 11 % protein. Whey cheese proteins are in the most easily assimilated state. Combining the biological value of yogurt and ricotta will create a balanced food with amino acid content.

Introduction

Ricotta cheese was traditionally made in Italy from the whey of sheep`s milk cheese. The whey was heated to denature and coagulate the whey proteins; the coagulated protein was scooped from the whey. The traditional process has been modified in some countries due to the increasing popularity of Ricotta, which is now often made from milk or milk/whey mixtures. Dried whey protein powders are also used in Ricotta manufacture. In the traditional (batch) process, whey (or milk/whey mixtures) is acidified to pH 5.6-6.0 with a starter culture or acid (e.g., acetic or citric) and heated to 80 ^{0}C by steam injection or by indirect heating of the vats. The flocculated protein rises to the surface where it is collected and separated from the whey. It is placed in forms for further drainage. Calcium chloride is sometimes added to improve flocculation. Curds may be homogenized to produce a smoother consistency (Weinzweig, 2018).

UF is now often used to concentrate the whey (or milk/whey mixtures) before or after acidification and the retentate is concentrated to ~ 30 % TS. The UF retentate is then heated and packaged in a hot environment. Acidification can

also be achieved by adding acidic whey powder to milk. The whey derived from Ricotta cheese can be acidified to pH 5.4 with citric acid and boiled at 80 ^{0}C to recover additional whey proteins and is used to make Ricottone cheese (sometimes whole milk is added to the whey). In a continuous Ricotta process, blends of full-fat milk and whey are heated to 90 ^{0}C and directly acidified with acid to pH 5.3 to 5.5, which results in protein precipitation. The curd is then separated from the whey on a conveyor belt and hot packaged. Ricotta is often used in baking and confectionery applications. Requesón is a spreadable Hispanic cheese similar to ricotta (John, 2022; Cotter, 2017).

Soft cheeses like ricotta, in contrast to hard cheeses, are of increasing importance, as their production from 1 ton of raw materials increases by 10…20 % and labor costs decrease. The show of soft, especially fresh, and short-ripening cheeses can be widely implemented in existing farms and urban dairies without significant capital investment, which increases the volume of cheeses obtained and their production efficiency (Tetra Pak Processing Systems AB, 2003).

The purpose of the research was to find out the method of ricotta production with the properties we need. It was also to improve the technological parameters of ricotta production, and ensure a high food yield.

Materials and methods

These research activities were carried out with the support of the Innovative Agriculture Training and Learning Camp (AGRI CAMP) Program which is financed by The United States Agency for International Development (USAID) and implemented by the International Center for Agribusiness Research and Education Foundation (ICARE).

The contents are the responsibility of the author/s and do not necessarily reflect the views of USAID or the United States Government. Experimental ricotta were produced from reconstituted milk. Fat content: 3.2 %, dry fat-free substances: 8.2 %, density: 1027 kg/m^3, titratable acidity: not higher than 21 oT. The reconstituted milk was kept at 6-8 ^{0}C for 9-12 hours for hydration. Ricotta cheese was produced in three ways to find out which method is more convenient for us in terms of the quality of the finished product (Kristensen, 1999; Lucero, 2020).

During the first method (thermal acid method: precipitation of protein from pre-fermented milk coagulation), milk is heated to the coagulation temperature of 43-45 ^{0}C, and thermophilic bacterial culture STI-15 (*Streptococcus thermophilus*) is added and thoroughly mixed for 5 minutes. The dosage is 50 units per 500 liters of milk. Coagulation is carried out thermostatically until the formation of a dense clot without syneresis pH = 4.5-4.6. The finished coagulum is heated on steam at 85 to 87 ^{0}C until protein flakes appear. This temperature is kept for 15 to 20 minutes, and the clot is carefully collected from the surface and transferred to the serum separation mold. After 2 hours, the obtained protein mass is transferred to a refrigerator at +5 ^{0}C. Studies were performed 12 hours after placing the mass in the refrigerator (McSweeney, 2010).

During the second method (thermo-acid method of protein precipitation), milk is heated to 85...87 ^{0}C, and an aqueous solution of citric acid is added in the ratio of 30 g of citric acid per 100 liters of milk and mixed for 2 minutes. Protein flakes appear almost immediately, which we do not stir, but leave alone to grow. We keep it at this temperature for 15 to 20 minutes. The clot is carefully collected from the surface and transferred to the serum separation mold. At high temperatures, whey proteins are denatured, which, when acidified with food organic acids, causes casein coagulation. Unlike lactic acid cheeses, which coagulate at pH 4.6, with thermo acid processing, coagulation occurs at a higher pH greater than 5.3. After 2 hours, the obtained protein mass is transferred to a refrigerator at +5 ^{0}C. Studies were performed 12 hours after placing it in refrigerator (Bouchait, 2019).

In the third method (thermo-salt method of precipitation of proteins), milk is heated to 85 to 87 ^{0}C, and an aqueous solution of calcium chloride is added in the ratio of 120 g per 100 liters of milk, and mixed for 2 minutes. Protein flakes appear almost immediately, which we do not mix, but leave alone to grow. They are kept at this temperature for 15 to 20 minutes. A clot is carefully collected from the surface and transferred to a serum separation mold. After 2 hours, this protein mass is transferred to a refrigerator at +5 ^{0}C. Studies were performed 12 hours after placing them in the refrigerator (Robinson, et al., 2012). Organoleptic evaluation and physicochemical studies of experimental ricotta were performed using standard methods (Aghababyan, et al., 1988).

Results and discussions

The proposed research aims to develop and study the technology of a new lactic acid techologies. Our idea is to create a super product from yogurt and ricotta. Thanks to special technology, the updated product will not contain lactose. It will be useful for lactose intolerant patients, children, and athletes.

The second ingredient in the upcoming Yogric product being developed is ricotta, made from whole milk.

We have developed 3 ricotta samples using different protein precipitation techniques. The results of the organoleptic evaluation of the experimental ricotta (from 1 to 5 points), and the results of determining the product yield, are presented in Figures 1, 2.

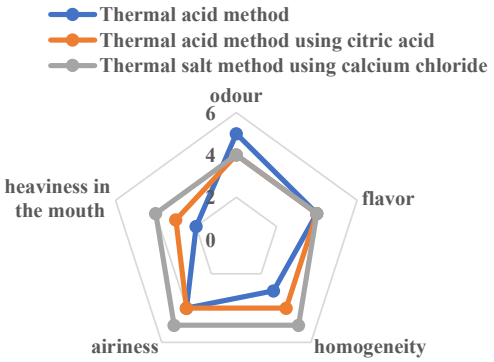

Figure 1. Organoleptic evaluation of the tested ricotta (*composed by the authors*).

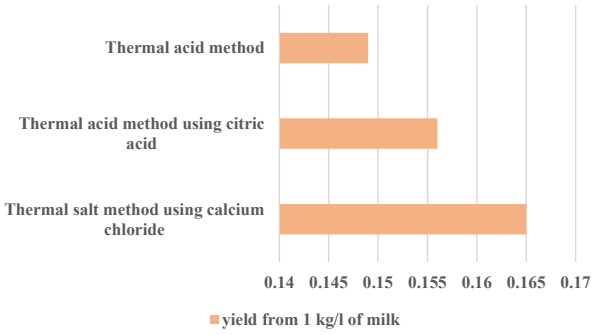

Figure 2. Yield of tested ricotta depending on the method of protein separation (*composed by the authors*).

We need ricotta with a pleasant taste and aroma, light, homogeneous, delicate, and without solid protein grains. It is also important to get a high intake of food. As can be seen from the results obtained in Figures 1, 2, the most suitable product under our development is ricotta produced by the thermo-salt method of protein precipitation.

The ricotta produced with thermo-acidic ricotta is delicately expressed in aroma and taste, but not so homogeneous and with low solubility in the mouth. The ricotta produced by the thermal-aza method is more homogeneous, delicate, highly soluble in the mouth, and has an aromatic and delicious taste. The sample obtained by the addition of citric acid with the thermo-acidic method has a pleasant taste, medium solubility in the mouth, and is inhomogeneous but delicate and aromatic.

Every producer, in addition to producing safe and tasty food, thinks about how to generate more food with minimal costs and thus get more income. According to Figure 2, the thermo-acidic method provides the lowest output of 0.148 kg from 1 kg of milk. In the case of the given weather, a bacterial mass is used and it is necessary to provide the necessary conditions for the development of microbes so that the acidity of the food will increase and the proteins will coagulate, which will further extend the duration of production. If the bacterial concentration is replaced by citric acid (120 g for 100 liters of milk), the duration of production will be reduced and the output of the product will be 0.156 kg from 1 kg of milk. Lime made using the thermal-salt method shortens production time. The amount of calcium chloride added to milk was 120 g per 100 kg of milk. Thanks to this production method, we got the highest yield: 0.165 kg from 1 kg of milk.

