JOURNAL OF SCIENTIFIC PERSPECTIVES

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JOURNAL OF SCIENTIFIC PERSPECTIVES JSP

ABOUT THE JOURNAL

Journal of Scientific Perspectives (JSP) is a **scholarly** and **international peer-reviewed journal**. It is published quarterly in *January, April, July* and *October,* in the fields of **basic sciences, engineering, natural sciences** and **health sciences**. All articles submitted for publication are evaluated by the editor-in-chief, field editor, editorial board and referees. The original research papers, technical notes, letter to the editor, debates, case presentations and reviews, only in *English,* are published in the journal. Thus, it aims to bring together the views and studies of academicians, researchers and professionals working in the fields mentioned above.

Journal of Scientific Perspectives is an *open access electronic journal*. All articles published in the journal, are assigned the *DOI number*. Article submission and follow-up process are carried out by the manuscript submission system.

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- Forestry
- Marine, Aquatic Sciences and Fisheries
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There are no limits to the fields in which the study will be accepted to the journal. The journal is open to all works aimed at contributing to the national and international developments of the professional organizations and individuals who follow the developments in the field of health, science and engineering and to create a resource in these fields.

JOURNAL OF SCIENTIFIC PERSPECTIVES JSP

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1. *Journal of Scientific Perspectives* has begun publication in July 2017. It is an internationally peer-reviewed and periodical journal published regularly in four issues per year in **January, April, July and October**, in the fields of **basic sciences, engineering, natural sciences and health sciences.** All articles submitted for publication are evaluated by the editor in chief, field editor, editorial board and referees.
2. Journal only accepts the studies written in **English.** Original research papers, technical notes, letters to the editor, discussions, case reports and compilations are published in our journal.
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18. Articles submitted for evaluation must not exceed 25 pages after they are prepared according to the specified template. Article summary should not exceed 300 words and minimum 3 and maximum 7 keywords should be written.

JOURNAL OF SCIENTIFIC PERSPECTIVES JSP

ETHICAL GUIDELINES

Journal of Scientific Perspectives (JSP) is committed to meeting and upholding standards of ethical behaviour at all stages of the publication process. It strictly follows the general ethical guidelines provided by the Committee on Publication Ethics (COPE) and the Open Access Scholarly Publishers Association (OASPA). Depending on these principles and general publication requirements, editors, peer reviewers, and authors must take the following responsibilities in accordance to professional ethic and norms. The proper and ethical process of publishing is dependent on fulfilling these resposibilities.

1.The Responsibilities of Editors

1.1. The General Responsibilities

- Editors should be accountable for everything published in their journals.
- The editor should make the efforts to improve the quality of and contribute to the development of the journal.
- The editor should support authors' freedom of expression.

1.2. Relations with Readers

- Readers should be informed about who has funded research or other scholarly work and whether the funders had any role in the research and its publication and, if so, what this was.
- The editor should ensure that the non-peer-reviewed sections of the journal (letters, essays, announcements of conferences etc.) are clearly identified.
- The editor should make efforts to ensure that the articles published align with the knowledge and skills of the readers.

1.3. Relations with Reviewers

- The editor should match the knowledge and expertise of the reviewers with the manuscripts submitted to them to be reviewed ensuring that the manuscripts are adequately reviewed by qualified reviewers.
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- The editor should provide necessary information about the review process to the reviewers about what is expected of them.
- The editor must ensure that the review process is double blind and never reveal the identities of the authors to the reviewers or vice versa.
- The editör encourage reviewers to evaluate manuscripts in an objective, scientific and objective language.

- The editor should develop a database of suitable reviewers and update it on the basis of reviewer performance and timing
- In the reviewer database; It should be attentive to scientists who evaluate the manuscripts objectively, perform the review process on time, evaluate the manuscirpt with constructive criticism and act in accordance with ethical rules.

1.4. Relations with Authors

- The editor should provide clear publication guidelines and an author guidelines of what is expected of them to the authors and continuously review the guidelines and templates.
- The editor should review the manuscript submitted in terms of guidelines of the journal, importance of the study, and originality and if the decision to reject the manuscript is made editor should explain it to the authors with clear and unbiased way. If the decision is made that the manuscript should be revised by the authors in terms of written language, punctuation, and/or rules in the guidelines (spacing, proper referencing, etc.) the authors should be notified and given time to do the corrections accordingly.
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- Editor should provide publication policies and guidelines to the editorial board members and explain what is expected of them.
- Editor should ensure that the editorial board members have the recently updated publication guidelines and policies.
- Editor should review the editorial board members and include members who can actively contribute to the journal's development.
- Editorial board members should be informed about their roles and responsibilities such as
    - Supporting development of the journal
    - Accepting to write reviews in their expertise when asked
    - Reviewing publication guidelinesand improving them consistently
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2.The Ethical Responsibilities of Reviewers

- The reviewers must only agree to review manuscripts which align with their expertise.
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- Salami slicing, producing more than one article from a single study.
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AUTHOR GUIDELINES

INSTRUCTION FOR AUTHORS

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The editorial board of JSP welcomes original novel contributions and reviews in word format. By submission of a manuscript an author certifies that the work is original and is not being considered simultaneously by other journals. All articles are subjected to critical reviews by referees.

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EVANS, W.A., 1994, Approaches to Intelligent Information Retrieval, *Information Processing and Management*, 7 (2), 147-168.

Conferences:

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SILVER, K., 1991, Electronic Mail: The New Way to Communicate, *9th International Online Information Meeting*, 3-5 December 1990, London, Oxford: Learned Information, 323-330.

Thesis:

SURNAME, NAME , Publication Year, Name of Thesis, Master's Degree/Doctorate, Name of Institute

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Maps:

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MASON, James, 1832, Map of The Countries Lying Between Spain and India, 1:8.000.000, London: Ordnance Survey.

Web Pages:

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HOLLAND, M., 2002, Guide to Citing Internet Sources [online], Poole, Bournemouth University, http://www.bournemouth.ac.uk/library/using/guide_to_citing_internet_sourc.html, [Date Accessed: 4 November 2002].

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CONTENTS

Basic Sciences and Engineering

Health Science

Natural Sciences

Journal of Scientific Perspectives

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DEVELOPMENT OF ELECTROCHEMICAL SENSORS FOR QUANTITATIVE ANALYSIS OF METHYLDOPA AT MODIFIED-GCE AND PGE ELECTRODES BY VOLTAMMETRY

Suzan YANIK* & Saliha B. KURT & Betul ARI*** & Sahin DEMIRCI**** & Selehattin YILMAZ*****

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ABSTRACT

Methyldopa is one of the important drugs used in the treatment of high blood pressure (hypertension). In addition to various methods such as chromatographic and spectrophotometric methods, electrochemical methods are used for the determination of methyldopa. However, poly (p-aminobenzene sulfonic acid), pen-tip graphite electrode (PGE) study was not found in the literature search. Modified electrodes are important because they increase the sensitivity of the analysis. Furthermore, electrochemical methods have advantages such as being faster and cheaper than other instrumental analysis methods, being more sensitive, not requiring long pretreatments in the preparation of samples. In this study, the glassy carbon electrode (GCE) was modified with poly(p-

aminobenzene sulfonic acid) to prepare poly (p-aminobenzene sulfonic acid) -modified glassy carbon electrodes. Cyclic voltammetry (CV) technique was used for the electropolymerization process. Methyldopa was selected in various concentrations of phosphate pH 7.40 buffer, anodic and cathodic voltamograms were taken and oxidation and reduction properties were investigated. Measurements were taken at different scanning rates by CV technique and the current type of methyldopa was determined. Peak flow-concentration graphs were drawn from the measurements taken by Differential Pulse Voltammetry (DPV) technique and the linearity range was 0.020- 2.500 µM for modified-GCE and 0.020-2.820 µM for PGE. The limit of detection (LOD) was calculated as 0.006 µM for modified-GCE, 0.012 µM for PGE. The limit of quantification (LOQ) was calculated as 0.020 µM for modified-GCE and 0.040 µM for PGE.

Keywords: *methyldopa, voltammetry, modified electrode, pen tip graphite electrode, glassy carbon electrode.*

1. INTRODUCTION

Methyldopa, a catechol derivative, is an ancient antihypertensive agent. It has been used to treat high blood pressure since the 1960s. It is a structural analogue of the anti-Parkinsonism drug dopa (dihydroxyphenyl alanine). It has a catechol group and an amino acid skeleton with a methyl group and α-carbon of the side chain on it. Methyldopa converts to methyl norepinephrine through biotransformation at adrenergic nerve terminals. L-DOPA recognized as levo-dopa and ʟ-3,4-Dihydroxyphenylalanine is an amino acid and can be produced in human body (Bastide et. al., 2015; De Deurwaerdere et. al., 2017). L-DOPA is an endogenous precursor for dopamine which is a neurotransmitter and moreover, L-DOPA produced by biosynthesis of L-tyrosine (De Deurwaerdere et. al., 2017; Hauser, 2009; Mercuri and Bernardi, 2005). L-DOPA is highly hydrophilic due to its hydroxyl and amino groups (Azari and Zou, 2012; Mu et. al., 2017). Because of this unique feature it can be utilize for modification reactions (Di Giovanni et. al., 2019), (Gholivand and Amiri, 2009).

Methyldopa is one of the anti-hypertensive agents, especially during the pregnancy period (Figure 1.). It has been also found that methyldopa would inhibit the enzyme DOPA decarboxylase that consequently would convert L-DOPA into dopamine precursor of epinephrine and norepinephrine. Thus, determination of methyldopa in pharmaceutical and biological samples is very important.

Various techniques have been used to detect methyldopa in bulk and drug preparation like spectrophotometric (Norouzi et al., 2009) and chromatography (Fouladgar and Karimi, 2013) methods. Such methods need mathematical analysis, utilization of the organic solvents, costly tools, and laborious procurement process of the samples. Put differently, researchers considerably attended the electrochemical procedures owing to simplified operations, satisfactory accuracy, inexpensiveness, environmental-friendly, quickness, and higher sensitivity for detecting methyldopa, and diverse analytes free from other difficult pre-treatments.

Undesirable events of the electrode can be controlled by changing the chemical structure of the electrode surface. Electroanalytical chemists have been used to carbon, gold, platinum and mercury electrodes until the mid-1970s (Stradiotto *et. al.,* 2003).

In the literature, some chemical reagents were chemically bonded to electrode surface to improve electrode properties with the unique properties of chemical reagents (Murray *et al.,* 1987). Electrodes are usually prepared by modifying a conductive substrate. Thus, modified

substrate possessed desirable functions which is different from the unmodified substrate. The substrate surfaces are prepared by changing them in many different ways as adsorption, chemically modification, and so on (Stradiotto *et al.,* 2003).

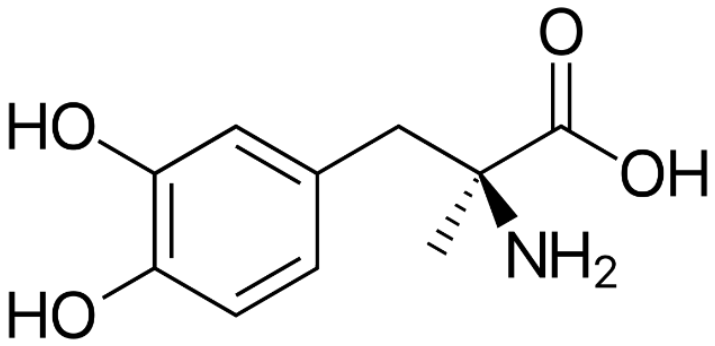
All electrodes are primarily electronically conductive materials they are modified. Carbon, metals, semiconductors, a conductive polymers and organometals can be use as a substratematerial (Ozdemir, 2006). In making electrodes covered with polymer film, either pre-coat with chemically synthesized polymers or directly monomer polymerized at the electrode surface (Murray *et al.,* 1987).

Due to the limited number of electrodes used in electroanalytical chemical analysis working conditions by changing the chemical or electrochemical properties of the electrodes developed. The desired reaction on the carbon electrode may be blocked after a while due to oxidation and contamination on the surfaceor different mechanisms. Top revent this, the surfaces of the solide electrodes are changed by modification (Shahrokhian and Ratsgar, 2011). Fabrication of modified electrodes their controllable morphology and better surface functionalization offer ultra sensitive and selective analysis for electrochemical detection.

The aim of this study is to determine the sensitivity of methyldopa in low concentration by developing electrochemical nanobiosensors (Shahrokhian and Ratsgar, 2011). Electrochemical methods are faster, cheaper and more sensitive than their methods such as Chromatography and UV. In addition, It has advantages such as not requiring long pre-treatment in preparation.

In this study, GCE was modified with poly (p-aminobenzene sulfonic acid) and p-ABSA modified glassy carbon electrodes were prepared. Electrochemical behavior of the active substance has been examined in PGE, modified-GCE electrode. The stability and repeatability of the electrodes were investigated. Current type of oxidation of methyldopa in the chosen supporting electrolyte system determined. The lower limit of detection (LOD) and the quantitative limit of detection (LOQ) were calculated. The accuracy of the two electrodes can be restored with methyldopa obtained from commercially available tablets. The recovery values were determined by calculating the results.

Figure 1. Chemical formula of *Methyldopa*



2. MATERIAL AND METHOD

2.1 Apparatus

AUTOLAB 12 potentiostat/galvanostat device (EcoChemie, Netherlands) was used for all electrochemical measurements and raw voltammograms were treated with a Savicky and Golay algorithm using GPES 4.9 software program by moving average method (peak width 0.01 V). The three electrode system was comprised of a pencil graphite electrode (PGE), Ag/AgCl/3M KCl reference electrode and a platinum wire as the auxiliary electrode. The

Tombow 2B pencil lead of 0.5 mm diameter and length of 60 mm was used as PGE for the investigation. GCE as working electrode (GCE; ∂ = 3 mm, Metrohm), a platinum wire as auxiliary electrode and Ag/AgCl (KCl 3M, Metrohm) as reference electrode. The GCE electrode was polished with alumina (prepared from ∂ = 0.01 µm aluminum oxide) on an alumina polish pad before each experiment and then rinsed with ultra-pure deionized water and ethanol. All experiments were performed at room temperature (22.0-25.0˚C).

2.2 Reagents and materials

Acetate and phosphate buffers were used as buffer solutions in the experiments. 1 M acetic acid solution was prepared for the acetate buffer and reached the desired pH values with 5 M NaOH. pH 3.50 - 5.50 has been studied with this buffer.

Preparation of 0.50 M acetate buffer solution (ABS) (pH 4.50):To prepare of 0.50 M ABS (pH 4.50) 0.2722 g (0.002 mol) sodium acetate per liter of 0.50 M ABS used acetate trihydrate and 0.1154 mL acetic acid. The pH of the solution is adjusted to 4.80 by pH meter using 0.1 N NaOH or 0.1 N HCl. To provide ionic strength 1.168 g of NaCl was added to solution.

Preparation of PBS (pH 7.40): To prepare of PBS firstly 0.2 M $NaH_2PO_4.2H_2O$ and 0.2 M Na_2HPO_4 aqueous solutions were prepared and suitable for the desired pH adjusted with conjugated base solutions.

Electrode surface was cleaned with 0.3 µm, 0.1 µm and 0.05 µm alümina (Al_2O_3) before modification. Later alumina residues remaining on the electrode surface were also cleaned with HNO_3 (1:1), acetone and distilled water. GCE was modified by electropolymerization technique in 0.1 M NaH_2PO_4-Na_2HPO_4 (pH 7.40) buffer containing p-aminobenzene sulfonic acid. The coated surface was activated by cylic voltammetry technique and methyldopa analysis was carried out in 0.1 M NaH_2PO_4-Na_2HPO_4 (pH 7.40) buffer solution.

The activation process for the PGE was performed in pH 4.70 ABS in volving 0.5 M glacial acetic acid.

Methyldopa stock solution and support electrolyte were prepared in PBS at pH 7.40. All aqueous solutions have prepared by using TKA2 Smart (0.055 µS/cm conductivity) distilled water.

In this study, methyldopa active substance and drug form was procured from the pharmaceutical company (İ.E Ulugay) (alfamet®).

2.3 Calibrationgraph for the quantitative determination of methyldopa

The diluted methyldopa solutions were prepared by using diluting with Britton-Robinson (B-R) buffer solution from the stock solution (Reddaiah *et al.*, 2012). A linear calibration curve was created in the B-R buffer solution (pH 7.40) with the DPV method in the concentration range of 0.020 µM and 70 µM methyldopa.

2.4 Recovery works

Five tablets were weighed and powdered in a mortar to determine the amount of methyldopa in Alfamet® tablets. The 30.4 mg of each tablet contains 20 mg methyldopa An appropriate amount of each sample was dissolved and sonicated for one hour to prepare equivalent molar stock solutions. So that check the validity of the developed method, the amount of methyldopa in alfamet® tablet forms by determining the recovery studies were performed in accordance with the linear response of the calibration graphs.

2.5 Preparation of sensor

The graphite pencil electrode used in the studies was prepared by cutting Tombo brand pen tips to a size of 3.0 cm. The pen tips were placed in the pen so that they were 1 cm inside the electrochemical cell. The 1.0 cm surfaces of the electrodes were activated in PBS under the application of +1.40 V potential for 30 seconds. (Subak and Ozkan, 2018). With this process, the groups on the surface of carbon electrodes was oxidized to carboxyl groups.

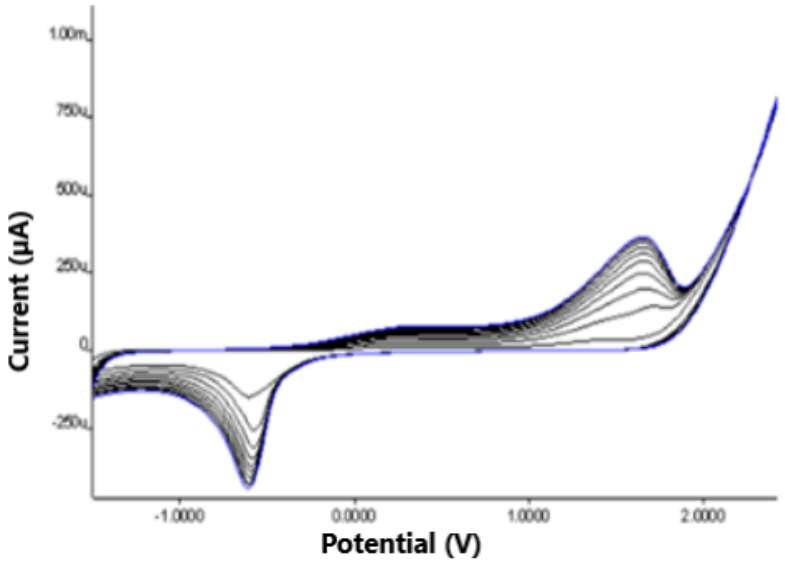
GCE: Before starting the modification, the first electrode was cleaned with 0.1 μm and 0.3 μm diamond paste and 0.05 μm alumina on cleaning pads. Then washed in an ultrasonic bath with HNO_3: distilled water (1:1 by the volume) and finally acetone and distilled water. After cleaning the electrode surface, it was prepared based on the method given in the literature (Mo and Ogorevc, 2005), (Sağlıkoğlu, 2011), (Yang *et al.*, 2006). GCE was modified with $2.0x10^{-3}$M p-aminobenzene sulfonic acid at 200 mVs^{-1} in the potential range of -1.5 V and +2.4 V in the phosphate buffer solution by electropolymerization technique (Jin *et al*., 2005). It was coated, taking 10 cycles at scan speed. Coated surface conversion voltammetry technique and analysis of methyldopa was made after the activation process.

3. RESULTS AND DISCUSSION

3.1 Modification and Activation of Glassy Carbon Electrode

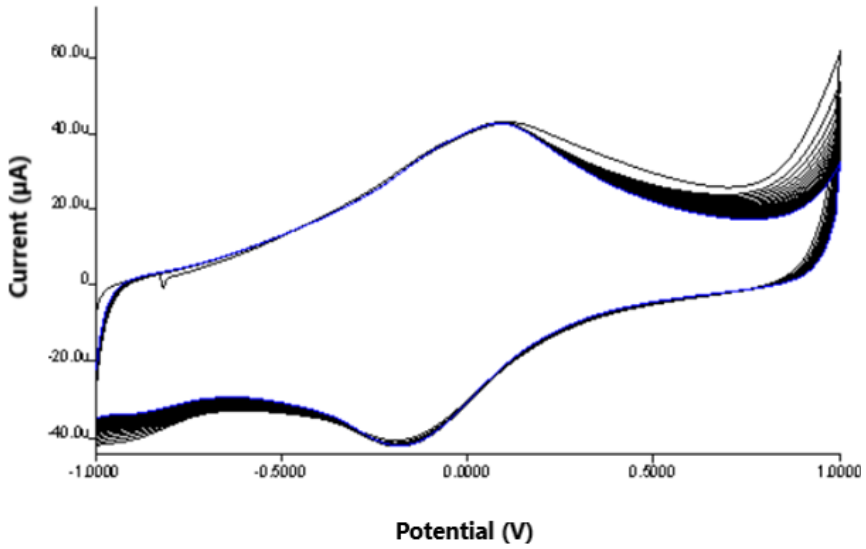
First of all, the surface of electrode 1 was cleaned before modification, after cleaning by cleaning pads with 3 μm diamond paste and 0.05 μm alumina (Al_2O_3), respectively. HNO_3 was washed in an ultrasonic bath with acetone and distilled water (1: 1 by volume). The electrode was cleaned in this way, the surface electrode was coated using the method in the literature (Huang *et al.*, 2008). GCE was covered by electropolymerization technique in 0.1 M PBS tampon solution (pH=7.4) that contained $2x10^{-3}$ M aminobenzene sulfonic acid at the potential range -1.5 V and +2.4 V at 200 mVs^{-1} at scan rate for 10 cycles in Figure 2.

Figure 2. The electrochemical polymerization of $2x10^{-3}$ M p-aminobenzene sulphonic acid in 0.1 M PBS (pH 7.40) on GCE surface, 200 mVs^{-1}voltammogram at scan rate.



The modified electrode was activated with 20 cycles in the range -1 / + 1V (Figure 3).

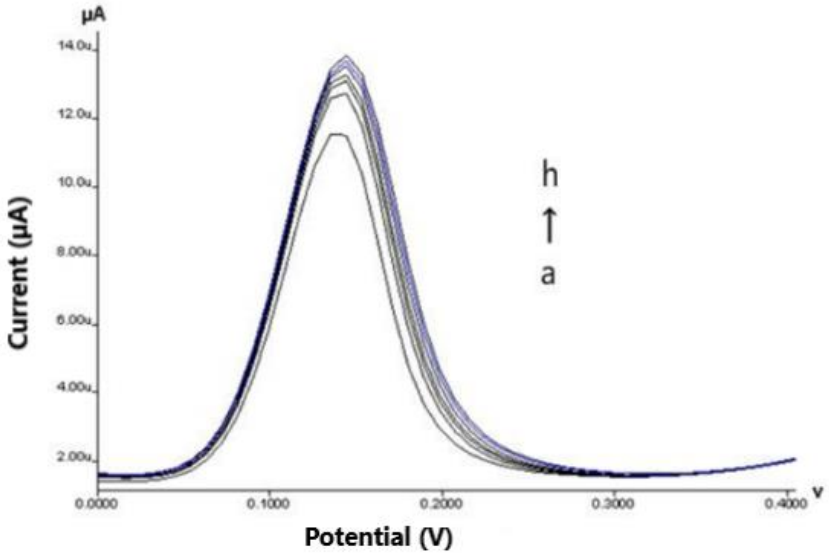
Figure 3. Voltammogram of the activation of the modified- GCE (scanning rate = 200 mVs^{-1}).



3.2 The Analysis of Methyldopa at Glassy Carbon Working Electrode

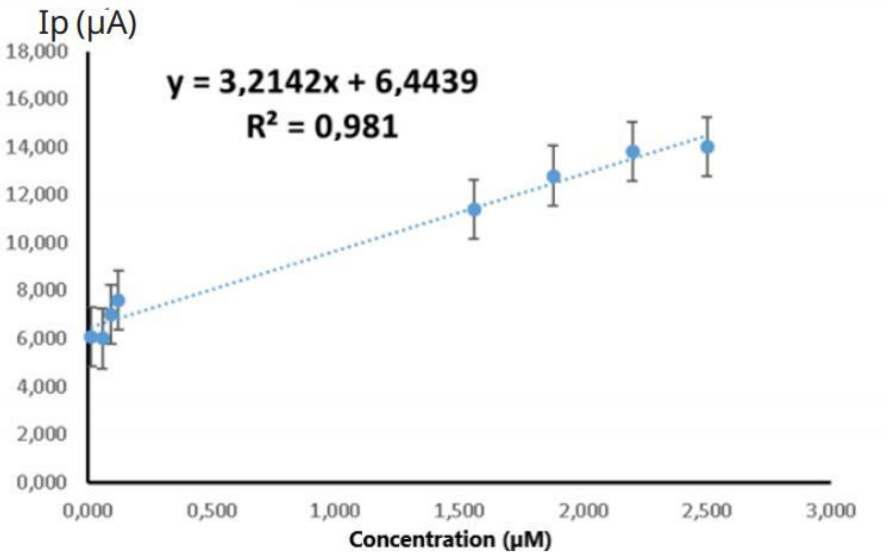
The modified electrode was actived at pH 7.40 according to the method given in the literature (Mo and Ogorevc, 2005). It was calibrated due to the standard addition method of methyldopa at pH 7.40 in PBS. The voltammograms of the calibration are shown in Figure 4.

Figure 4. Voltammograms for calibrations of methyldopa at poly ABSA modified-GCE (a: 0.015µM, b: 0.06 µM, c: 0.094 µM, d: 0.125 µM, e: 1.56 µM, f: 1.88 µM, g: 2.20 µM, h: 2.50 µM) (scanning rate: 200 mVs^{-1})



In phosphate buffer (pH 7.40) peak of current-concentration (C-Ip) graph is shown in Figure 5.