Table 1. Calculation of energy value of finished food*

Components	Energy separated from 1g of the component, kJ	Content of the component in 100 g of food, %	Energy value, Kj/100 g of food
Protein	16.747	11.02	184.6
Fat	37.681	3.2	120.6
Carbohydrates	15.909	3.7	58.9
Total	-	-	364.1

*Composed by the authors.

The most suitable product we are developing is ricotta, which is produced by the thermo-salt method of protein precipitation. It is this method that allows excellent product output. The resulting product has a pleasant taste and aroma, is light, and homogeneous. We also calculated the nutritional value of the produced food, which is presented in Table 1.

The technological scheme for the production of the developed ricotta is as follows: milk recovery at a temperature of 35 to 40 ^{0}C > exposure of the recovered

milk for 9 to12 hours at a temperature of 6 to 8 ^{0}C > milk heating to 83 to 85 ^{0}C > addition of calcium chloride 120 g per 100 liters of milk > milk heating to 85 to 87 ^{0}C > milk stirring for 2 minutes > release of protein mass in 10 minutes > transfer of the released milk curds into a mold > whey drainage in 2 hours > cooling to +5 ^{0}C in 4 to 5 hours > storage and sale.

Conclusion

The results of the research allowed for the development of a technology to obtain ricotta from milk with a fat content of 3.2 %, which should become the second component of an upcoming product called Yogrik. Studies have shown that ricotta obtained by thermo-salt protein coagulation is easy to dissolve in the mouth, homogeneous, and has a shorter production time, so it is suitable for the product we are developing. It was found out that the significant technological parameters affecting the yield of the product are: the amount of calcium chloride – 120 g per 100 liters of milk, the temperature of calcium chloride addition – 83 to 85 ^{0}C, the temperature of milk coagulation is 85 to 87 ^{0}C. At this temperature, milk is mixed for 2 minutes. This method provided a high yield of 0.165 kg from 1 kg milk. We calculated Ricotta's energy value, wich makes 364.1 kJ.

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The Use of Milk and Flour Made from Almonds in the Production of Lactic Foods

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ABSTRACT

A nation's health and mental strength depend on its food consumption which is one of the main conditions for the full functioning of the human body. In modern times, the demand for sour milk products has increased significantly due to their dietary and medicinal features. The research object was pure cow's milk, almond milk, almond flour, and samples of sour milk products. During the work, a study of the physicochemical and sensory indicators of the raw materials added almond milk, and the finished product was performed. Based on the sensory and physicochemical indicators of the product, the optimal dosages are as 70 % of cow's milk, 30 % of almond milk, and 1.5 % of almond flour were determined. Based on the above, it is recommended to introduce the developed technology of lactic acid products in production, which will complement the assortment of sour milk products, enrich the chemical composition of the product, and increase the body's resistance and digestibility.

Introduction

Recently, lactase enzyme deficiency, an enzyme disease characterized by the ability to break down milk sugar (lactose) due to a decrease in activity or lack of lactase enzyme, has been recorded mainly in adults and children. The expediency of using almond milk to increase the biological value of sour milk products is scientifically and theoretically justified. The technological parameters for dairy product production using almond milk and flour have been developed. The introduction of the developed technology of a new type of lactic acid production in production will expand the range of sour milk products. This will complete the product's chemical composition, and increase the body's resistance and digestibility. Almond milk is a rich source of biologically active substances; it has a rich chemical composition. (Kasyanov, 2001; Margaryan and Shaninyan, 1976).

Materials and methods

Sour milk products are prepared by technology specific to the dairy product. Sour milk products have dietary

and medicinal properties due to the action of lactic acid bacteria, lactic acid gases, vitamins, ethyl alcohol, flavoring substances, enzymes, proteins, fatty acids, and others, which destroy pathogenic microbes. Sour dairy products are also prepared using curds containing lactic acid bacteria and bifid bacteria. Such dairy products acquire functional properties that benefit human health (Galstyan, 2018; Granato et al., 2010).

Almond milk is a drink made from unroasted and ground almonds and water. It tastes like protein drinks, soy milk and other plant-based milk. In the Middle Ages, almond milk was often used in confectionery as a substitute for cow's milk. This is because it has a longer shelf life than cow's milk without refrigeration. Almonds are commonly called nuts, but they are kernels. Walnut contains a record amount of choline, 52.1 mg per 100 g of raw material, which is responsible for the youth and general health of the body. In addition, walnuts contain vitamins of groups *B, E, C,* and *PP*. Potassium, phosphorus, calcium, and magnesium stand out as the macro elements (Diagram 1).

Taking into account the traditional and advanced technologies for the production of yogurts, their production based on almond milk was proposed. Almond milk is rich in vitamins and minerals, such as vitamin *E* – 28.3 %, and manganese – 16.7 %. Vitamin *E* has antioxidant features, is necessary for the work of gonads, and heart muscle, and is a universal stabilizer of cell membranes. Almond milk improves vision, promotes weight loss, strengthens bones, and improves heart health. It also strengthens muscles, normalizes blood pressure, and helps the kidneys work properly (www.health-diet.ru).

Based on the above-mentioned circumstances, the problem is topical and has both theoretical and practical significance. The work aims to obtain sour milk products with rich chemical composition using almond milk and flour. To achieve the goal, it is necessary to solve the following problems (Diagram 2, 3):

- to study the chemical composition of almond milk, its properties, significance, and effect on the manufactured product
- to determine the optimal amount of almond milk addition
- to define the optimal parameters of technological regimes of food production
- to study sensory, physicochemical, and biochemical indicators of prepared food.

The research object was pure cow's milk, almond milk, almond flour, and samples of sour milk products. During the research, a study of the physicochemical and sensory indicators of raw materials added to almond milk and the finished product was performed. Test and check samples were prepared using classic drinkable yogurt technology (Beglaryan R., Beglaryan A., 2003).

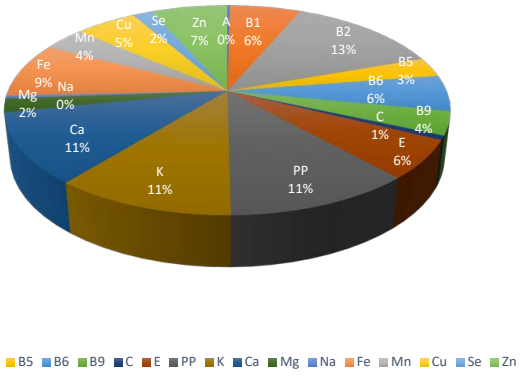

Diagram 1. Vitamins, minerals (https://productplanet.ru).

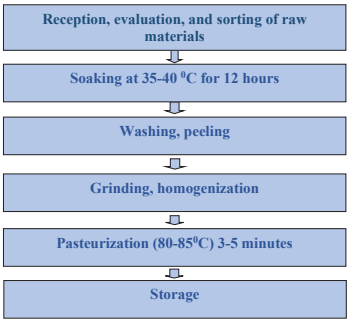

Diagram 2. Almond milk preparation diagram (*composed by the authors*).

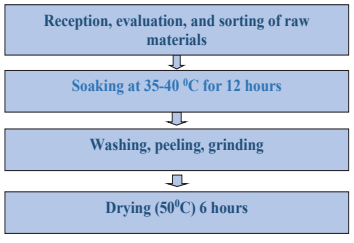

Diagram 3. Almond flour preparation diagram (*composed by the authors*).

Results and discussions

The research was carried out under laboratory and semi-production conditions. The experimental works were carried out in the laboratory of the Chair of Livestock Products Processing Technologies (Krus, et al., 2006).

Accepted scientific research methods were used during the investigation. Batches of almond milk and almond flour (experimental) and traditional (control) were produced by traditional biotechnology (Aydinyan and Chatinyan, 2009).

The subjects of the study were cow's milk, almond milk, almond flour DVS-type dry mass, and products made with sour milk technology.

The sensory and physicochemical indicators of cow's milk and almond milk were studied. (Rodionov, et al., 2020).

Sensory parameters of cow's milk are as follows: color – white to slightly yellowish, taste – naturally sweet, without extraneous taste and smell, consistency – certain thickness, without sediment. Sensory indicators of almond milk: color – white, taste – typical of almond milk, without extraneous taste and smell, consistency – a certain thickness, with a slightly expressed sediment. Composition of almond milk: water – 1000 g, almonds – 190 g.

Table 1. Physicochemical parameters of the studied cow's milk*

	Mechanical pollution	Microbial pollution	Titratable acidity, ^{0}T	Density, kg/m^3	Protein, %	Fat, %
Experimental	I	I	19	1030	3.2	2.5
Control	I	I	19	1030	3.2	2.5

Table 2. Physicochemical parameters of almond milk that are under investigation*

	Mechanical pollution	Titratable acidity ^{0}T	Density kg/m^3	Fat %
Experimental	I	10.5	1020	9.1
Checker	I	10.5	1020	9.1

*Composed by the authors.

Table 3. Determining optimal amounts of almond milk addition*

Sample	Amount of almond milk, %	Amount of almond flour, %	Amount of cow's milk, %	Hour
Control	0	0	100	5
Experimental 1	30	1.5	70	4.5
Experimental 2	50	1.5	50	4.5
Experimental 3	30	1.0	70	5
Experimental 4	50	1.0	50	5

Table 4. Indicators of technological processes of food produced with the developed technology*

Technological indicators	Experimental	Control
The fat content of the mixture, %	3.2	3.2
Titratable acidity ^{0}T	18	18
Density kg/m^3	1028	1028
Homogenization temperature ^{0}C Pressure, MPa	60-65	60-65
Pasteurization temperature ^{0}C for 5 minutes	90-95	90-95
Condensation temperature,	43	43
Amount of clod to be added YF-L811	50 units per 500 liters of milk	50 units per 500 liters of milk
Amount of almond flour to be added, %	1.5	0
Duration of coagulation, hours	4.5	5
Acidity at the end of coagulation, ^{0}T	80	90

*Composed by the authors.

Physicochemical parameters of cow's milk are as follows: mechanical contamination is determined according to standard I class, microbial contamination – according to reductase test I class, fat content – 2.5 %, titratable acidity – 19 ^{0}T, density – 1030 kg/m^3, protein content – 3.2 %.

Physicochemical indicators of almond milk are as follows: mechanical pollution – according to standard I class, fat content – 9.1 %, titratable acidity – 10.5 ^{0}T, density – 1020 kg/m^3. Accepted scientific research methods were used during the investigation.

The research was conducted to determine the optimal dosage of almond milk. In the next phase of the research, experiments were carried out to determine the optimal amount of almond flour addition (Table 3).

Conclusion

Taking into account the results of the scientific research, theoretical and experimental works, the following conclusions and recommendations were made. The composition of cow's milk is enriched with easily digestible carbohydrates, vitamins, and minerals. It has been theoretically substantiated and experimentally confirmed that the composition of dairy products is enriched with carbohydrates, vitamins, and minerals due to almond milk and flour. It is mainly useful for people with lactase enzyme deficiency, a fermentopathy characterized by the inability to decompose lactose. As a result of the research, it was discovered that with the addition of the optimal amount of almond milk and flour, the experimental drinkable lactic acid product acquires a unique taste, smell, homogeneous fine consistency, and high digestibility. Taking into account the above, we can say that the use of vegetable raw materials allows for expanding the assortment, improving the quality of the product, and using it in therapeutic prophylactic food. The use of almonds in the dairy industry contributes to the increase of nutritional and biological value, obtaining a product with appropriate physicochemical indicators and a relatively long shelf life.

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Investigating the Impact of Yeasts Additives on the Quality of the Red Wines: Autolyzes of Yeast and Yeast Derivatives Introduced During Alcoholic Fermentation

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ABSTRACT

The second part of the article series is about the study of the influence of yeast and autolyzed yeast derivative on red wines' qualitative and quantitative characteristics. Wine samples in which the preparation process was explained in the first part of the article were investigated. Wine coloring and phenolic compounds were measured. Organoleptic properties of the wines are presented as spider graphs with tasting notes.

Introduction

One of the main quality parameters of red wine is color and phenolic compounds. As mentioned in the study's first article (Kazumyan and Mikaelyan, 2022), Armenian grape varieties have been chosen: Tigrani, Karmrahyut, and Charentsi which are selective grapes with red-colored pulp (juice). These grape verities have been slightly investigated and our research will be useful for many Armenian winemaking companies. Winemakers use these verities to enhance the color and taste of red wines. Many winemaking companies refuse to use the Karmratut grape because it has unstable color and coloring matter sediment. Phenolic compounds in red wines also influence the wines' quality, color stability, and aging potential.

Materials and methods

The OIV and EAEU GOST methods were used to assess wine physicochemical indicators. Phenolic compounds are measured using the photocolorimetry method (Fracassetti and Gabrielli, 2017; Anikina, 2019; Gerzhikova, 2009; Jacobson, 2006; Ljiljana, 2016; Marnie, 2013; Moreno, 2012).

Armenian wine industry experts at the EVN Yerevan Wine Academy at Armenian National Agrarian University evaluated the organoleptic characteristics of the wines at the tasting room of the EVN Yerevan Wine Academy in collaboration with www.winenet.am/en/, the online resource.

Results and discussion

The corresponding spider diagrams (Aroma wheels) were drawn up based on the tasting results. The sensory analysis of wine quality involves assessing how the wine tastes, smells and looks (Marnie, 2013). The fermentation and aging processes first determine a wine's physicochemical composition. According to the matrix principle, sensory evaluation was conducted to determine the relationship between organoleptic assessment and physicochemical indicators of young wine composition. A total of 11 indicators were used to assess aroma perception, five indicators to assess taste perception, and four indicators to assess general perception. Based on all the information obtained, the spider graph of the wine-tasting matrix reflects the aroma wheels (Figure 1 and Table 1). It can be seen that Karmrahyut wine has the strongest flower smell at 3.2, Tigrani wine has the strongest smell of red and black fruits inherent in red wines in general, Karmrahyut wine has a spicy smell at 2,3, yeast smell is about the same as 1.7-1.8; the oak smell is lower in Karmrahyut wine, at 1.4.

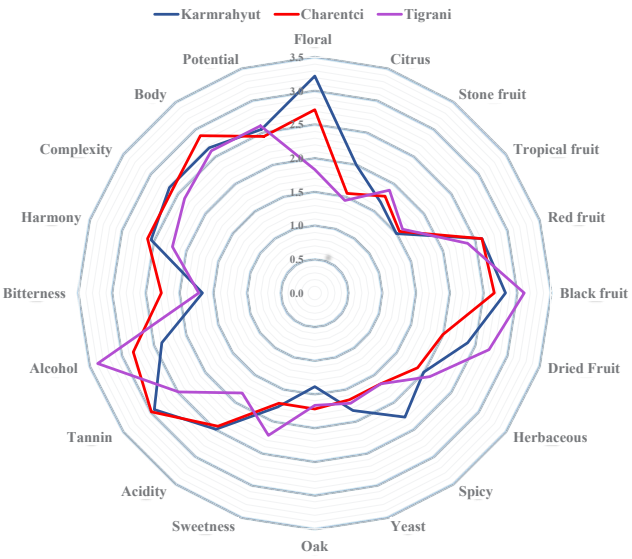

Figure 1. An aroma wheel and tasting graph for wines made from Tigrani, Karmrahyut, and Charentsi grape varieties (*composed by the authors*).

The characteristic of taste according to the results of tasting is distributed sweet, most pronounced in Tigrani wine – 2.2. The yeast race used has the property of increased accumulation of glycerol (up to 8 g/l); acidic taste prevails in Karmrahyut and Charentsi wines – 2.5-2.4; tannin taste is expressed in all samples and prevails in Charentsi wine 3.0 (Vidal et al., 2004); the taste of alcohol is expressed in Tigrani wine – 3.4; the taste of bitterness manifests itself in Charentsi wine – 2.3.

The general impression of the harmony taste, according to the characteristics of Tigrani (2.2) is noted below; against 2.6 in Karmrahyut and Charentsi wines, in terms of complexity the Tigrani is inferior to 2.4; in Karmrahyut and Charentsi, respectively 2.7 and 2.6.

Table 1. The wine-tasting results are drawn from Tigrani, Karmrahyut and Charentsi grapes*

Odour							
	Karmrahyut	**Charentsi**	**Tigrani**		**Karmrahyut**	**Charentsi**	**Tigrani**
Floral	3.2	2.7	1.8	Dried Fruit	2.4	2.0	2.7
Citrus	2.0	1.6	1.4	Herbaceous	2.0	1.9	2.1
Stone fruit	1.7	1.8	1.9	Spicy	2.3	1.7	1.7
Tropical fruit	1.5	1.6	1.6	Yeast	1.8	1.7	1.7
Red fruit	2.6	2.6	2.4	Oak	1.4	1.7	1.7
Black fruit	2.8	2.7	3.1				
Taste				**Overall Impression**			
Sweetness	1.8	1.7	2.2	Harmony	2.6	2.6	2.2
Acidity	2.5	2.4	1.8	Complexity	2.7	2.6	2.4
Tannin	2.9	3.0	2.5	Body	2.7	2.9	2.6
Alcohol	2.4	2.8	3.4	Potential	2.6	2.4	2.6
Bitterness	1.7	2.3	1.7	max = 5			

*Composed by the authors.

According to the characteristics of the body of the wine, the prevailing wine sample is Charentsi 2.9. The development potential is estimated at 2.6 for Tigrani and Karmrahyut wines and 2.4 for Charentsi wine.

Phenolic compounds play a significant role in plant metabolism. Grapes and wine contain phenolic compounds and their balanced complex determines red wine quality. Grape compounds, especially flavonoids, are significant in high-quality red wines. Flavonoids are mainly concentrated in the solid parts of the grape (Ribereau-Gayon, et al., 2006; Ribereau-Gayon, et al., 2006).