Figure 5. Graph of peak current vs concentration (C-Ip) of methyldopa in PBS (pH 7.40) at poly ABSA modified GCE

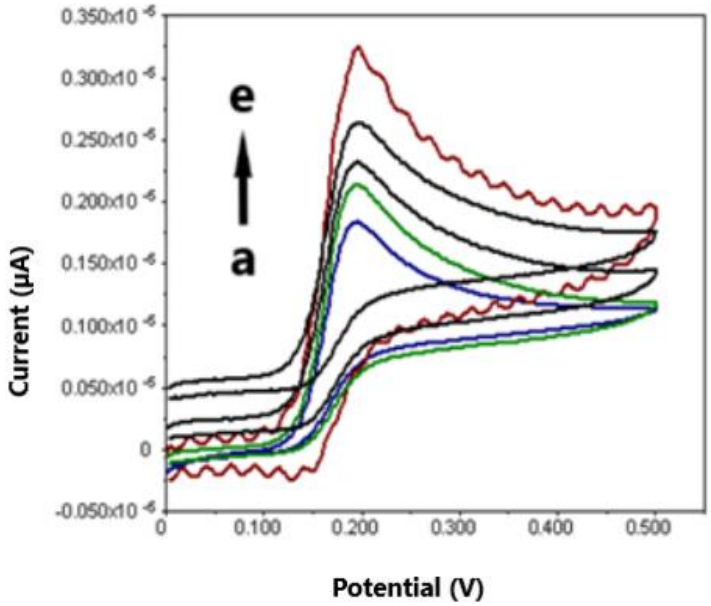


As a result, the calibration graph has been drawn based on outcomes. The slope of the graph was determined as 3.2142 and the regression coefficient was found as 0.981. The linearity of calibration was determined between 0.015µM-2.50 µM from the equation of the graph.

3.3 Analysis of Methyldopa Using Pen Tip Graphite Electrode

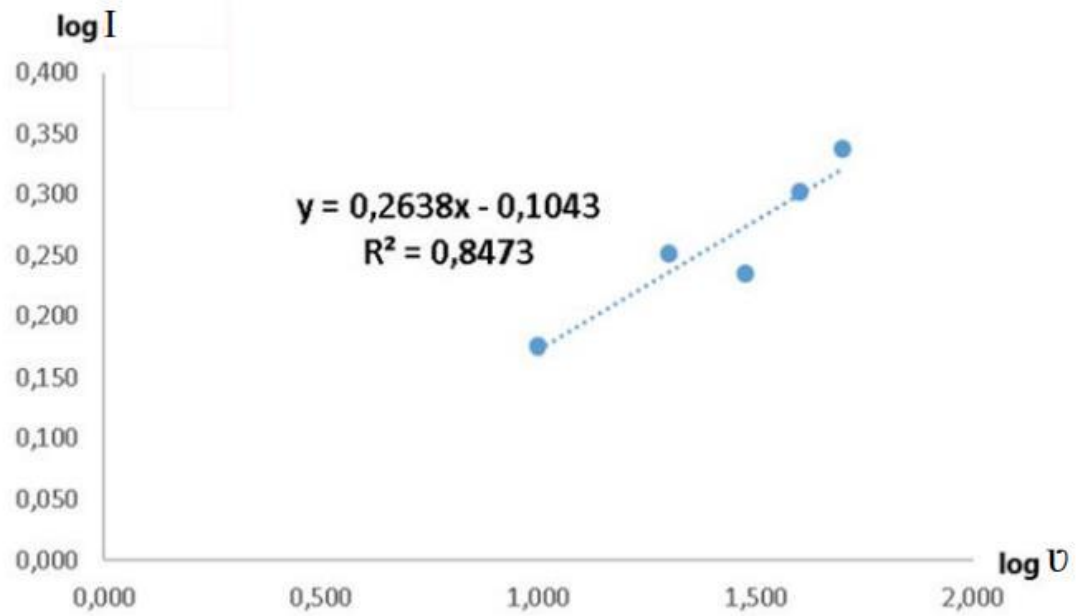
Scanning rates study was done between 10-50 mV/s to determine the current type of methyldopa at activated electrodes (Figure 6).

Figure 6. Voltammogram of scanning rates study of methyldopa at PGE (Scan rate = a) 10; b) 20; c) 30; d) 40; e) 50 mVs^{-1}**)**



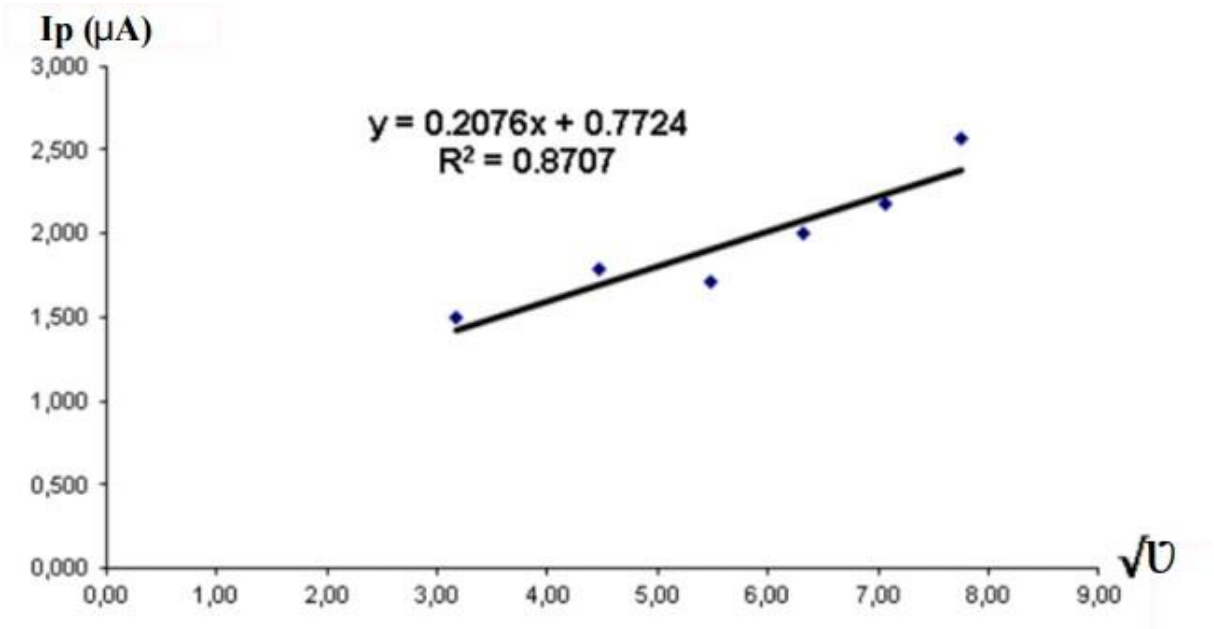
In order to determine the type of current in the oxidation reaction of methyldopa, voltammograms were taken at different scanning rates by cyclic voltammetry technique. This scan rate of voltammograms were (a) 10; b) 20; c) 30; d) 40; e) 50 mVs^{-1})). The graph of logarithm of the current vs log scan rate was given in Figure 7. According to this, slope of the graph was found far from 0.5, indicating that shows the current adsorption was controlled (Rezaei, *et al.*, 2013).

Figure 7. Log scan rate-log peak current obtained by cyclic voltammetry technique of methyldopa using PGE current (logv-logI_P) graph.



It is also obtained from the increase of the oxidation peak current linearly with the square root of the scan rate vs current was that was given Figure 8. Correlation coefficient (R^2) was obtained 0.8473 (far from 1). It is an important indicate or that the current was controlled by adsorption (Skrzypek et al, 2005).

Figure 8. Peak current vs square root of scan rate of methyldopa at PGE



For methyldopa, whose current type is determined with adsorption control, DPV measurements in anodic direction between 0.0 V-0.45 V by accumulating 100 seconds before each analysis has been taken. Voltammograms of the calibration where linearity was determined in Figure 9. and Figure 10. Graph of peak current-concentration (C-Ip) in the phosphate (pH 7.40) buffer solution of methyldopa in 4.9. specified.

Figure 9. Voltammograms of calibration of PGE and methyldopa (a: 0.002µM, b: 0.005 µM, c: 0.007 µM, d: 0.010 µM, e: 0.012 µM, f: 0.034 µM, g: 0.060 µM, h: 0.100 µM) (scanning rates: 16 mVs^{-1})

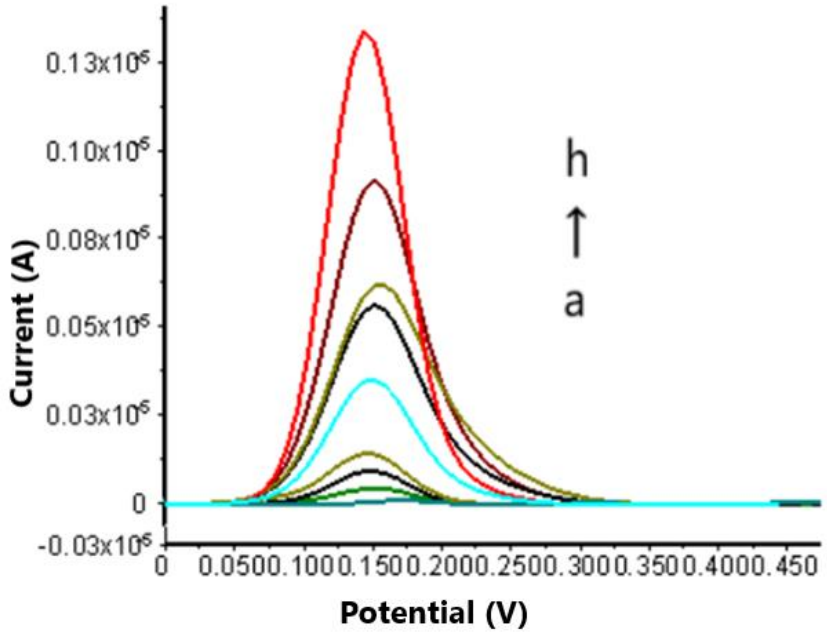
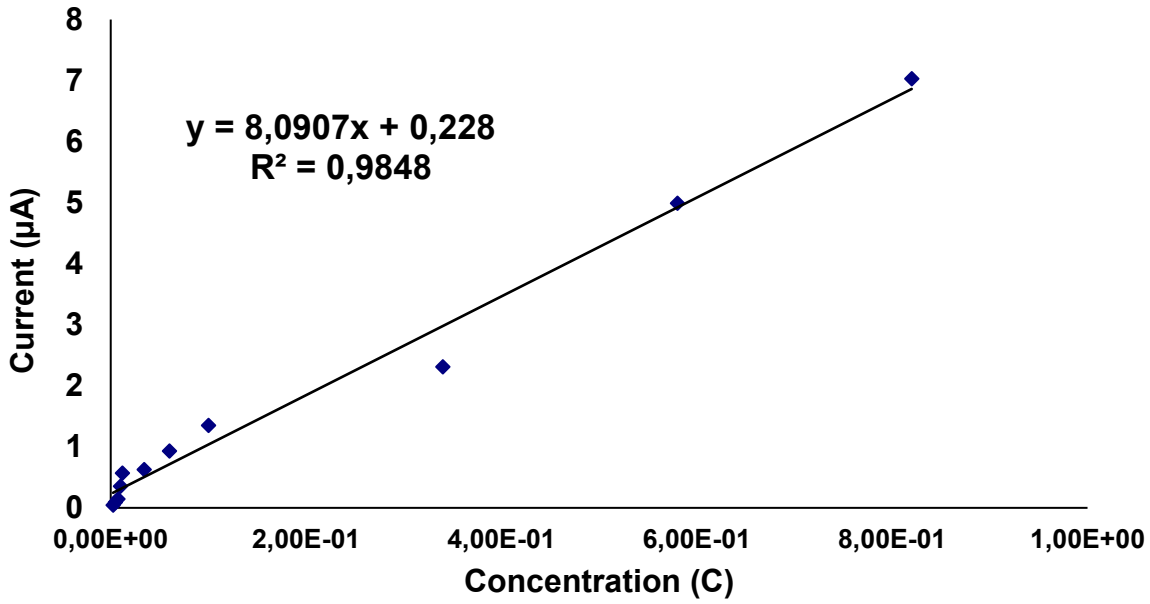


Figure 10. The graph of peak current vs concentration (C-Ip) of methyldopa in PBS (pH 7.40) at PGE.



As a result, the calibration graph has been drawn based on outcomes. The slope of the graph was determined as 8.0917 and the regression coefficient as 0.9848. The linearity was determined from the equation of the graph between 0.02µM-0.820µM. For quantitative analysis of methyldopa, obtained using modified- GCE and PGE electrodes. Comparison of the analytical determination parameters with those given in Table 1.

Table 1. The Comparison of parameters to determination of methyldopa at modified-GCE and PGE electrodes

PARAMETERS	MODIFIED - GCE	PGE
Potential, V	0.144	0.151
Linearity range of concentration, µM	0.020-2.500	0.020-0.820
Slope, µAM	3.214	8.091
Intercept,	6.444	0.228
Number of measurements	5	5
R^2	0.981	0.985
LOD (µM)	0.006	0.012
LOQ(µM)	0.020	0.040
%RSD	0.286	0.735

3.4. Determination of the Amount of Methyldopa in Commercial Drug Forms and Validity of Applied Voltammetric Method

To determine the amount of methyldopa in ALFAMET tablets by voltammetric method, 5 tablets of alfamet tablets are weighed and powdered and its solution containing 0.1 µM methyldopa prepared. Voltammogram of this solution was measured and the current value to concentration graph was drawn. The amount of methyldopa in 1 tablet was 245.25 mg for the modified-GCE and it was 243.75 mg for PGE. This value was specified on the tablet as 250 mg compared with Table 2.