Processing of grapes and the subsequent alcoholic fermentation: after maceration of the grape pomace, the must is enriched with these valuable substances from the solid parts of the grape. According to the degree of oxidation, flavonoids are divided into groups. The mutual transformation of one group into another is possible through oxidation and reduction processes.

Tannins or proanthocyanidins (polymers of flavan-3-ol) are the most common phenols in grapes. Red wines are astringent due to tannins extracted from the skin and the soft parenchyma of the seed (Vidal, 2004; Yakimchenko, 2012). The diffusion coefficient, i.e. the rate at which tannin is extracted into fermenting must, depends on the size of the tannin molecule. Grapes contain monomers, dimers, trimers, oligomers, and polymers. The degree of polymerization reaches significant levels, and the molecular weight is up to 3500. These are catechin (condensed) tannins. Seed tannins consist of catechin and epicatechin. The degree of their polymerization is 10 units. Tannins from skins and seeds are extracted at different speeds. Extraction from the seeds is more intensive, easier, and in greater quantities than from the skins (Ribereau-Gayon, 2006). During alcoholic fermentation, tannin accumulation peaks on the 7-10th day (Kazumov, 2013). Tannins are easily dissolved in alcohol. Therefore, during alcoholic fermentation with the accumulation of ethyl alcohol, the dissolving ability must increase tannins (Yanniotis, 2007). After fermentation of sugars and enzymatic transformations, tannins polymerize, yielding macromolecules of proteins and polysaccharides. This forms complex compounds that become colloidal and precipitate. The tannins of grape skin and seeds have different effects on the wine taste (Busse-Valverde, 2011). Seed tannins define the wine's structure and body, while skin tannins make the wine soft and smooth (Marnie, 2013).

Catechins, tannins, and anthocyanins are the most concentrated natural antioxidants (Maleti, 2009). Tannins are the first oxidized substance of wine. Other compounds also can be involved in oxidative reactions (Mazza, 1995; Vidal, 2004; Yakimchenko, 2012).

Table 2. Tigrani, Karmrahyut, and Charentsi wine phenolic compounds and color composition*

№	Phenolic compounds				
	Parameters	**unit**	**Charentsi**	**Karmrahyut**	**Tigrani**
3.1.	Total Flavonoids	mg/l	2263.96	2261.88	1569.72
3.2.	Total anthocyanins	mg/l	368.73	508.45	658.30
3.3.	Total phenols (Gallic acid)	mg/l	2808.93	2740.71	2019.54
3.4.	Folin-Cicalteu Index		64	62	46
4.	Chromatic characteristics				
4.1.	A-420		0.37	0.35	0.45
4.2.	A-520		0.42	0.45	0.57
4.3.	A-620		0.11	0.12	0.19
4.4.	Color Intensity		8.96	9.17	12.19
4.5.	Color Shade		0.88	0.77	0.79
4.6.	Color composition, %				
4.6.1.	A-420 yellow	%	41.22	37.76	37.17
4.6.2.	A-520 red	%	46.79	49.00	46.99
4.6.3.	A-620 blue	%	13.63	15.25	18.82

*Composed by the authors.

Using the results of our tests shown in (Table 2), we determined the total amount of anthocyanins, flavonoids, and phenolic compounds in wine. These compounds are significant for evaluating tannin-anthocyanin reserves to prepare high-quality red wines.

The total amount of phenolic compounds is in the range of 2019.54 mg/l in Tigrani (the minimum amount), in Karmrahyut: 2740.71 mg/l, in Charentsi: 2808.93 mg/l (maximum amount). Total flavonoids – condensed phenolic substances recorded in samples: Tigrani – 1569.72 mg/l or 77.73 % (of the content); Karmrahyut – 2261.88 mg/l or 82.53 %; Charentsi – 2263.96 mg/l or 80.6%. Folin & Ciocalteu′s is an international index of total phenolic compound content in wines (International Organisation of Vine and Wine, 2021). The index is for Charentsi – 64, the highest and Tigrani – 46, the smallest.

A grape's anthocyanins determine the color of its future wine (Monagas, M. 2009; Wrolstad, 2005; Burns, 2002). Winemaking using the red method extracts the maximum amount of color pigments from the grape skin during the maceration process in the ongoing alcoholic fermentation and their subsequent preservation in wine (Ribereau-Gayon, 2006). Anthocyanins are polyphenols present in grapes and wine in the form of glycosides and diglycosides. Depending on the grape variety, the anthocyanin composition and total amount in wine differ. The total amount of anthocyanins was recorded as 368.73 mg/l in Charentsi, the lowest amount, 508.45 mg/l in Karmrahyut, and 658.30 mg/l in Tigrani, the highest.

Grapes contain anthocyanins, which cause wine's red color. Additionally, they are found in the thick epidermal layer of the skin. They absorb light and represent many colors ranging from red to blue (Anikina, 2012; Ribereau-Gayon, 2006). The color of young wine obtained through maceration depends on the minerals present in the wort. Anthocyanins (anthocyanidins) are associated with sugar molecules. Externally, anthocyanins are free in grapes and wine and can be linked by ester bonds with acetic and coumaric acids, with substituted hydroxyl and individual groups. The color and shade of anthocyanins are determined by their qualitative and quantitative states. The color composition in the samples was determined on a UNICO 2802 UV/VIS photoelectric colorimeter at 420, 520, and 620 nm, in a 1 mm thick cuvette (International Organisation of Vine and Wine, 2021; Heredia, 1998). As a result of the research, color intensity was obtained: the largest in Tigrani – 12.19; Karmrahyut – 9.17; the smallest in Charentsi – 8.96.

The highest color shade indicator was recorded in the Charentsi sample – 0.88, and in the Karmrahyut and Tigrani samples, respectively – 0.77 and 0.79.

There are three colors in the color composition, each determined by wavelengths of 420 nm (yellow), 520 nm (red), and 620 nm (blue). With over 65 % of the samples being red and blue, the Tigrani sample has the highest combination of these colors. As a result, the color intensity index is 12.19.

Conclusion

Based on the results of this study, we can conclude that the selected active dry yeast and yeast derivatives are beneficial for the production of red wine. Wines made from investigated grapes meet all quality requirements. Based on the tasting results, all wines display a body, typically red and black fruit aromas.

While the wine samples are in their middle stage of complexity and harmony, they were bottled in the spring of 2021 without being seasoned. The wine samples have excellent anthocyanins and the phenolic content is high. The highest total anthocyanins content was found in the Tigrani wine sample and the greatest total phenols were found in the Charenci wine sample.

About 50 % of the total wine color was determined in the Karmrahut wine sample. The aging potential of red wine is crucial for evaluating it. For reliable and valid research findings, this aspect must be assessed accurately, as well as yeast derivatives should be considered. Following a substantial period of bottle maturation, we plan to perform an aging potential analysis. Typically, this process takes several years.

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Assessing Health Risks Associated with Antibiotic Residues in Armenian Honey

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ABSTRACT

This study assessed potential risks associated with antibiotic residues in Armenian honey. Honey sample analysis revealed multiple antibiotics, in varying concentrations. Estimated daily intakes of antibiotics were calculated for different consumer clusters. The margin of exposure was determined based on acceptable daily intake values. The findings indicate that there are no risks to consumers regarding antibiotic residues in honey. However, it highlights the importance of controlling antibiotics in beekeeping practices to ensure honey safety.

Introduction

Natural honey has long been known for its nutritional value and health benefits. Honey possesses antimicrobial properties and antioxidant activity due to its rich content of polyphenols, enzymes, and a combination of high osmolarity and low pH (Almasaudi, 2021; Nolan, et al., 2019). Additionally, honey has an attractive taste and high energy value due to the rich mixture of sugars (Ashagrie Tafere, 2021).

Honey composition varies depending on factors such as geographical and botanical origin, honey bee health and species, as well as honey processing and storage methods (Almasaudi, 2021; Mama et al., 2019; Nolan et al., 2019). Armenian honey, in particular, is unique due to the mountainous terrain, and special plant composition, including endemic plants. Different authors have mentioned the high quality and beneficial properties of Armenian multi-floral honey (Belyaeva, et al., 2020; Pipoyan, et al., 2019), leading to its growing popularity in the international market. Notably, Armenia's natural honey export has experienced substantial growth, with exports reaching $126 thousand in 2020 and $3.28 million in 2021 (Trend Economy, 2022).

The mentioned characteristics and increasing demand for Armenian honey emphasize the importance of its safety. While honey is generally considered a nourishing and beneficial product, there are growing concerns regarding antibiotic residues (Lima, et al., 2020). Antibiotics in various fields of agriculture, including beekeeping practices for disease prevention and treatment, raise issues related to the potential impact on product safety. Excessive

or improper use of antibiotics can result in residues potentially posing risks to the population consuming contaminated food (Kumar, et al., 2020; Kim, et al., 2021; Ghimpețeanu, et al., 2022; Arsène, et al., 2020).

The global community is actively seeking optimal solutions to address the issue of substance residues in honey. It is also seeking to minimize the use of antibiotics along with the associated risks. There is a growing emphasis on regulatory measures to monitor and control antibiotic use in beekeeping. Governments and organizations are implementing guidelines and standards to prevent the occurrence of antibiotic residues in honey. Regular monitoring and testing of honey samples for residue detection play a crucial role in maintaining honey purity and consumer safety (Bonerba, et al., 2021; Lima, et al., 2020). The results of the annual residue analysis can provide comprehensive insights, enabling the identification and characterization of potential risks induced by antibiotic residues. Therefore, this study aims to assess the potential risks associated with antibiotic residues identified in Armenian honey within the framework of the national monitoring program.

Materials and methods

Consumption data collection and processing

The honey consumption data was collected via population survey conducted by the Informational-Analytical Center for Risk Assessment of the Food Chain, CENS in 2018. A Food Frequency Questionnaire (FFQ) was used, which included inquiries about portion size, consumption frequency, and demographic information. A total of 1040 residents from different districts of Yerevan, aged between 18 and 65 years, participated in the survey (Stepanyan, et al., 2022). The *FFQ* methodology ensured accurate data collection (Pipoyan, et al., 2020). The collected data were statistically analyzed using *SPSS* software (version 22.0). The cluster analysis method was employed to classify consumers into homogeneous clusters.

Analysis of samples

As part of the Armenian monitoring program on residues in animal-origin products, 32 multi-floral honey samples were collected in November 2019. These samples were obtained from various producers of natural multi-floral honey in Armenia, with each sample weighting between 0.5 and 1 kg. The samples were tested at the Republican Veterinary-Sanitary and Phytosanitary Laboratory Services Center. Honey samples were analyzed for the presence of residues of 10 antibiotics, namely dihydrostreptomycin (DHSTM), oxytetracycline (OTC), tetracycline (TC), sulfadiazine (SDZ), penicillin G (PenG), enrofloxacin (ENR), terramycin (TM), streptomycin (STM), sulfadimethoxine (SDMO), salinomycin (SM). The initial analyses were conducted using the ELISA method with MaxSignal ELISA Kits and a BioTek ELx800 analyzer. LC-MS/MS was used to analyze the primary screening results.

Risk assessment

The risks associated with antibiotic residues were assessed based on the Margin of exposure (*MOE*) approach using the following equation:

$$MOE = \frac{HBGV}{EDA}, \quad (1)$$

where *HBGV* is the toxicologically established health-based guidance value (mg/kg/day); and the *EDA* is the estimated daily intake of antibiotics through honey consumption (mg/kg/day).

The *EDI* of antibiotics was calculated for each cluster of honey consumers, using the following equation:

$$EDI = \frac{C_{honey} \times C_{antibiotic}}{BW}, \quad (2)$$

where C_{honey} is the mean daily intake (consumption) of honey (kg/day); $C_{antibiotic}$ is the mean content of antibiotic residue in food (mg/kg). In the case of content data on antibiotic residues below the Limit of Detection (*LOD*), commonly called "left-censored", the value of *LOD/2* was used. *BW* is the body weight of consumers, averaged at 65 kg per population survey. For *PenG*, the *EDI* was calculated by multiplying the content with the honey consumption, without dividing by the body weight (*BW*), since the acceptable daily intake, presented as *HBGV*, is expressed in "mg/person/day".

The worst-case scenario

Risk assessments commonly incorporate various scenarios, including the worst-case scenario, to ensure a comprehensive evaluation and avoid underestimating risks. In this study, the worst-case scenario refers to a hypothetical situation where all variables, such as residue and consumption data, are the highest when calculating the potential risk associated with antibiotic exposure. By considering this scenario, the study aims to evaluate the maximum possible risk. This provides valuable insights into the upper limit of risks associated with antibiotic exposure.

Results and discussions

Honey consumption

Through analysis of the survey data obtained from the Food Frequency Questionnaire (*FFQ*), three consumer clusters were identified based on their daily honey consumption. The first cluster, comprising 80 % of respondents, has an average daily consumption of 0.006 kg of honey. In contrast, the second cluster, representing 15 % of respondents, showed higher daily honey consumption at 0.028 kg/day. The third cluster, consisting of 5 % of respondents, exhibited the highest honey consumption daily, amounting to 0.059 kg/day.

Antibiotic residues in honey

Analyses showed that the studied honey samples did not contain residues of *DHSTM, OTC, ENR, TC*, and *SDMO* antibiotics. Therefore, exposure to these antibiotics and associated risks were not addressed in this research paper.

The study results revealed that out of the 32 honey samples analyzed, 8 tested positive for *TC*, 28 for *SDZ*, 31 for PenG, 32 for *STM*, and 6 for *SM*. Table 1 shows the minimum and maximum antibiotic concentrations as well as the calculated means and standard deviations (*SD*).

Table 1. Antibiotic residues in the studied honey samples*

Antibiotics	Antibiotic residues (µg/kg)			
	Min	Max	Mean	SD
TC	3.3	195.9	14.7	45.3
SDZ	4.7	40.9	10.5	8.1
PenG	1.9	4.5	3.2	0.7
STM	11.9	554.5	50.1	106.3
SM	1.2	1.4	0.8	0.3

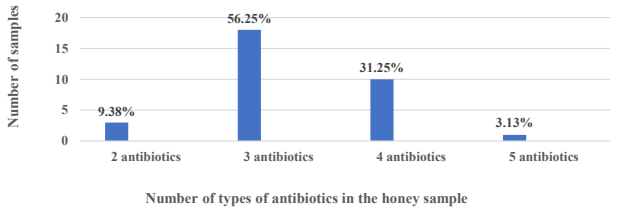

Figure. Number of types of antibiotics and percentage of honey samples (*composed by the authors*).

The honey samples analyzed contained varying numbers of antibiotic types (Fig.). Out of the 32 honey samples analyzed, none were "antibiotic-free". Each sample simultaneously contained residues of varying numbers of different antibiotics.

Approximately 9.38 % of the samples contained 2 different antibiotics *PenG* and *STM*. The residues of three different antibiotics were found in 56.25 % of the analyzed honey samples (18 samples), with 17 samples containing *PenG*, *STM*, and *SDZ*, and one sample containing *PenG, STM*, and *TC*. In 31.25 % of the samples (10 samples), four types of antibiotics were detected. The antibiotic composition varied among these samples, with five samples containing *TC, SDZ, PenG*, and *STM*, four samples containing *SDZ, PenG, STM,* and *SM,* and one sample containing *TC, PenG, STM,* and *SM*. Finally, all five detected antibiotics (*PenG, STM, SDZ, SM, and TC*) were present in only one sample.

Estimated daily intake (EDI) and Margin of exposure (MOE) of antibiotic residues

*EDI*s of antibiotics from honey for three consumer clusters are represented in Table 2.

Acceptable daily intake (*ADI*) values were taken as *HBGV* for the studied antibiotics. The estimated daily intake of all studied antibiotic residues was much lower than the established *ADI*s.

Table 2. The *EDI* of antibiotic residues through honey consumption, mg/kg/day*

Consumers	*TC*	*SDZ*	*PenG***	*STM*	*SM*
Cluster 1	1.36E-06	9.68E-07	1.90E-05	4.63E-06	7.70E-08
Cluster 2	6.33E-06	4.52E-06	8.86E-05	2.16E-05	3.59E-07
Cluster 3	1.33E-05	9.52E-06	1.87E-04	4.55E-05	7.57E-07

***is expressed in "mg/person/day"*

*Composed by the authors.

The *ADI* for TC is 0.03 mg/kg/day (JECFA, 1998). Due to its poor absorption from the intestinal tract, the toxic effects of *TC* on the body are relatively low. *ADI* is primarily associated with the risk of resistant bacterial strains (JECFA, 1998). The *ADI* for *SDZ* is 0.02 mg/kg bw/d (NRA, 2000). This *ADI* was derived by applying a safety factor of 2000 to the No-Observed-Adverse-Effect Level (NOAEL) of 37.5 mg/kg bw, which indicated fetotoxic effects in rats (NRA, 2000). The *ADI* for *PenG* is 0.03 mg/person/day (JECFA, 1990). The most common adverse effect of *PenG* is hypersensitivity reactions, but such cases are not associated with residual drug levels in food (WHO/JECFA). The *ADI* for *STM* is 0.05 mg/kg/day (JECFA, 2002). The *ADI* is obtained by applying a safety factor of 100 to NOAEL (5 mg/kg), which is based on the decreased body weight gain in rats in a two-year dietary study. And although these studies were performed with dihydrostreptomycin, the results apply to streptomycin due to their close relationship (NCBI-a). The *ADI* for *SM* is 0.01 mg/kg/day (APVMA, 2023). A study in human cell cultures showed the cytotoxic effect of *SM*. Scientific research and investigation of domestic cases with various animals revealed that high doses and long-term use of *SM* in animals have a neuropathic effect, can reduce the growth rate and reproductive functions of animals, and damage the skeletal and cardiac muscles. The toxicity of *SM* has not been extensively studied, but due to its identified antitumor activity, it is the subject of research (NCBI-b). Since the studied substances are medicines, they have undergone numerous studies and tests, as a result of which hazard categories have been identified for each drug. We referred to data from the European Chemicals Agency (ECHA) and the National Center for Biotechnology Information (NCBI-c) to compare antibiotic hazard statements (Table 3).