The method was developed to check the validity (accuracy and precision) of results. Determination of the amount of methyldopa in alfamet tablet forms and recovery studies. The results was shown in Table 2.

Table 2. Determination of the amount of methyldopa from its Alfamet tablets and Recovery % (average of 5 experiments)

PARAMETERS	MODIFIED-GCE	PGE
Labeled methyldopa, mg	250.00	250.00
Amount found, mg	245.25	243.75
Relative Standard deviation (RSD / %)	0.286	0.735
Added methyldopa, mg	10.00	10.00
Found methyldopa, mg	9.87	9.75
Recovery percentage (%)	98.75%	97.50%

A recovery study was performed to check the accuracy and precision of the two different voltammetric methods applied. For this purpose, the peak current of 0.1 µM methyldopa was measured. The current value was placed in the equality of the calibration graph and the amount of methyldopa as an active ingredient in the alfamet tablet was calculated. Then, 10 mg of

methyldopa was added to the same solution. The recovery of methyldopa was calculated 98.70% in modified-GCE and 97.50% in PGE in Table 2. by comparing the amount of methyldopa that was added and the amount of methyldopa that was found. Since the recoveries of both different electrodes were 98.70% and 97.50%, it was concluded that the accuracy of the two voltammetric methods applied and the drug additives did not affect our analysis method.

4. CONCLUSIONS

Neurological diseases are related to the amount of dopamine in the body. Since dopamine is a molecule secreted by brain cells, the reduction of dopamine secretion as a result of damage to these cells causes neurological disease such as Schizophrenia and Parkinson's (Mo and Ogorevc, 2005).

The most common method used for dopamine determination is electrochemical methods. Although dopamine sensors based on electrochemical responses are constantly being developed, studies on the development of more reliable and effective sensors based on low detection range analysis of dopamine are still interesting topics. Therefore, the selection of the material to be used in electrode modification is very imporant in the design of sensors with excellent performance.

In this study, the electrochemical oxidation of methyldopa at 4-(ABSA)-modified-GCE by differential pulse and cyclic voltammetry techniques. In the voltamograms were taken at pH 7.40 in PBS, an oxidation peak was obtained between 0-0.45 V. 4-ABSA modified- GC electrode was prepared by coating the GCE surface with electropolymerization technique. It was observed that the modified electrode was conductive and enabled to oxidize of the methyldopa. It was determined that the oxidation current peak of methyldopa prepared in this modified electrode and gave much higher signals compared to the pencil tip electrode. For same concentration (0.1µM), a current of 1.35µA was obtained at the PGE electrode, while a current of 7.60 µA was obtained modified-GCE. When the two electrodes are compared, current was observed more 5 times high at modified-GCE than at PGE.

Electron transfer is facilitated as a result of the electroanalytic effect of the GCE surface covered by polymeric film. This shows that the modified electrode is more sensitive. It has been concluded that the applied voltammetric methods are more preferable than HPLC and UV spectroscopy due to their advantages as fast, economical and sensitive, working with small amount of samples and being able to analyze without need for time-consuming processes as separation. For the validity of the voltammetric method, recovery studies indicated that the drug additives did not affect the determination of methyldopa.

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Research Article

PRODUCTION AND CONSUMPTION TRENDS OF NATURAL GAS OF TURKMENISTAN THE YEARS FROM 2009 TO 2019

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ABSTRACT

In this study, natural gas production and consumption of Turkmenistan has been mentioned for years. Leaving the Soviet system in 1991, Turkmenistan started to take the first steps to move its economy from a closed system to a free market system. To raise its economy to the level of developed states, Turkmenistan has come by implementing new regulations in social and industrial areas within 10 years.

Turkmenistan has chosen an international, open, and pluralist economic model since its independence. This model has projected the "10-Year National Development Program" to ensure the economic development of the country and to be carried out according to the targets and plans. The main goal of the development program can be shown as placing the country among the first world countries, a social market economy, managing the country within its means, and encouraging international investors to structural and economic investments.

Today, World trade represents the liberalization trend. Liberalization of trade and markets provides the necessary foreign savings and foreign exchange needs to finance economic development with the help of foreign investments in developing countries such as Turkmenistan. Being a country with very rich natural resources, Turkmenistan aims to improve the country's economy by processing these resources and presenting them to the world markets. According to data in 2009, Russia is the largest importer of natural gas, the country's most important export product, while Iran is the second. During this period, exports to China are at a low level. However, while gas exports to Russia and Iran decreased to very low levels over time, China became the biggest consumer of Turkmenistan natural gas. In this, the relations of countries, energy needs, and investments made by demanding countries and the geographical distribution trends of the natural gas consumption-supply demand balance in the world. 2009-2019 is the economic advancement decade in the energy sector of Turkmenistan as a result of exploring and producing new gas reserves in the country, after which consumers gained conscious consumption as well as concluding new agreements with investors.

Keywords: *Turkmenistan, Natural gas, Energy.*

1. INTRODUCTION

Turkmenistan, located in the east of the Caspian Sea between 53-66 degrees east longitude and 36-43 degrees north latitude, has an area of 488,100 km^2. Turkmenistan, which has the largest surface area after Kazakhstan among the Central Asian countries, is washed by the Caspian Sea in the west and the Amuderia River in the East. The Karakum Desert occupies four-fifths of the area of this country. Turkmenistan, about 80% of which is desert in nature, borders with Uzbekistan in the east and northeast, the Caspian Sea in the west, Kazakhstan in the north, Iran in the south, and Afghanistan in the southeast (Hojamuradov, 2005) (Arxiv, 2020).

Turkmenistan was a colony of the Russian Empire and after the Union of Soviet Socialist Republics (USSR) from 1881. In this colonial system, each colony had its mission to provide its products to governance, Moscow. The mission of Turkmenistan was the production and maintenance of organic cotton because of its agricultural infrastructure. For many years, the country thought that its main economical "wealth" is cotton. Russian Empire and the Soviet Union were exploring petroleum and natural gas by their scientists.

After the collapse of the USSR, all ex-colonial countries had an economical-crisis because of the countries' focus on their "mission products." Turkmenistan governance didn't have enough technical and theoretical knowledge and background about its natural reserves. Coastal countries of the Caspian Sea: Iran, Azerbaijan, and Kazakhstan started to utilize site petroleum earlier.

After several years Turkmenistan started to explore new natural reserves: natural gas in the Karakum desert and petroleum in the Caspian Sea. After the discovery of petroleum in the Caspian Sea, the route of natural crystal salt and iodine was turned up. Currently, the country's crucial natural resources are petroleum, natural gas, sulfur, and natural crystalline salt.

2. ECONOMIC STRUCTURE OF THE COUNTRY AND ITS TRADE POLICY

In the period of the Union of Soviet Socialist Republics (USSR), the economic structure of Turkmenistan, like other Central Asian Republics, was based on supplying processed agricultural products and cotton to the Center and transferring the obtained raw materials to Russia following the economic agreement policies of the USSR period. The focus of the economy on the production of raw materials and the use of these raw materials in the industries and other republics caused other sectors to fall behind (Hojamuradov, 2005).

The country has large-scale gas and oil resources, and the government attaches great importance to the development of the energy sector and conducts a series of projects to unlock the sector's potential. The success of these projects depends to a great extent on the entry of foreign resources into the country (DTM, 2002) (Rutka, 2017).

The fact that the economy is primarily a raw material production, prevented the economy from deteriorating after independence. The main branch of the economy is associated with the extraction of natural gas and oil (DTM, 2002).

Turkmenistan has refused to take corrective measures because it considers social stability a necessary condition for economic growth. The years after 1991 saw a sharp decline in the country's gross domestic product (GDP). The cumulative decline in GDP between 1992 and 1996 was 50% (DTM, 2002).

General trends in the economic activity of Turkmenistan are being shaped by changes in the energy sector, which is the cornerstone of the economy (DTM, 2002). However, the economy of Turkmenistan did not benefit from the rise in energy prices. The reason for this is

that the unpaid and accumulated debt of some countries of the Commonwealth of Independent States (CIS) to Turkmenistan increased as a result of their non-payment for gas imports, and thus, gas production in Turkmenistan decreased. The suspension of gas exports to Ukraine due to payment problems in 1997 also led to a decline of 11.3% of GDP in the same year (Özsu, 2003).

However, there has been a continued economic recovery since 1997, and after independence, GDP grew for the first time in the period 1997-1999. This growth was driven by improved yields for cotton and wheat, as well as increased production of gas and oil. The growth that continued in 2000 was largely due to the increase in gas exports to Russia. At the end of 1999, Turkmenistan signed an agreement with Russia on the sale of gas for 20 billion cubic meters (bcm), later this volume was increased to 30 billion cubic meters. An agreement on the sale of 30 bcm of gas was signed with Ukraine for 2001 (Özsu, 2003).

In the early years of independence, the geographical distribution of goods exported by Turkmenistan and the main structure of the commodity composition had a very complex structure based on the export of cotton and gas, represented in the main markets of the former republics of the Soviet Union. However, in 1992, an obligation to produce international prices and payments in foreign currency was introduced in commercial transactions with the CIS. After the authorities of Turkmenistan were obliged to make payments in foreign currency, there were constant problems with the prices for gas exports between Turkmenistan and these countries (Odabaş, 2001).

In addition to non-payment of debts related to gas exports to the former Soviet republics at world prices, failure to pay the accumulated debt on time caused significant cash problems in 1994. Russia's refusal to allow Turkmen gas to flow into markets that will generate foreign currency has exacerbated these problems. However, the inflow of money into the country began with Ukraine's payments in foreign currency and give goods in return in April 1995. A pledge from countries such as Azerbaijan, Georgia, and Armenia to pay off the gas debt in 1998 brought relief. In 1998, the same problem was faced with Ukraine, and the government of Turkmenistan at the time did not export natural gas in protest. However, in the following period, a mutual agreement was reached between the two countries, and the export of natural gas began (Odabaş, 2001).

The main objectives of Turkmenistan in trade policy; diversification of the country's export markets and an increase in added value in the process of obtaining raw materials that it exports domestically (Odabaş, 2001).

Another factor that is taken in increasing Turkmenistan's export earnings is infrastructure investment, which will provide some of the opportunities needed for significant investment. Turkmenistan is also making various efforts to enter new export markets where payments are made in foreign currency, especially in Eastern Europe (Odabaş, 2001).

3. PRODUCTION AND CONSUMPTION TRENDS OF NATURAL GAS OF TURKMENISTAN

3.1. Natural Gas Production Companies in Turkmenistan

Former President of Turkmenistan Saparmurat Niyazov (Turkmenbashi), after discovering natural gas reserves in the country, concluded the first agreements with Turkey and the Chinese government for the production and operation of gas. From the Turkish company, Calik Enerji Sanayi ve Ticaret AS (Çalık Enerji) and the China National Petroleum Corporation (CNPC) came to Turkmenistan and continued to work on the exploitation of natural gas. In 2008, companies such as Gaffney, Cline & Associates also came to the country and were the

first to edit natural gas for other companies. Subsequently, companies such as Hyundai Engineering and Petrofac came to the country and invested.

3.2. Natural Gas Reserve Distribution, Production, and Consumption of Turkmenistan and Trade Relation with the other Asian Countries by Years

Turkmenistan, which has the largest natural gas reserves in Central Asia, is also the country with the largest export potential. Natural gas exports to Russia, the mainstay of Turkmenistan's export revenues, have recently been replaced by the Chinese market with 30-35 Bcm / year exports. Exports to Russia have completely ended in 2015 (CAGP, 2020).

With the transition from Russia to China as its main export destination, Turkmenistan's gas sector has changed in two important ways. First, Turkmenistan, which shuns away from international oil companies and is reluctant to work with foreign investors, now works with two foreign companies (China National Oil Company and Petronas) for more than a quarter of its gas production. Second, after many years of planning, Turkmenistan started a large petrochemical factory in Kiyanli, which will contribute significantly to the economy in 2018 (CAGP, 2020). It is expected that pipeline construction works for transporting the country's natural gas to other countries will continue in the 2020s. The possibility of increasing exports of Turkmenistan gas to China, a major consumption area, will be possible with the construction of the 4th part of the Central Asia - China pipeline system planned to be made between two countries (CAGP, 2020) (OE, 2020).

As seen in Table 1, Turkmenistan exported natural gas to Russia from 2010 to 2015 but has ended natural gas sales since 2016. Likewise, Turkmenistan, which exports natural gas to Iran until 2016, has gradually decreased its exports to the country and approached zero in 2018. While it did not export gas to its neighbor in the west, Azerbaijan until 2017, it has initiated 1 Bcm natural gas sales since 2017. On the other hand, while there was no export in 2010 in Kazakhstan, which is its close neighbor, the natural gas export continues with a small increase in 2014, starting from 2011, then pausing in 2012 and reaching up to 1.5 Bcm in 2018. Finally, between 2010 and 2018, natural gas exports to China are expected to continue to increase while reaching 14.5 Bcm, increasing by approximately 10 times. China alone is the region that consumes almost all the natural gas produced in the country.