The statements listed in Table (3), provide information about the potential hazards associated with each antibiotic. SDZ appears to have the highest number of hazard statements among the studied antibiotics. The hazard statements for antibiotics include two types: health hazard (H3--) and environmental hazard (H4--). While *TC* and *SDZ* both have these hazard classifications, *SDZ* has a more significant number of them. In general, the most common hazard classes are related to ingestion, dermal effects, and reproductive toxicity. It is important to note that antibiotic *ADI*s may not always be directly associated with reported hazards, as these hazards are often linked to long-term therapeutic doses or overdoses. However, it should be recognized that these substances can have negative effects on the body. Furthermore, individual consumers may vary in terms of their metabolism and sensitivity to specific substances, potentially experiencing adverse effects at lower doses.

Table 3. Hazard statements for antibiotics*

GHS** Code	Hazard Statements	Antibiotics				
		TC	*SDZ*	*PenG*	*STM*	*SM*
H300	Fatal if swallowed					+
H301	Toxic if swallowed	+				
H302	Harmful if swallowed	+	+	+		
H312	Harmful in contact with skin			+		
H315	Causes skin irritation	+	+	+		
H317	May cause an allergic skin reaction		+	+		
H319	Causes serious eye irritation		+	+		
H332	Harmful if inhaled			+		
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled		+	+		
H335	May cause respiratory irritation		+	+		
H341	Suspected of causing genetic defects		+			
H351	Suspected of causing cancer				+	
H361	Suspected of damaging fertility or the unborn child				+	
H361(d)	Suspected of damaging the unborn child	+	+			
H362	May cause harm to breast-fed children	+	+			
H400	Very toxic to aquatic life		+			
H410	Very toxic to aquatic life with long-lasting effects		+			
H411	Toxic to aquatic life with long-lasting effects	+	+			
H412	Harmful to aquatic life with long-lasting effects		+			

*Note: GHS** - Globally Harmonized System of Classification and Labeling of Chemicals*

*Composed by the authors.

Table 4. *MOE* of antibiotic residues*

Honey consumption	*TC*	*SDZ*	*PenG*	*STM*	*SM*
Cluster 1	$2.21*10^4$	$2.07*10^4$	$1.58*10^3$	$1.08*10^4$	$1.3*10^5$
Cluster 2	$4.74*10^3$	$4.43*10^3$	$3.39*10^2$	$2.32*10^3$	$2.78*10^4$
Cluster 3	$2.25*10^3$	$2.10*10^3$	$1.61*10^2$	$1.1*10^3$	$1.32*10^4$

*Composed by the authors.

We calculated the Margin of Exposure (*MOE*) to antibiotics (Table 4) in honey for the three consumer clusters using the above-mentioned *ADI*s. While Table 3 suggests the presence of suspected genotoxicity in *SDZ* and carcinogenicity in *STM*, it is important to note that there is currently no definitive or confirmed data available regarding these specific risks. Moreover, the *ADI*s of all studied antibiotics are not associated with genotoxic or carcinogenic risks. Therefore, the threshold for risk assessment is set at $MOE \geq 10^2$. If the *MOE* is less than 10^2, it indicates a potential risk to consumers (Chem Safety PRO, 2018; Scientific Committee, 2019).

The *MOE* values for the studied antibiotics in the different consumer clusters ranged from $1.61*10^2$ for *PenG* in the third consumer cluster to $1.3*10^5$ for *SM* in the first consumer cluster. The *MOE* of *PenG* in the third consumer cluster is the closest value to 10^2. It is worth noting that none of the *MOE* values is lower than 10^2. This indicates that there is no potential risk associated with the studied antibiotics for consumers.

The worst-case scenario

For the worst-case scenario, the highest value of each antibiotic residue and the consumption data from the third consumer cluster were used to calculate the Estimated Daily Intake (*EDI*) and Margin of Exposure (*MOE)* values (Table 5). The results from the worst-case scenario analysis indicated that all *MOE* values remained above 10^2, suggesting no significant risk associated with the studied antibiotics. However, it should be noted that the *MOE* value for *STM* is slightly below 10^2. Hence, it is plausible to assume that an increase in honey consumption and/or the presence of antibiotic residues could potentially raise concerns regarding consumer health.

Table 5. EDI and MOE in the worst-case scenario*

Antibiotic	Max residue (mg/kg)	*EDI* (mg/kg/day)	*MOE*
TC	0.1959	$1.78*10^{-4}$	$1.69*10^2$
SDZ	0.0409	$3.71*10^{-5}$	$5.39*10^2$
PenG	0.0045	$2.66*10^{-4}$●	$1.13*10^2$
STM	0.5545	$5.03*10^{-4}$	99.3
SM	0.0014	$1.27*10^{-6}$	$7.87*10^3$

Note: ●*– mg/person/day*

*Composed by the authors.

Conclusion

The growing demand for Armenian honey emphasizes the critical importance of its safety, particularly regarding antibiotic residues. The absence of some studied antibiotics in the honey samples indicates a favorable outcome in terms of consumer safety. However, the presence of multiple antibiotic types in some samples raises concerns about their potential impact on consumer health. The study findings highlight the importance of antibiotic usage control in beekeeping practices to ensure the safety of honey products.

Overall, the MOE values, which were assessed for each antibiotic in different consumer clusters, exceeded the threshold of 10^2. This indicates a low likelihood of adverse effects. Nevertheless, it is crucial to consider the worst-case scenario, where increased honey consumption and/or higher levels of antibiotic residues could raise concerns regarding consumer health. Moreover, the potential long-term effects and uncertainties associated with certain antibiotics, such as suspected genotoxicity and carcinogenicity, should be acknowledged.

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Preparation of Functional Cookies with Red Grape Pomace Bio-Dust

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ABSTRACT

The influence of secondary raw materials in primary winemaking on the organoleptic and physico-chemical parameters of butter cookies made of wheat and oat flour has been studied. It was revealed that cookies with this additive have appropriate consumer properties and physicochemical parameters that meet accepted standards. It was shown that the processed product was enriched with the elements necessary for the body. These elements include *Na, C, Mg, Ca, P, Fe, Zn,* and *Cu*, and the powerful antioxidant resveratrol. Obtaining food additives by processing grape pomace and their use in confectionery products will contribute to primary winemaking with minimal waste.

Introduction

Studies of secondary raw materials for winemaking began in the 80s of the last century (Kondratiev, 2009). The secondary raw material of wine production is about 40 % of processed grapes (Tagirova and Kasyanov, 2015), and according to A. N. Tikhonova makes 20 % (Tikhonov, 2017), out of which 8.0-17.0 % is fermented pomace (Tikhonova, 2015). For the first time, in 1999 in the Theodosia region juice was removed from dark grapes and a dark dispersion mass was produced from pomace bio-dust, which contains resveratrol, vitamins *A* and *E*, and fiber (Tikhonov, 2015; Sedrakyan, 2017). Grape bunch, peel/skin, seeds and remaining juice are included in the grape pomace (Kondratev, 2007). The red grape peel contains twice more polyphenols than the white grape. The red grape peel contains anthocyanins, and antioxidants, including resveratrol, vitamin, dihydrocretin, and trace elements; due to the high content of copper and cobalt salts, they have a positive effect on metabolic processes, and the extract provides antioxidant protection of the cell. Resveratrol is found as well as in blueberries, peanuts, cranberries, and Sakhalin's gorse, with large amounts – in black mulberries (Tagirova and Kasyanov, 2015). For the first time, resveratrol was written about in 1939 by Japanese researcher M. Takaoka noting that it is found in the peel of red grapes, and therefore in red wine. This substance accounts for the positive effect of red wine on blood vessels and low cholesterol levels. There is also an opinion that a large amount of these substances can protect a person from obesity and diabetes (www.mfarm.ru). Many researchers suggest that moderate consumption of red wine reduces the risk of developing cardiovascular disease. This phenomenon is known as the "French paradox".

It has been found out that resveratrol helps treat certain types of cancer, as it kills up to 65 % of melanoma cells when used with radiotherapy. The cardioprotective, anti-inflammatory, immunomodulatory, antitumor, antimicrobial, and neuroprotective effects of resveratrol are currently confirmed. The obtained data make it possible to select resveratrol as a promising therapeutic agent (Moiseeva, et al., 2012). The research aims to increase the efficiency of wine production through the processing of red grape pomaces as secondary raw materials for winemaking, producing food additives, and using them in the production of cookies, which will increase the usefulness of cookies and contribute to the development of waste-free production in primary winemaking.