Table 1. Gas Balance of Turkmenistan (BP, 2020) (OE, 2020)

	YEARS **Numbers are given in Bcm (billion m^3)**								
	2010	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**
Production	40.1	56.3	59.0	59.0	63.5	65.9	63.2	58.7	61.5
Total Gas Balance	40.1	56.3	59.0	59.0	63.5	65.9	63.2	58.7	61.5
Domestic Consumption	18.9	20.7	17.6	18.7	20.0	27.0	25.5	22.9	24.5
Export	21.2	35.6	41.4	40.3	43.5	38.9	37.7	35.8	37.0
To / Through Russia	10.7	11.2	10.9	10.9	11.0	3.1	0	0	0
To İran	7.0	10.0	9.0	5.0	6.0	7.0	7.0	0	0
To Azerbaijan	0	0	0	0	0	0	0	1	1
To Kazakhstan	0	0.3	0	0.3	1	1	1.3	1.5	1.5
To China	3.5	14.1	21.5	24.1	25.5	27.8	29.4	33.3	34.5

Figure 1. Turkmenistan's Gas Infrastructure (OIES, 2015)

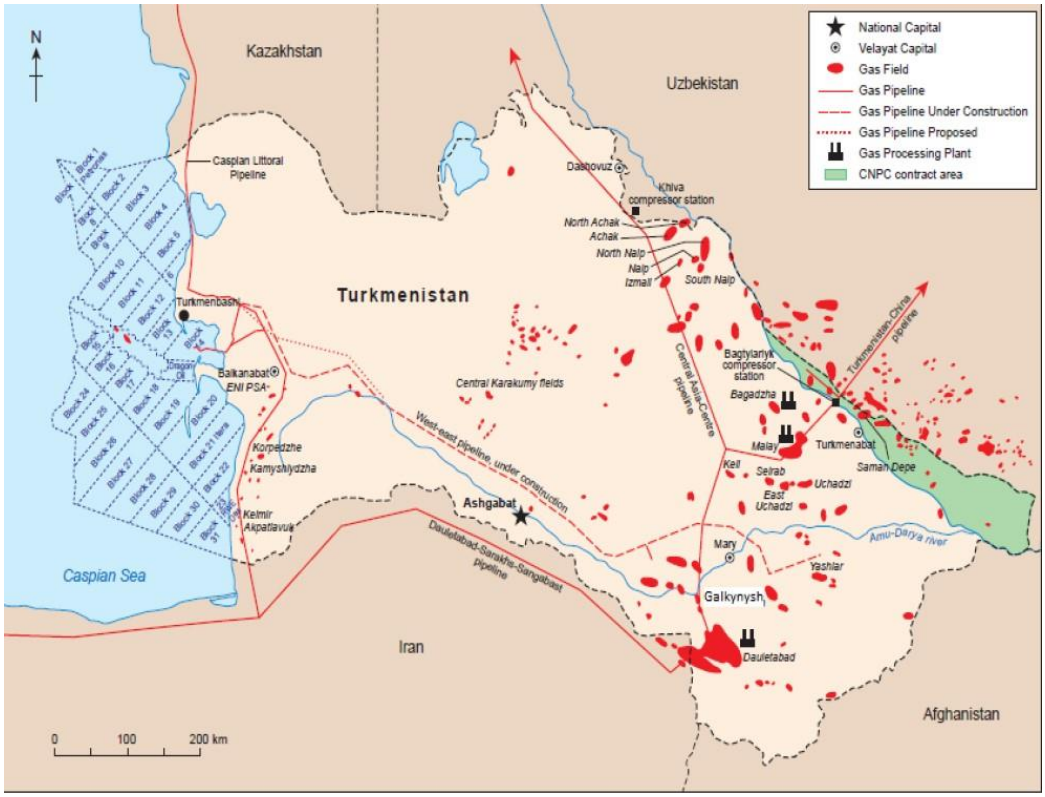


Figure 2 shows the trade balance of Turkmenistan since 2003. Especially after a period when revenues from gas exports to Russia increased, there was a decrease in 2009-2010. However, the start of export trade to China and high oil and gas prices resulted in a renewed increase in export revenues that reached record levels in 2012-2014. There was a sharp decline after 2014, with export revenues in 2016 at the lowest level in a decade, followed by a renewed increase (IMF, 2019) (OE, 2020).

Figure 2. Turkmenistan's Exports and Imports Of Goods (Million Dollars 2003-2018) (IMF, 2019**)**

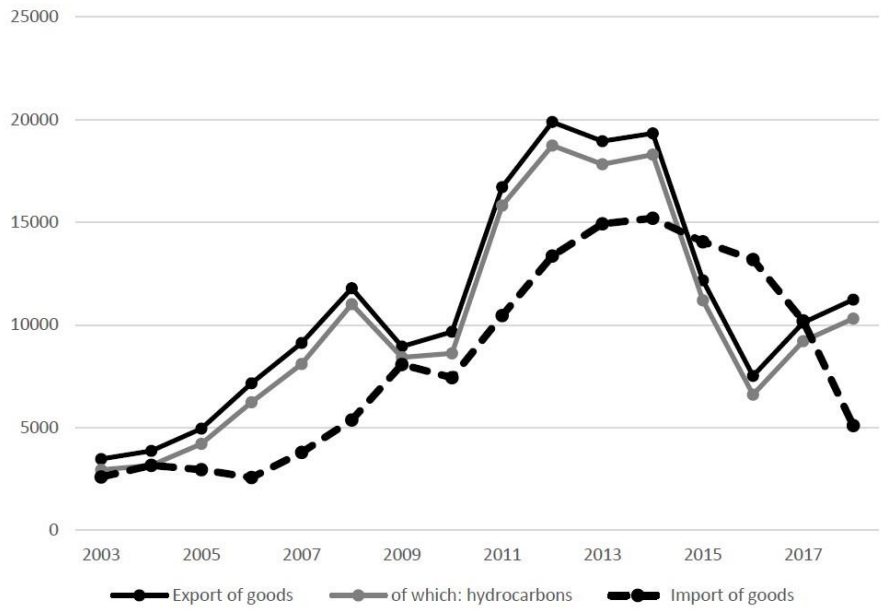


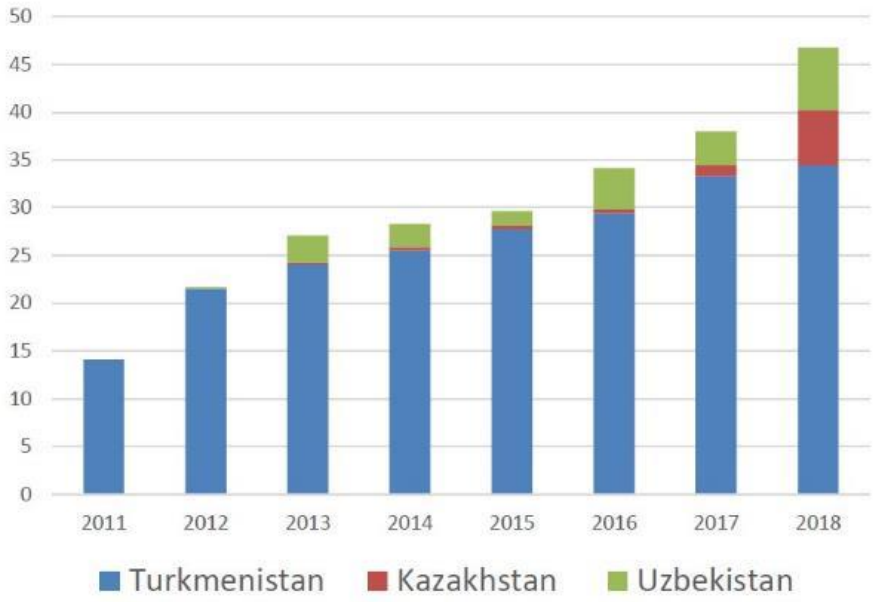
Table 2 presents the available production information by company and country of destination. In particular, it divides the production of the state-owned Turkmengaz into natural gas exported to China and gas used on other export routes (Russia, Iran, and Kazakhstan), as well as on the domestic market. In the 2010s, the Galkynysh field became the main source of exports to China. When these exports began in 2010, Galkynysh was not yet producing gas, and China directed its route to Devletabad, Turkmenistan's second-largest field, and continued to export natural gas from other quarries in the southeast of the country. Until 2014, the capacity of Galkynysh was 10 billion cubic meters per year, and by 2019 it increased to 30 billion cubic meters, which is 3 times higher than its capacity.

Table 2. Turkmenistan Gas Production By Company and Destination (BP, 2020)

Bcm	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019 est
Total Production (sales gas)	33.3	40.1	56.3	59.0	59.0	63.5	65.9	63.20	58.7	61.5	62
Turkmengaz and other state-owned companies											
To China, İncluding from Galkynysh	0	1.0	11.0	18.1	19.6	19.2	15.5	17.8	20.5	25.7	25.8
For the export routes and the domestic market	33.2	35.5	40.2	34.1	32.7	33.4	36.2	30.4	23.0	19.5	18.9
Exports to Russia	11.8	10.7	11.2	10.9	10.9	11.0	3.1	0	0	0	0
Exports to İran	7.0	7.0	10.0	9.0	5.0	6.0	7.0	7.0	0	0	0
Exports Kazakhstan	0	0	0.3	0	0.3	1	1	1.3	1.5	1.5	1.5
For the domestic market	14.4	17.8	18.7	14.2	16.5	15.4	25.1	22.1	21.5	18.0	17.4
Private Companies											
CNPC: under PSA at Bagtyarlyk	0.1	3.6	4.6	5.5	5.5	9.1	12.5	12.8	13.0	13.0	13.2
Petronas: Offshore Caspian Block No. 1			0.58	1.28	1.23	1.75	1.69	2.22	2.16	3.33	4.15
Private companies, as % of total	0.03	8.9	9.2	11.4	11.4	17.1	21.5	23.8	25.8	26.5	28

The steady growth of gas exports from Turkmenistan, Uzbekistan, and Kazakhstan to China is shown in Figure 3. As can be understood from the figure, Turkmenistan is the largest natural gas exporter country to China in Central Asia compared to other countries (Pirani, 2012) (OE, 2020).

Figure 3. Exports Of Central Asian Gas To China. (CAGP, 2020).



4. CONCLUSION

Turkmenistan, after gaining its independence, has become a rapidly rising and developing country since 1991. It has introduced itself to the world by ranking 4th after Qatar with its underground wealth such as natural gas and oil. Considering the natural gas production, consumption, and export between 2009 and 2019, the country's economy is improving significantly with the determination of more reserves year by year, increase in production, conscious consumption, and new agreements in exports. In recent years, China has become the country where Turkmenistan gas is used the most, and the natural gas supplied to Russia and Iran in the last 10 years has gradually decreased to a zero. Many parameters such as international relations, changes in the energy policies of countries, the effect of the geography where energy is used are main contributors.

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Research Article

TIME-BASED DEVELOPMENT PLANS FOR DISTRIBUTION NETWORKS IN THE PRESENCE OF DISTRIBUTED GENERATORS AND CAPACITOR BANKS

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ABSTRACT

In this paper, a time-based model for distribution network development planning is proposed, considering the possibility of using distributed electricity generation technologies and the existence of capacitor banks. The proposed model specifies the location, capacity, and timing of the use of distributed generation technologies and capacitor banks as well as the schedule for increasing the capacity of the grid lines. The Genetic Enhanced Algorithm is used to solve the stated problem to optimize the network development plan including the time, location and capacity of DG and capacitor banks in the distribution network as well as to optimize the investment cost and operating cost. It was also implemented in a MATLAB programming environment to validate and evaluate the effectiveness of the proposed solution to the problem of distribution network development planning on a 17-bus radial distribution network.

Keywords: *Development of Distribution Networks, Distributed Generation Resources, Capacitor Bank, Genetic Algorithm*

1. INTRODUCTION

The purpose of planning the development of the distribution system is to strengthen it by adding new equipment to meet the growth in load consumption at the lowest possible cost and with the most reliable reliability. In the overall planning process of power systems integrated at the production, transmission and distribution levels, network load growth must first be anticipated in the coming years so that network development can be done correctly. After performing load prediction, the amount of power generation increase is studied to increase the capacity of existing plants or build new ones if needed. For this purpose, finding the right points for deployment of new power plants is of great importance because if the new plants are not deployed in the right places, the cost of operating the network will be higher. One of the important points to note after this step is to increase the capacity of the new posts and lines. For this purpose, each of the stages of development of substation and transmission line planning is carried out with the aim of minimizing the cost of development and meeting network needs [1-4]. Given the significant advantages of electric power over other energies, it is predicted to be simple and convenient for long-distance distribution and transferability, with the largest energy consumption in the next century being electricity and the distribution network responsible for providing electricity. Consumers, as one of the main components of the power system, are of great importance and value. In a power system, it typically accounts for half of the losses in the distribution network, and distribution networks are expanding as demand for power increases. The annual investment in this field amounts to billions of dollars. Inadequate financial resources in this sector, inappropriate design, and operation strategy, as well as the prevalent culture of craftsmanship, have made the country's distribution networks unsuitable. Thus, when the two factors, namely large-scale investment and losses, come together, it will be clear that reforming even a small part of the design methods of this system will lead to a fundamental change in power distribution companies.