Materials and methods

The study objects are bio-dust processed from grape pomaces produced in the wine industry from the red grape varieties of "Tigrani" and "Kakhet" common in the winemaking sector of the Republic of Armenia, 9 samples of wheat flour cookies with biological additives (Table 1) and 5 samples of oat flour cookies (Table 2). The following raw materials were used in the cookie production: "Baghramyan" wheat high-grade baking flour – GOST 26574-2017, oatmeal – GOST 31945-2012, white sugar – GOST 33222-2011, "NAZ" margarine – GOST 32188-2013, sodium bicarbonate – GOST 32802-20147, ammonium carbonate – GOST R 55580-2013, table salt – AST 239-2005, drinking water – SanPin N2-III-A 2-1-02, "Kakhet" and "Tigrani" grape fermented pomaces bio-dusts produced via processing by convective drying with the technology developed by A. Sedrakyan (Sedrakyan, 2017). "Kakhet" and "Tigrani" are late-ripening Armenian grape varieties (www.files.stroyinf.ru; www.vinograd.info). Sensory indicators of baked butter cookies were assessed according to GOST 5897-90. The tasting committee has evaluated the quality of cookies with different contents of red grape wine fermented pomaces bio-dust with a 10-point grading system developed by our research group, per the sensory indicators normalized by the standard. Following the physicochemical alanysis, the indicators were normalized and not normalized. Based on the evaluation data provided by the tasting committee, the cookies with the highest score were selected for further examination. The quality indicators of cookies were determined in the laboratories of YSU (Yerevan State University) and the "National Institute of Health Named after Academician S.Kh. Avdalbekyan" CJSC. Per the physicochemical indicators of cookies, the mass fraction of moisture was measured according to GOST 5900-2014, the wetness index – according to GOST 10114-8013, the basicity – according to GOST 5898-8714, the mass fraction of total sugar – according to GOST 5903-8916, and the mass fraction of ash – according to GOST R 51411-99 (ISO 2171-93). The chemical elements were determined through flame photometric, voltammetry, and photo colorimetric methods, and the resveratrol content was measured by high-performance liquid chromatography at the Institute of Horticulture, Viticulture and Winemaking of Georgia (www.pubmed.ncbi.nlm.nih.gov).

Results and discussions

The preparation of functional cookies includes the following technological processes: raw material preparation, dough making, dough formation, baking, cooling, packaging, labeling, and storage.

In the first stage of dough making the fat mass is prepared, in case of which margarine is mixed with white sugar with the help of a mixer.

In the second stage sifted wheat flour (or oat flour) is taken separately, to which the bio-dust of fermented grape pomace was added in different amounts: 2; 3; 5; 10 %, and in amounts of 2; 3; 5; 10; 20 % (by weight of flour) to obtain functional cookies of different compositions; sodium bicarbonate, ammonium carbonate, and table salt were then added and mixed for 1-2 minutes until all ingredients were evenly mixed.

In the third stage, the flour mass was added to the whipped fat mass and mixed for 6-8 minutes until a crumbly dough was obtained so as it should not stick to the hands. Balls are made from the dough, which is opened in the form of a layer of 2-3 mm thickness, shaped by cutting into a shape, and placed in the oven of the stove. The baking process was conducted at 180-190 ^{0}C for 17-18 minutes, thereafter cooling of the finished baked cookies was implemented to a temperature of 22-25 ^{0}C. Cookies without a grape pomace bio-dust were taken as control samples. The evaluations of the tasting committee are presented in Table 1.

As a result of the sensory analysis of functional cookies with biodust of wine pomace from "Tigrani" and "Kakhet" varieties (Table 1), the maximum score was given to the cookies containing 3 % bio-dust (sample N 3 and sample N 7). Samples N 3 and N 7 were selected for further testing. The low scores of samples N 4, N 5, N 8, and N 9 containing red wine pomace bio-dust are due to the low indicator in the color score.

To exclude the significant effect of bio-dust, especially on the color index, wheat flour was replaced with oat flour. The data are presented in Table 2.

As a result of the average scores of the tasting committee, the highest score – 9.5 points – was given to the cookies containing 10 % red grape pomace bio-dust.

Table 1. Scoring the sensory analyses of functional cookies made from wheat flour with wine pomace bio-dust produced from red grape varieties "Tigrani" and "Kakhet"*

Cookie samples	Scoring per sensory analysis, points				
Sensory analyses	**appearance**	**color**	**taste and smell**	**appearance in a fracture**	**total**
Maximum score, points	**1.0**	**3.0**	**5.0**	**1.0**	**10.0**
"Tigrani" with grape pomace bio-dust					
Control Sample - N 1	1.0	3.0	4.0	0.7	8.7
Sample N 2 - 2% bio-dust	0.8	2.8	4.4	0.7	8.7
Sample N 3 - 3% bio-dust	0.8	2.8	5.0	0.8	9.4
Sample N 4 - 5% bio-dust	0.8	1.0	5.0	0.5	7.3
Sample N 5 - 10 % bio-dust	0.8	0.7	4.9	0.3	6.7
"Kakhet" with grape pomace bio-dust					
Sample N 6 - 2 % bio-dust	0.8	2.6	4.7	0.6	8.7
Sample N 7 - 3 % bio-dust	0.8	2.8	5.0	0.7	9.3
Sample N 8 - 5 % bio-dust	0.8	1.3	4.9	0.6	7.6
Sample N 9 - 10 % bio-dust	0.8	0.9	4.8	0.4	6.9

Table 2. Scoring functional cookies made from oat flour with grape variety "Tigrani" pomace bio-dust*

Sensory analyses	Cookie samples, scoring points					
	maximum score, points	**control sample N 1**	**sample N 2 3 % bio-dust**	**sample N 3 5 % bio-dust**	**sample N 4 10 % bio-dust**	**sample N 5 20 % bio-dust**
Appearance	1.0	0.8	0.8	1.0	1.0	0.7
Color	3.0	3.0	3.0	2.7	2.7	2.5
Taste and smell	5.0	4.3	4.4	4.5	4.8	4.5
Appearance in a fracture	1.0	0.8	0.8	1.0	1.0	0.3
Total	10.0	8.9	9.0	9.2	9.5	8.0

*Composed by the authors.

Table 3. Chemical composition of wheat and oat flour cookies prepared with red grape pomace bio-dust*

Index Name	Number of the indicator testing method	Index value				
		bio-dust content of red grapes with wheat flour			bio-dust content of red grapes with oat flour	
		control sample N 1	sample N 3 "Tigrani"	sample N 7 "Kakhet"	control Sample N 1	sample N 10 "Tigrani"
Mass fraction of moisture, %	GOST 5900-2014	15	15	15	15	15
Mass fraction of protein, %	GOST 26889-96	7.77	7.41	8.10	7.10	7.20
Mass fraction of carbohydrates, %	GOST 5903-89	58.6	59.89	59.82	58.3	58.5
Mass fraction of total sugars, %	GOST 5903-89	21.94	22.10	21.50	21.90	22.15
Mass fraction of oil, %	GOST 31902-89	18.0	17.9	16.5	18.3	18.1
Mass fraction of total ash, %	GOST 5901-99	0.614	0.632	0.585	1.30	1.45
Mass fraction of 10 % insoluble HCL ash, %	GOST 5901-99	0.04	0.05	0.05	0.07	0.09

Table 4. Chemical composition of functional cookies made from wheat and oat flour with grape variety "Tigrani" pomace bio-dust*

Index Name	Number of the indicator testing method	Measuring unit	Index value			
			wheat flour		oat flour	
			control	3.0 % bio-dust	control	10 % bio-dust
Potassium	GOST 30504-97	mg/%	113.0	115.6	182.0	195.4
Sodium	GOST13496.1-98	mg /%	4.2	4.3	10	12
Calcium	GOST 26570-95	mg /%	8.0	8.5	22.0	23.1
Magnesium	GOST 26570-95	mg /%	11.0	13.2	74.5	75.8
Phosphorus	GOST 26657-97	mg /%	156.0	166.0	201	212
Iron	GOST 26928-86	mg /%	1.20	1.30	1.80	2.02
Zinc	GOST 51301-99	mg /%	0.78	0.80	1.30	1.85
Copper	GOST 51301-99	mg /%	0.26	0.29	0.20	0.25
Total resveratrol	HPLC/ 9/	mg /kg	0.00	0.03	0.00	0.08

*Composed by the authors.

As a result of the examination it was disclosed, that normative indicators of butter cookies were defined by GOST 24901-2014 (GOST 24901-2014. Cookie. General Specifications), i.e., mass fractions of moisture, oil, ash content insoluble in 10 % *HCl*, humidity, and solidity meet the requirements of the standard. The chemical composition of the wheat and oat flour cookies was determined through the physicochemical analysis: the total and 10 % mass fractions of carbohydrates, sucrose, and oil, chemical elements, and the amount of resveratrol were determined (Tables 3, 4).

The data in Table 3 show that the cookies have a high nutritional value.

It can be seen from Table 4 that the functional cookies made from wheat, and oat flour with the bio-dust of the fermented pomace of "Tigrani" grape variety were enriched with the elements *Na, K, Mg, Ca, P, Fe, Zn, Cu,* and antioxidant resveratrol required for the human body.