The purpose of the design principles of distribution networks is to provide a design that guarantees the growing need for electric power in a technically and economically acceptable manner. Thus, the design of the distribution system, on the one hand, is related to load growth parameters, spatial distribution of consumption points and on the other hand, to technical factors such as the values of lines and feeders, the capacity and location of the over-distribution substations, the desired voltage levels, and reliability levels, and Takes into consideration the other.

Also, economic aspects such as the cost of purchasing and installing equipment, the cost of annual energy losses, interest rates, and so on, must be taken into account to be viable. To thoroughly examine the distribution networks that ultimately lead to the design of the proper principles and methods for their design, it is necessary to determine the structure of the network throughout the power system [5-9]. In a power grid, the power generation capacity passes through the transmission grid to the transmission grid through substations, where it travels to the grid-connected to the grid after crossing the grid. The total power passing through each post should not exceed the maximum permissible capacity of the equipment installed in the post, such as motor transformers, switches, rails, etc. On the other hand, the growth of loads or the construction of DGs may cause problems for existing posts.

In this case, the development of existing posts or the construction of new posts on the network can improve the network status. The amount of load that is delivered by each distribution post depends on how the distribution network is arranged and the layout of the substation. It is also dependent. Therefore, scheduling the development of posts will not be complete without the limitations of the lines. In the development of substation planning, it

should be specified how much and at what time the equipment capacity of the network substations should be constructed and added to the existing set of networks [10].

2.THE OBJECTIVE FUNCTION

The objective function of the proposed model to solve the stated problem is to minimize the total investment and operation cost of the distribution network and DG units and capacitor banks over a specified planning period. Project investment costs include the cost of DG unit's investment, the cost of capacitor banks investment and the cost needed to increase the capacity of the distribution lines. Operating costs also include the cost of energy purchased from the upstream grid and the cost of operating the DG. In short, the objective function (OF) is:

$$OF = INC + OC \tag{1}$$

In this respect, INC is the total investment cost including DG investment cost, capacitor investment cost, and feeder reinforcement cost, and total operating cost (OC) includes the cost of purchasing power from the upstream grid and the maintenance cost of DGs in Equation (1). INC details are as follows:

$$\begin{aligned} INC = \sum_{t=1}^{T}\sum_{i=1}^{N_{LB}} \beta(t) \times \{ & [IN_{DG} \times \left(S_{DG,i}^{M} + BK\right) \times \sigma_{DG,i} \times \left(M(t - IY_{DG,i} + 1\right) \\ & - M\left(t - IY_{DG,i}\right))] + [IN_{CAP} \times \left(C_{CAP,i}^{M}\right) \times \sigma_{CAP,i} \times \left(M(t - IY_{CAP,i} + 1\right) \\ & - M\left(t - IY_{CAP,i}\right))] + [B_{r,i} \times \sigma_{R,i} \times (M(t - VY_i + 1) - M(t - VY_i)]\} \end{aligned} \tag{2}$$

This relationship consists of three parts, the first part of which is the cost of DG investment. The second part deals with the cost of investing capacitors and the third part about the cost of reinforcing feeders. In relation (2), the decision variables $\sigma_{DG\ i,t}$ and $\sigma_{CAP\ i,t}$ represent the presence or absence of DG in the bus *i* and the presence or absence of capacitor *C* in bus *i*, which are binary variables. Also, the variables $S_{DG,i}^{M}$ and $C_{CAP,i}^{M}$ indicate the DG installation capacity of bus *i* and the capacitance (*C*) installed in bus *i*, which are integers. These decision variables are determined by the optimization algorithm. IN_ constant (DG) is the investment cost of DG and IN_{CAP} is the capacitor investment cost. The investment cost is obtained by adding the annual investment cost over the planning period. A DG is also considered as an extra backup (*BK*) (for emergencies). Also, the function $\beta(t)$ of the financial cost-conversion function in year *t* is equivalent to its present value as follows

$$\beta(t) = \frac{1}{(1+d)^{t}} \tag{3}$$

If $IY_{DG\ i}$and $IY_{CAP\ i}$ are determined by the algorithm as DG installation year and capacitor installation year, then the DG investment cost and capacitance will be as follows:

$$\beta\left(IY_{DG,i}\right) = \frac{1}{(1+d)^{IY_{DG,i}}} \tag{4}$$

$$\beta\left(IY_{CAP,i}\right) = \frac{1}{(1+d)^{IY_{CAP,i}}} \tag{5}$$

In the third part of the equation, which relates to feeder reinforcement cost, VY_i represents the year of increasing the capacity of the bus line *i* and $B_{r\,i}$ the cost of increasing the capacity of the bus line *i* and also the variable $\sigma_{R,i}$ The choice of whether or not to select bus *i* is to increase capacity, which is also a binary variable. The details of the *OC* operation part in relation (6) are as follows:

$$\mathrm{OC} = \sum_{t=1}^{T} \beta(t)[\sum_{k=1}^{N_{kk}} (US_{t,k} \times LD_k \times CS_{t,k}) + \sum_{i=1}^{N_{LB}} \sum_{k=1}^{N_{kk}} (OPC_{DG} \times S_{i,t,k}^{DG} \times LD_k)] \quad (6)$$

$$US_{t,k} = SS_{t,k} + Sloss_{t,k} - \sum_{i=1}^{N_{LB}} (S_{i,t,k}^{DG}) \qquad \forall k \in N_{kk}, \forall t \in NY \quad (7)$$

$$Sloss_{t,k} = \sum_{i=1}^{N_{LB}} \sum_{j=i+1}^{T} Lf((V_{i,t,k} - V_{j,t,k}) \times I_{ij}^{*}(t,k)) \qquad \forall k \in N_{kk}\,, \forall t \in NY \quad (8)$$

Relation (6) also consists of two parts, the first part being the cost of purchasing energy from the upstream grid. Each DG unit is assumed based on its operating costs compared to other available power sources (such as upstream or other power supplies), where $US_{t,k}$ and $CS_{t,k}$ the active power purchased from the upstream grid, respectively, and the price of electricity at the load level *k* of year *t*, and LD_k is the time constant of the load level *k*, expressed in hours. In the next section of this relationship, OPC_{DG} shows maintenance costs for DG units. Backups will be excluded from this calculation assuming maintenance costs are free and used only in emergencies. $S_{i,t,k}^{DG}$ Represents the output power of DG at bus *i* and at the load level *k* of year *t*, expressed in KW [11-13].

3. THE DEMANDED CONSTRAINTS

The equilibrium point of production and load in the slack bus must be established that this equation (9) and (10) Described for active power and reactive power. The amount of energy consumed by the load shall be equal to the amount of power produced, which equations for the active and reactive power are described in Equations (11) and (12), respectively. The voltage value in each bus must be within the acceptable range and not exceed its permissible range. This constraint is described by Equation (13) where V^{min} is the minimum voltage limit and V^{max} is the maximum voltage limit. The power throughput of the grid lines must be within its permissible range and ithe f excess investment is exceeded. It will be necessary to strengthen these feeders. In relation (14), $F_{i,t,k}$ is used as the throughput of bus *i* at the voltage level *k* of year *t*, and F_i^{max} and VY_i are the maximum bus throughputs, respectively. *i* and the year the feeder is fed to the bus feeder *i*. The DG output power must be within the minimum and maximum permissible limit per bus. So that $S_{i,t,k}^{DG}$ and $Q_{i,t,k}^{DG}$, respectively, the active and reactive DG power produced at the load level *k* in the bus i of t must be less than $S_{DG,i}^{min}$ and $Q_{DG,i}^{min}$ are the maximum active and reactive power produced by DG and greater than $S_{DG,i}^{min}$ and $Q_{DG,i}^{min}$ the minimum active and reactive power permitted by DG, which are the constraints on the relationships. (15) and (16) are described.

$$US_{t,k} = \sum_{j=1}^{T} |V_{1,t,k}||V_{j,t,k}||AD_{1\,j}| \cos(\theta_{1,t,k} - \theta_{j,t,k} - \delta_{1\,j}) \qquad \forall k \in N_{kk}.\ \forall t \in NY \tag{9}$$

$$UQ_{t,k} = \sum_{j=1}^{T} |V_{1,t,k}||V_{j,t,k}||AD_{1\,j}| \sin(\theta_{1,t,k} - \theta_{j,t,k} - \delta_{1\,j}) \qquad \forall k \in N_{kk}, \qquad \forall t \in NY \tag{10}$$

$$S_{i,t,k}^{DG} - S_{i,t,k}^{dem} = \sum_{j=1}^{T} |V_{i,t,k}||V_{j,t,k}||AD_{ij}| \cos(\theta_{i,t,k} - \theta_{j,t,k} - \delta_{ij}) \qquad \forall i \in N_{LB}, \forall t \in NY \tag{11}$$

$$Q_{i,t,k}^{DG} - Q_{i,t,k}^{dem} = \sum_{j=1}^{T} |V_{i,t,k}||V_{j,t,k}||AD_{ij}| \sin(\theta_{i,t,k} - \theta_{j,t,k} - \delta_{ij}) \tag{12}$$

$$\forall i \in N_{LB}, \forall k \in N_{kk}\,, \forall t \in NY$$

$$V^{min} \le |V_{i,t,k}| \le V^{max} \qquad \forall i \in T, \forall k \in N_{kk}\,, \forall t \in NY \tag{13}$$

$$|F_{i,t,k}| \le (1 + \sigma_{R\,i} \times M(t - VY_i + 1)) \times |F_i^{max}| \qquad \forall i \in N_{LB}, \qquad \forall k \in N_{kk}\,, \forall t \in NY \tag{14}$$

$$\sigma_{R\,i} \times M(t - IY_{DG,i} + 1)) \times S_{DG,i}^{min} \le S_{i,t,k}^{DG} \le \sigma_{R\,i} \times M(t - IY_{DG.i} + 1) \times S_{DG.i}^{min} \qquad \forall i \in N_{LB}, \qquad \forall k \in N_{kk}\,, \forall t \in NY \tag{15}$$

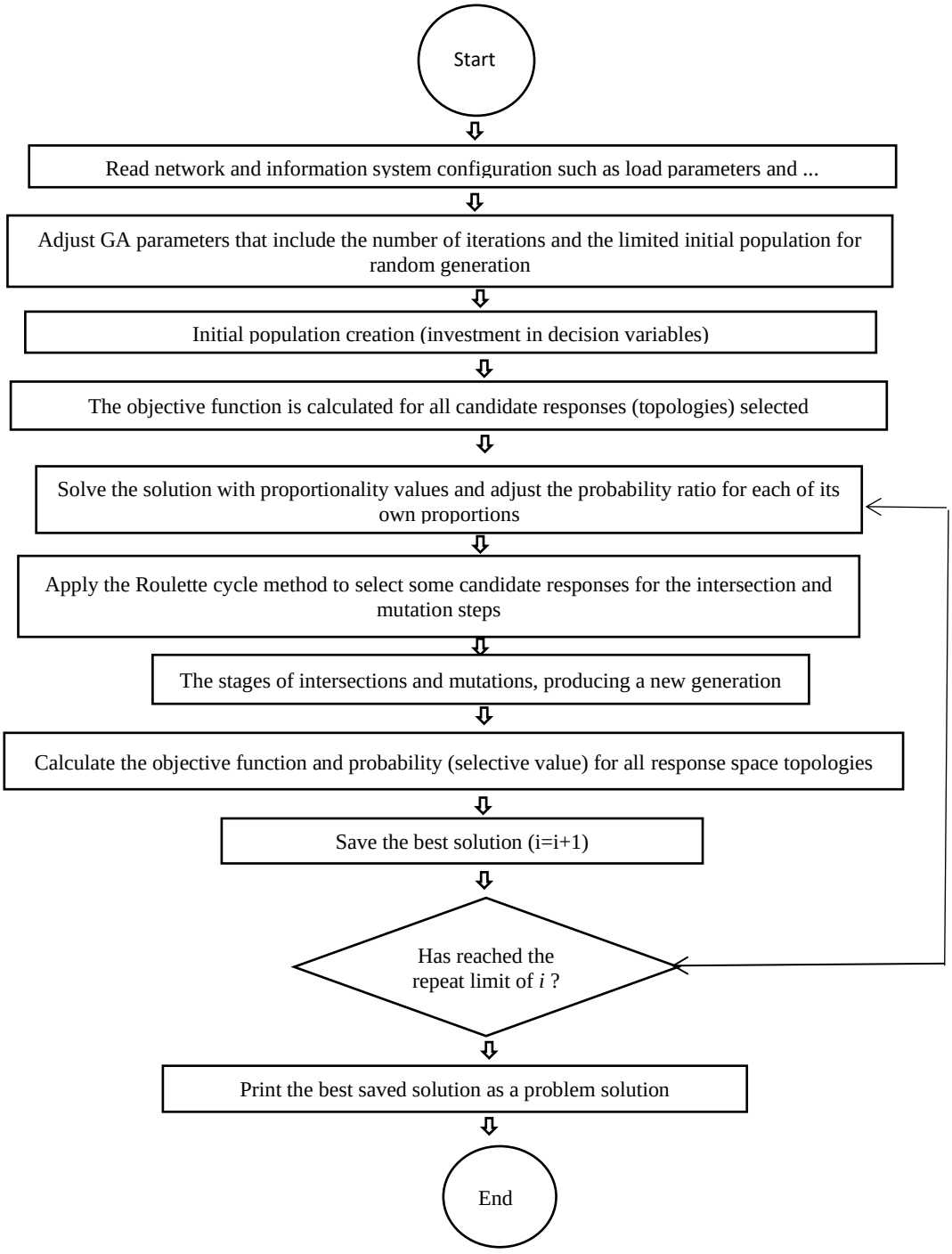
$$\sigma_{R\,i} \times M(t - IY_{DG,i} + 1)) \times Q_{DG,i}^{min} \le Q_{i,t,k}^{DG} \le \sigma_{R\,i} \times M(t - IY_{DG,i} + 1) \times Q_{DG,i}^{min} \qquad \forall i \in N_{LB}, \qquad \forall k \in N_{kk}\,, \forall t \in NY \tag{16}$$

4. PROPOSED ALGORITHM OPTIMIZATION METHOD

In this algorithm, they are first classified as primary populations by generating a set of random variables, including DG locations and capacitor locations as well as their installation time. These decision variables then move on to the next optimization to obtain their optimal capacity. This step is accomplished using the proposed model by adding a compound cost value (objective function value) to each decision variable, which represents the proportion and best DG capacities and capacitors capacities. Obviously, since the algorithm used in this problem processes binary variables, after changing the binary variables and the integer available for each algorithm response, the integer variables are converted to their equivalent binary form. Here the constraints of the problem are examined, and if the obtained parameters apply to the defined constraints, the values obtained are accepted and entered into the next step of the algorithm, otherwise they are removed from the set of possible problem solvers and then proceed to the next step of the algorithm. they do not. In this way, the algorithm continuously searches to satisfy all constraints defined in the problem and minimizes the objective function without violating the constraints. As stated above, after generating acceptable initial populations, they are ranked based on their fitness values and some are selected for intersection and mutation stages. After the intersection and jump operations, a new generation is obtained that is referred to the optimization process for the best value and evaluation of the fit value. New solutions are

selected by combining the best of them into new and old populations. This method is repeated frequently and the best response is stored at the end of each iteration so that the last iteration can be solved. Figure (1) shows the structure of the optimization algorithm.

Fig. 1. Optimization algorithm structure



5. SYSTEM STUDIED

In this paper, a 17-bus radial distribution network is intended for simulation. In this grid, a 23KV feeder with 17 bus, including 16 bus and a slack bus, is provided from the 63.23kV substation shown in the figure (2). The specifications of this system are presented in Table (1). Also, the planning period and annual growth rate are 4% and 5%, respectively. Gas generators are used as DG units with DGs installed capacity of 3, 2, 1 and 4MW combined with one MW units and their investment costs 0.89 M$/MVA at a rate of 10 $/MWh. It is assumed for the maintenance costs of the units. Also, a 1 MW unit is considered as a reserve unit that does not include maintenance costs. The annual cost of 5.5 $/KVar capacitance and the cost of generating active power at 120 $/KW peak as well as the cost of power purchased from the upstream grid 0.7541$. The heat capacity of grid feeders is 12 MW for 0.15 $M/km to strengthen each feeder.

Fig. 5. Single-line diagram of 17-bus distribution system

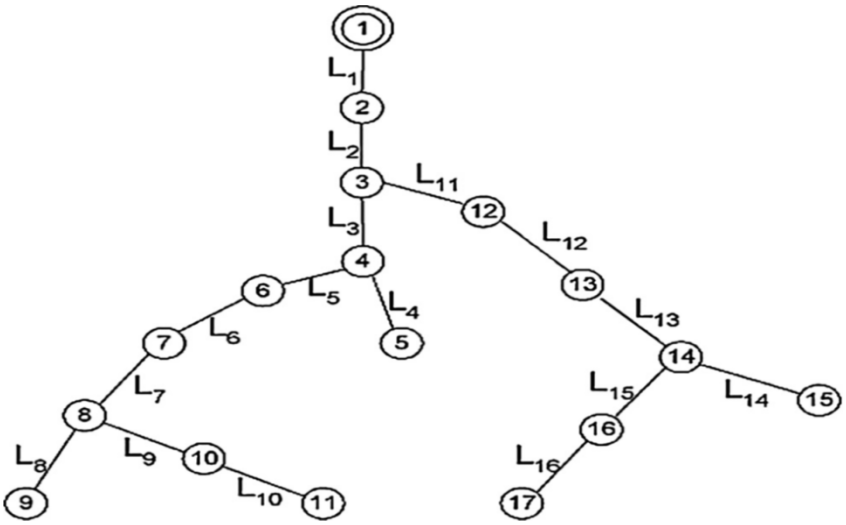


Table 1. Parameters used for 17-bus network

Bus Number	From	To	R(ohm)	X(ohm)	P(MW)	Q(MW)
1	1	2	0.05	0.05	0.8	0.6
2	2	3	0.11	0.11	0.8	0.6
3	3	4	0.15	0.11	0.8	0.6
4	4	5	0.08	0.11	0.8	0.64
5	8	6	0.11	0.11	1.2	0.16
6	6	7	0.04	0.04	0.8	-0.16
7	7	8	0.80	0.11	0.6	0.48
8	8	9	0.075	0.10	1.6	1.08
9	8	10	0.09	0.18	2.0	0.72
10	10	11	0.04	0.04	0.4	0.36
11	3	12	0.11	0.11	0.24	-0.20
12	12	13	0.04	0.04	1.8	0.80
13	13	14	0.09	0.12	0.4	0.36
14	14	15	0.11	0.11	0.4	-0.44
15	14	16	0.08	0.11	0.4	0.36
16	16	17	0.04	0.04	0.84	-0.32

6. ANALYZE THE RESULTS OF THE PROPOSED ALGORITHM

The numerical results obtained for the best response of the proposed 17-bus distributed programming problem-solving method are shown in the following analysis. In the simulation performed in this section, the effectiveness of the proposed genetic algorithm for solving the problem of distribution network development planning is evaluated. For this purpose, the results of the proposed algorithm are implemented for the 17-bus system. Table 2 shows further details of the optimal solution algorithm for the 17-bus system. In the development planning problem, DGs determine the capacity and position of the DG for each year from the planning horizon and the capacity and position for each of the capacitor banks as well as the feeder reinforcement time along the development planning horizon.

Table 2. Numerical results of the best response by proposed genetic algorithm solution in 17-bus distribution network

The cost of increasing the capacity of the lines	0.8474
Cost of electricity purchased from upstream grid	7.3651
DG Investment Cost	1.5621
DG operating cost	0.08712
Capacitor bank investment cost	0.7214
Price losses	0.9632
Location planning, capacity, and optimal year of installation with DG	First year: -- Second year:-- Third year:-- Fourth year: 2MW at bus 5 and 11
Optimal location, capacity, and year of installation of the capacitor bank in the planning horizon	First year:-- Second year:-- Third year:-- Fourth year: 1.2 and 0.83MVAr in line 4 and 7
Schedule for increasing the capacity of the lines	First year: Lines 3 and 8 Second year: Lines 6 and 14 Third year: Line 12 Fourth year: Line 7, 9, 10 and 15
The objective function (OF)	10.2631

7. CONCLUSION

This paper presents a new model for the problem of distribution network development to determine the optimal design of distribution network development over a specified period using distributed generation technologies and the existence of capacitive banks. Planning the development of distribution networks is a multivariate optimization problem involving both a spectrum of discrete and continuous decision variables. As such, the objective function of the proposed model is equivalent to minimizing the total investment and operational costs of the project using the proposed solution method. Also, the technical constraints governing the network, capacitor banks and DG units make the above model a nonlinear, rugged and complex integer optimization problem. So solving this problem with conventional analytical methods would be a complicated task. For this reason, in this paper, a genetic algorithm solution is used to minimize the total cost by choosing the best solution. The proposed model and solution method was implemented on a 17-bus distribution network.

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Research Article

EFFECT OF FTO rs9939609 POLIMORPHISM ON OBESITY IN TURKISH POPULATION

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ABSTRACT

Obesity is a disease that is affected by environmental conditions as well as genetic predisposition. This is a case-control study that aimed to investigate the relationship between FTO rs9939609 polymorphism and obesity. The relation between the rs9939609 polymorphism and obesity in 80 ower-weight (BMI≥30) and 131 under/normal weight (BMI<30) subjects was examined. The allele and genotype frequencies of each group were determined by the allele counting method. The relationship between single nucleotide exchange and obesity risk was calculated using the odds ratio. Statistical analyzes were performed with SPSS 18.0.

Results: The frequency of T allele was 0.58 and 0.45 respectively in the healthy and overweight group when allele frequencies of both groups were compared. The change in allele frequency increased the obesity rate by 1.8 fold. The rs99399609 polymorphism increases obesity risk by2.7 fold in the homozygotes model.

Conclusions: In this research, we found statistically significant results in allele frequency difference and allele positivity. The relationship between rs9939609 polymorphism and obesity will be highlighted by larger population studies.

Keywords: *Adiposity, FTO, rs9939609, BMI; Body composition.*

1. INTRODUCTION

Obesity is a major problem in the 21st century affecting the public health andis directly related to increased risk of premature death, coronary heart diseases, diabetes mellitus, cancer, hypertension, dyslipidemia and shock (Finkelstein et la. 2009). Obesity case havebeen increasing in the last three decades, the reason for this rise pointed as malnutrition, unhealthy lifestyles, immobility, and overeating (Hu, 2003; Rizzi et al., 2016).

Usually, lifestyle changes enhance treatment success and decrease obesity. However, with nutritional genomics, the importance of lifestyle changes gradually decreased. The discovery of obesity-related genes inspirits in an enhanced personalized treatment regime(Doaei et al., 2017).It's predicted that genetic factors are responsible for 40-90% of patients who hasbody mass index (BMI) variation. (Fawcett and Barroso, 2010).

One of these genes is the FTO gene, which exists onlyvertebrates and marine algae. The expression of the FTO gene is associated with food intake and energy balance. (Doaei et al., 2017, Guifang et al., 2008). FTO proteins were seen to have similar sequences with E. Coli enzyme AlkB (enzyme which hydroxylating DNA methyl groups and repairing DNA methylation damages) family proteins and its eukaryotic homologs. FTO gene proteins, oxidatively demethylates dsDNA3-meT in the presence of iron(II), dioxygen and 2-oxoglutarate. Therefore the nucleic acid demethylase activity on DNA and RNA was noted (Fawcett and Barroso2010; Speakman, 2015). Its affinity is primarily on RNA and thiamine /uracil due to its crystal structure (Speakman, 2015). Its thought that reversing the methylation by FTO can be a signal for gene regulation (Fawcett and Barroso, 2010; Guifang et al., 2008) and nucleic acid demethylase activity of FTO can regulate expression of metabolism enzyme genes and this dysregulation process can result in obesity (Fawcett and Barroso, 2010). FTO levels were less in starved animals while high lipid intake animals had increased FTO levels. An increase in FTO levels negatively affect food intake while a decrease in FTO levels induce food intake(Yeo, 2011).In rodents, FTO expression was bidirectional sensible to nutritional state and physical activity. Starvation state decrease FTO mRNA levels and FTO immunopositive cell numbersin the hypothalamus. This effect was recovered by intraperitoneal glucose. Functional coupling analysis has revealed that this issue may be in relationship with Brain-derived Neurotrophic Factor(BDNF) taking the role of food intake regulation (Speakman, 2015)

FTO gene has 9 exons and is located in the 16thChromosome (16.q12.2). The most strong signal of obesity is 1st and 2ndintrons of FTO gene. This region consists of 89 variants and approximately 47000 (Clausnitzer et al, 2015). In GWAS studies, in 2007, it was declared that a common variant of FTO gene (rs9939609) plays a predisposing role for Type 2 diabetes mellitus patients in the European population and that this relationship is seen to be mediated with BMI (Fawcett and Barroso 2010; Yang 2010).

Numerous evidences showed FTO SNPs are in relationship with apetite ratings, satiety, loss of eating control, obesity, diabetes and metabolic syndrome. In this study, we examined FTO rs9939609 polymorphism and obesity in Çanakale population.

2. MATERIAL AND METHOD

2.1. Ethics Committee Approval

This research has beenapproved by Canakkale Onsekiz Mart University Faculty of Medicine, Canakkale, Turkey ethics committee (Approval number 2011-KAEK-27/2016-E.26510) Written and verbal consent was btained from all the participants. This research was conducted with consideration of Declaration of Helsinki(revised-2000) principles.

2.2. Study design and implementation

Case-control study is conducted for genotyping and allelic profiles of polymorphic FTO gene in general population.

2.3. Patient profile

This study includes 131 control and 80 obese patient. Including criterias were BMI <30 kg/m^2 for control groups and BMI ≥30 kg/m^2 for case group.Excluding criterias were having any medical problem such as hypertansion, diabetes, metabolic syndrome, psoriasis etc. For both groups. A questionnaire was conducted for getting personal and familial medical history. Obesity diagnosis was made according to Body Mass Index (BMI).

2.4. Genotyping

All the blood samples were collected in EDTA tubes for genotyping. Genotype analyses were conducted with peripheral blood with Real-time PCR reaction after genomic DNA analysis made with commercial isolation kit according to supplier's instructions.FTO rs9939609 polimorphism was genotyped in case and control groups by real time PCR. Real time PCR reaction was conducted with 50 ng of genomic DNA,7.4 µl PCR-grade fluid, 1.6 µl Mg^{+2} solution, 4 µl probes/primers mix and 2 µl Master mix real-time PCR in a total volume of 20 µlreaction mixture. Thermal cycling was performed under the following conditions; 10 min at 95°C(hold step), denatauration step as 45 cycles at 92°C for 15 second followed by annealing/extension60°C for 1 min. FTO rs9939609 T>A polymorphism was geneotyped by TaqMan allelic discrimination assay.