Conclusion

It is necessary to produce pomace bio-dust from the secondary raw materials generated in primary winemaking and use it in the production of cookies to enrich them with mineral elements: *Na, K, Mg, Ca, P, Fe, Zn, Cu,* and antioxidant resveratrol, using red grape pomace bio-dust with 3 % in wheat flour cookies and 10 % – in oat flour cookies. Wine production efficiency will surely increase by producing food additives from winemaking secondary raw materials and using them in confectionery production.

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ՑՈՒՑՈՒՄՆԵՐ ՀԵՂԻՆԱԿԻՆ УКАЗАНИЯ АВТОРУ AUTHOR GUIDELINES

ՊԱՐԲԵՐԱԿԱՆԸ ՆԵՐԱՌՎԱԾ Է ԴՈԿՏՈՐԱԿԱՆ ԵՎ ԹԵԿՆԱԾՈՒԱԿԱՆ ԱՏԵՆԱԽՈՍՈՒԹՅՈՒՆՆԵՐԻ ԱՐԴՅՈՒՆՔՆԵՐԻ ԵՎ ԴՐՈՒՅԹՆԵՐԻ ՀՐԱՊԱՐԱԿՄԱՆ ՀԱՄԱՐ ՀՀ ԿԳՄՍՆ ԲՈԿ-Ի ԿՈՂՄԻՑ ԸՆԴՈՒՆԵԼԻ ԳԻՏԱԿԱՆ ՀԱՆԴԵՍՆԵՐԻ ՑԱՆԿՈՒՄ:

ИЗДАНИЕ ВКЛЮЧЕНО В ПЕРЕЧЕНЬ ВЕДУЩИХ НАУЧНЫХ ЖУРНАЛОВ ВАК МНОКС РА, В КОТОРЫХ ДОЛЖНЫ БЫТЬ ОПУБЛИКОВАНЫ ОСНОВНЫЕ РЕЗУЛЬТАТЫ И ПОЛОЖЕНИЯ ДИССЕРТАЦИЙ НА СОИСКАНИЕ УЧЕНОЙ СТЕПЕНИ ДОКТОРА И КАНДИДАТА НАУК.

THE JOURNAL IS INVOLVED IN THE LIST OF SCIENTIFIC PERIODICALS RELEVANT FOR PUBLICATIONS OF THE RESULTS AND PROVISIONS OF DOCTORAL AND PHD THESES AND APPROVED BY THE HIGHER EDUCATION QUALIFICATION COMMITTEE OF THE RA MoESCS.

ՀՈԴՎԱԾՆԵՐԻ ԸՆԴՈՒՆՄԱՆ ԿԱՐԳԸ

1. Հոդվածներն ընդունվում են հայերեն, ռուսերեն և անգլերեն լեզուներով:
2. Հոդվածի առավելագույն ծավալը չպետք է գերազանցի 10 համակարգչային էջը (ներառյալ ամփոփագրերը):
3. Հեղինակների թիվը չպետք է գերազանցի չորսը:
4. Հեղինակների տվյալներում պետք է ներառվեն հեղինակ(ներ)ի անունը, ազգանունը, հայրանունը, գիտական աստիճանը, աշխատավայրը, էլ. հասցեն:
5. Հոդվածը ներկայացվում է տպագիր և էլեկտրոնային (WORD ձևաչափով) տարբերակներով:
6. **Հոդվածը շարադրվում է հետևյալ կառուցվածքով.** վերնագիր, 5 բանալի բառ, «Նախաբան», «Նյութը և մեթոդները», «Արդյունքները և վերլուծությունը», «Եզրակացություն», «Գրականություն»:
7. Գրականության հղումները կատարվում են տեքստում՝ փակագծում նշվում են հեղինակը և հրատարակման տարեթիվը:
8. Հոդվածները պետք է ունենան ամփոփագրեր. հայերենով և ռուսերենով ներկայացված հոդվածների դեպքում՝ հայերեն (50-60 բառ), ռուսերեն (50-60 բառ) և անգլերեն (150-250 բառ), անգլերենի դեպքում՝ անգլերեն լեզվով (50-60 բառ):
9. Հայերեն և ռուսերեն հոդվածների վերնագրերը, հեղինակ(ներ)ի տվյալները և բանալի բառերը ներկայացվում են հայերեն, ռուսերեն և անգլերեն լեզուներով:
10. Գրականության ցանկը ներկայացվում է այբբենական կարգով:
11. Էլեկտրոնային հղումը որպես աղբյուր մեջբերելիս գրականության ցանկում նշվում է դիտման ամսաթիվը:

Հոդվածներին ներկայացվող տեխնիկական պահանջներն են. անգլերեն և ռուսերեն հոդվածների տառատեսակը՝ Times New Roman, հայերեն հոդվածներինը՝ GHEA Grapalat, տառաչափը՝ 12, միջտողային տարածությունը՝ 1.5, վերնագիրը՝ մեծատառերով, գծապատկերները՝ Word, Excel ծրագրերով, աղյուսակները՝ ուղղահայաց դիրքով (Portrait), բանաձևերը՝ Microsoft Equation 3.0 ձևաչափով:

Կարգին չհամապատասխանող հոդվածները չեն ընդունվում: Հոդվածներն ուղարկվում են գրախոսման: Մերժված հոդվածները չեն վերադարձվում հեղինակին: Հոդվածները չեն հրատարակվի, եթե ամբողջությամբ կամ համառոտ տպագրված լինեն այլ պարբերականում:

ПОРЯДОК ПРИЁМА СТАТЕЙ

1. Статьи принимаются на армянском, русском и английском языках.
2. Объём статьи не должен превышать 10 компьютерных страниц (включая аннотации).
3. Число авторов не должно превышать четырёх.
4. В сведениях об авторах должны быть включены имя (имена), фамилия, отчество, научная степень, место работы, эл.адрес.
5. Статья представляется в печатном и электронном (в формате WORD) вариантах.
6. **Статья должна быть изложена следующим образом:** заглавие, 5 ключевых слов, “Введение”, “Материал и методы”, “Результаты и анализ”, “Заключение”, “Литература”.
7. Ссылки на литературу производятся в тексте с указанием в скобках автора и год издания.
8. Статьи должны иметь аннотации: статьи, представленные на армянском и русском языках – на армянском (не менее 60 слов), на русском (не менее 60 слов) и на английском (150-250 слов). В случае статей, написанных на английском, аннотация должна быть на английском языке (не менее 60 слов).
9. Объём представленных аннотаций на каждом языке не должен превышать 600 знаков (без пробелов).
10. Заглавия, данные автора (авторов) и ключевые слова статей на армянском и русском языках представляются на армянском, русском и английском языках.
11. Список литературы представляется в алфавитном порядке, сначала на языке статьи, затем на иностранном языке.
12. При ссылке в статье на интернет-ресурс как источник информации, в списке литературы необходимо отметить дату просмотра.

Технические требования к статьям: для статей на английском и русском языках – шрифт Times New Roman, для армянского – GHEA Grapalat; размер букв – 12; межстрочное расстояние – 1.5; заголовок – прописными буквами; графические изображения – программой Word, Excel; таблицы – вертикально (Portrait); формулы – в формате Microsoft Equation 3.0;

Статьи, не отвечающие требованиям, не будут приняты. Статьи передаются на рецензирование. Статьи, не принятые к печати, не возвращаются автору. Статьи не будут опубликованы, если ранее были полностью или частично опубликованы в других периодических изданиях.

THE STANDARDS FOR SUBMITTING ARTICLES

1. The articles are accepted in Armenian, Russian and English languages.
2. The size of the article shouldn’t exceed 10 PC pages (including summaries).
3. The number of authors should not exceed four.
4. Full name, academic degree, workplace and e-mail of the author (s) should be included in the information about the authors.
5. The article is submitted in a hard copy and electronically (WORD format).
6. **The article should have the following structure:** title, 5 keywords, “Introduction”, “Materials and Methods”, “Results and Discussions”, “Conclusion”, “References”.
7. References to the literature should be indicated in the text (the author and the date of publication in the parentheses).
8. The articles should have abstracts: in case of Armenian and Russian articles, abstracts in Armenian (minimum 60 words), Russian (minimum 60 words) and English (150-250 words) languages should be submitted, while in case of English articles, abstracts in English (minimum 60 words) language should be submitted.
9. The volume of the abstracts presented in each language should not exceed 600 characters (no spaces).
10. The titles, information about the author(s) and keywords should be presented in Armenian, Russian and English languages.
11. The list of references should be arranged in alphabetical order.
12. When citing internet links as a literature source the date of access should be mentioned.

Technical requirements for articles: font for English and Russian articles: Times New Roman, for Armenian articles: GHEA Grapalat, font size: 12, interstitial spacing: 1.5, title: with capital letters, charts: with Word, Excel, tables: vertical (Portrait), formulas: in Microsoft Equation 3.0 format.

Articles that do not meet the requirements are not accepted. Articles are sent for review. Refused articles are not returned to the authors. The articles which are already published in other scientific journals (completely or partially) can’t be valid for publication in our journal.

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