2.5. Statistical Analysis

Allele and genotype frequences were determined according to allele counting method.Chi-square, pearson-chi-square, fisher exact test were used for comparing subgroups. All statistical analyses were conducted via SPSS statistical software. Lower than 0.5 of p value was expressed as statistically significant. Genotype relations and relative risk was evaluated with Odds ratio using Armitage test.

3.RESULTS

In this research, we evaluated the association between FTO rs99399609 polymorphism and obesity in Turkish population.Obese patients recruited in this study (n=80) had an avarage of 35.93±3.54 years, and the control group consisted of 131 normal-weight volunteers with an avarage of 35.08±15.17 years.The BMI distribution of obese and control groups were 35.01 ±2.49 and 25.40±4.49 respectively.We observed the distribution of the genotypes in obese group were as follows: out of 80 cases, 18 were determined to have the wild (TT) genotype, 36 the heterozygotous genotype (AT) and 26 the mutant genotype (AA). The wild allele frequency (T allele) for FTO rs9939609 polymorphism was calculated as 0.45±0.041 among obese individuals. Genotype distribution in the control groupas follow: out of 131 cases, 45 had the wild genotype (TT), 62 had heterozygous genotype (AT) and 24 had mutant genotype (AA). The frequency of the T allele was determined as 0.45 ± 0.04(Table 1).

Table 1. Genotypes and allele frequencies of the FTO gene rs99399609 polymorphism in obese individuals and healthy controls.

Genotype	**Obese (*n*: 80) (%)**	**Controls (*n*: 131) (%)**	***Odds Ratio* *P-value***
TT	18 (16.20)	45 (44.09)	
AT	36 (39.60)	62 (63.82)	AT vs AA OR: 1.058 95%CI: [0.35-3.198] P:0.92
AA	26 (24.20)	24 (23.09)	AA vs TT OR: 1.714 95%CI: [0.498-5.899] P:0.392
Allele frequency			**Allele freq. difference**
T allele	0.45 ± 0.041	0.58 ±0.031	Odds_ratio=1.689
	p=0.42 (Pearson)	p=0.74 (Pearson)	C.I.=[1.136-2.511] chi^2=6.76 p=0.009 (P)

We observed that mutant genotype was significantly higher in obese individuals then normal-weight ones and increased obesity risk was 2.7 times higher in in homozygotous model [OR: 2.71, C.I.=[1.24-5.90] and p-value:0.01]. In addition, allele possitivity increased the obesity risk 1.8 fold more [OR:1.80, C.I.=[0.953-3.407], chi^2=3.33 and p-value: 0.067]. In overal evaluation, the FTO rs9939609 increase the obesity risk 1.64 times in Turkish population. [OR:1.64, chi^2=6.33, p=0.012]. In contrast, there was no significant difference found in heterozygotous comparations. [OR: 1.45, C.I.=[0.733-2.876], chi^2=1.15, p=0.28](Table 2) .

Table 2. Comparison of FTO rs99399609polymorphism in different models among obese patient and control subjects.

Genotype	**Obese (*n*: 80)**	**Controls (*n*: 131)**	***P***
Dominant Model TT : AA + AT	**18 : 62**	**45 : 86**	OR: 1.802 95%CI [0.95-3.41] p=0.068
Recessive Model AA : AT + TT	**26 : 44**	**24 : 107**	OR: 2.14 95% CI [1.12-4.08] z-statistics:2.32 p: 0.020
Over-dominant Model AA + TT : AT	**44: 36**	**69 : 62**	OR:0.911 95%CI [0.52-1.59] z-statistics:0.329 p: 0.74

4. DISCUSSION and CONCLUSION

Numerous evidences showed FTO rs9939609 in relationship with apetite ratings, satiety, loss of eating control together with higher BMI across different populations (Frayling et al. 2007, Al-Serri et al 2018, Wardle et al. 2008, Hunt et al. 2008). Wardle et al. (2008) has conducted Satiety Responsiveness and Enjoyment of Food questionnaire to children and genotyped them for FTO rs9939609. The results showed that AA homozygote childen have a decreased satiety response score and increased adiposity. Tanofsky-Kraff et al (2009) investigated rs9939609 polymorphism and the eating behavior of the children. Their study showed that rs9939609 polymorphism didnot affect resting/basal metabolism rates but the loss of control during eating was declared frequently by adolescents. Children with AA/AT genotype preferred energy-dense, palatable foods more than those who have the TT genotype. Aside, 34,7% of AA/AT subjects has loss of control response while 18,2% of TT subjects shown loss of control response in 190 children (Tanofsky-Kraff et al., 2009) A meta analysis collecting data from 37 research revealedFTO rs9939609 genotype significantly effect total energy and carbonhydrate intake over large scale cohort including 177,330 subject (Qi et al., 2014). The relationship between energy intake, physical activity, and rs9939609 variant is researched in 1978 Afroamerican and Euroamerican subjects and significance were not noted and its seen that rs9939609 variant doesn't affect gender, energy intake, and physical activity in adiposity related phenotypes (Liu et al., 2010). In several studies FTO rs9939609 polymorphism is linked with different obesity related causes and rs9939609. Song et al (2008) have hown the cumulative effects rs9939609 risk-allele "A" with BMI and speculated that each copy of "A" increased 0.45 kg/m(2) in BMI and waist circumference.

FTO genetic variations are associated with obesity in several populations (Yang et al 2012, Wood et al, 2016). González-Sánchez et al have evaluated FTO rs9939609 gene and obesity and shown that the "FTO AA genotype" was more frequent and related to increased waist circumference in the obese individuals among the Spanish population. Merra et al (2020) have shown the association ofrs9939609 polymorphism with incerased BMI and android fat mass-FM% in Italian population. In another study, FTO rs9939609 is associated only with increased Body mass index but not obesity-related metabolic traits in Tawain population (Hsiao and Lin, 2016).

This research reveals interactions between FTO rs9939609 polymorphismwith BMI in Turkish population and our results were similiar to previous studies (Ağagündüz and Gezmen-Karadag, 2019; Solak et al., 2014). It could be speculated that genetic predispositionassociated with obesity. The relationship between rs9939609 polimorphism and obesitywas highlighted by larger population studies previously and we confirmed that FTO rs9939609 polimorphism increased the obseity risk in Turkish population.

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Research Article

EFFECTS OF SOME HEAVY METALS ON TOTAL PROTEIN AND PEROXIDASE ACTIVITY IN CLOVER AND VETCH PLANTS

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ABSTRACT

Forage crop farming is shown as the most important way of continuous and safe forage production. Clover and vetch are the most used forage plants. In this research clover (Medicago sativa) and vetch (Vicia sativa) plants were planted in large pots (48x18x18cm) containing peat: perlite mixture (3:1). The seeds were kept in distilled water for 3 hours before planting and swelling was ensured. After the peat perlite mixture prepared in pots was watered sufficiently with distilled water and then seeds were planted. 1000 ppm heavy metal stock solutions have been prepared for lead nitrate Pb(NO3)2, copper nitrate Cu(NO3)2.3H2O, and cadmium nitrate (CdNO3)2.4H2O. Prepared heavy metal solutions were given to the plants at once, with irrigation water, in 300 mL for each pot. Total protein amount and peroxidase activity were examined by spectrophotometrically 72 hours after the heavy metal application. Three different heavy metals applications to the forage plants, have been showed different physiological reactions in these plants. These reactions have been measured by comparing control group of plants. According to research results, it was determined that the maximum increase in the total protein amount in M. sativa compared with the control group was 100 ppm applied lead. The maximum increase in the total protein amount in V. sativa compared with the control group was 30 ppm applied cadmium, respectively. The increase in peroxidase activity has been determined in M. sativa and V. sativa plants compared with the control groups were 30 ppm applied cadmium. As a result of our research, it was determined that cadmium has been found to be more effective than lead and copper for stimulation of plant defense system.

Key words: *Clover, Vetch, total protein, peroxidase, forage plants.*

1. INTRODUCTION

The accumulation of heavy metals in the soil has important effects not only on soil fertility and ecosystem functions, but also on animal and human health through the food chain (Jiwan and Ajay, 2011). Some heavy metals in high concentrations affect animals, plants and who feed on plants adversely (Hejna et al., 2018). Heavy metals, which are poisonous in all respects, are released from various sources to the environment. It is one of the important causes of environmental pollution (Jaishankar et al., 2014). Plants being ion in soil solution that usually take the heavy metals in the form by their roots (Pinto et al., 2014).

Lead (Pb) is the first metal which causes the greatest damage to the ecological system with human activities. The passage of lead into the soil and atmosphere being in various ways. Among these ways, fumes from the chimneys of industrial establishments and vehicle exhaust, solder, battery, paint, waste from the electricity, petroleum industry and pesticides (Okcu et al., 2009). Cadmium (Cd), one of the heavy metals, is a highly toxic metal that has come to the fore with its important role in pollution today.

Important sources of cadmium which affecting human life; cigaret burning smoke, refined foodstuffs, water pipes, coffee, tea, coal, shellfish, fertilizers used in the seed stage and industrial production flue gases formed in stages (Seven et al., 2018). Copper (Cu) is an important element due to its involvement in plant enzyme activation, nitrogen fixation, protein metabolism, antioxidant activity, cell wall metabolism, carbohydrate and lipid metabolism (Emiroğlu et al., 2018). Copper toxicity is often found in plant root systems and protein synthesis, photosynthesis, respiration, ion causes disruption of some physiological events such as uptake and cell membrane stability (Sosse et al., 2004).

Forage crop farming is shown as the most important way of continuous and safe forage production (Ozkan, 2020). Forage crops agriculture, which has a very important place in agricultural activities, is the insurance of crop and animal production. Considering the situation of the agricultural feed plant in Turkey available forage crops are grown most clover plant within our acreage (36.6%) vetch with it as well (31.9%), corn (21.4%) and sainfoin (9.7%) is followed by plants (Yolcu and Tan, 2008).

When animal feeds contaminated with heavy metals are consumed by livestock, they may pass into products such as meat, milk and eggs and reach levels that threaten human health. There are not many scientific studies on the heavy metal levels of the feed consumed by the animal. Therefore, this research is important in terms of filling the gap in this field.

2. MATERIAL AND METHODS

2.1. Plant Materials

M. sativa and *V. sativa* seeds were planted in large pots containing soil perlite mixture (3:1) at the temperature of 24±2˚C, 28.000 lux light, under the conditions of 16 hours light and 8 hours dark. Plantlets were grown in controlled plant chamber.

The seeds are swollen before planting by soaking in pure water for 3 hours. The soil: perlite mixture prepared in pots was watered sufficiently with distilled water. After that seeds belonging to two plant species has been planted. Seeds has been planted forms the following groups;

- 1 = *M. sativa* control
- 2 = *M. sativa* application lead (100 ppm)
- 3 = *M. sativa* application cadmium (30 ppm)
- 4 = *M. sativa* application copper (50 ppm)

- 5 = *V. sativa* control
- 6 = *V. sativa* application lead (100 ppm)
- 7 = *V. sativa* application cadmium (30 ppm)
- 8 = *V. sativa* application copper (50 ppm)

2.2. Preparation of Heavy Metal Stock Solutions and Application to Plants

The following procedure has been followed in the preparation of stock solutions.

1000 ppm (1000 mg/L) stock solution was prepared for lead nitrate $Pb(NO_3)_2$, solution. The solution of 100 ppm was diluted with distilled water in 1000 mL each. 1000 ppm (1000 mg/L) stock solution was prepared for copper nitrate $Cu(NO_3)_2.3H_2O$ solution. The 50 ppm solution was diluted with pure water in 1000 mL each.1000 ppm (1000 mg/mL) stock solution was prepared for cadmium nitrate $(CdNO_3)_2.4H_2O$ solution. The 30 ppm solution was diluted with distilled water as 1000 mL each. Prepared heavy metal solutions were given to the plants at once 300 mL for each pot with irrigation water.

2.3. Harvesting Plants for Homogenization and Analysis

After 72 hours of heavy metal applications on *M. sativa* and *V. sativa* seed for four weeks, the above-ground parts of the plants were harvested and the standard homogenization method was used. After the homogenates belonging to all groups were centrifuged, their upper parts were taken and used for total protein and peroxidase analysis of the preliminary trials. All trials were carried out in triplicate.

2.4. Determination of Total Protein Amount

The protein standards used in this method were prepared from Bovine Serum Albumin (BSA) stock solution. For this purpose, 0.02 mg/mL from 2 mg/mL stock ampoule BSA; 0.04 mg/mL; 0.08 mg/mL; 0.12 mg/mL; 0.16 mg/mL and 0.20 mg/mL concentrations were taken and transferred to test tubes and the final volume was completed to 1000 µl. For the protein amount measuring, homogenates were transferred to the glass test tubes as 100 µl from the eppendorf and 5 mL of Protein Reagent Blue G-250 was added to each test tube. The amount of total protein was measured by according to Bradford (1976) method. All trials were carried out in triplicate.

2.5. Determination of Peroxidase (POD) Activity

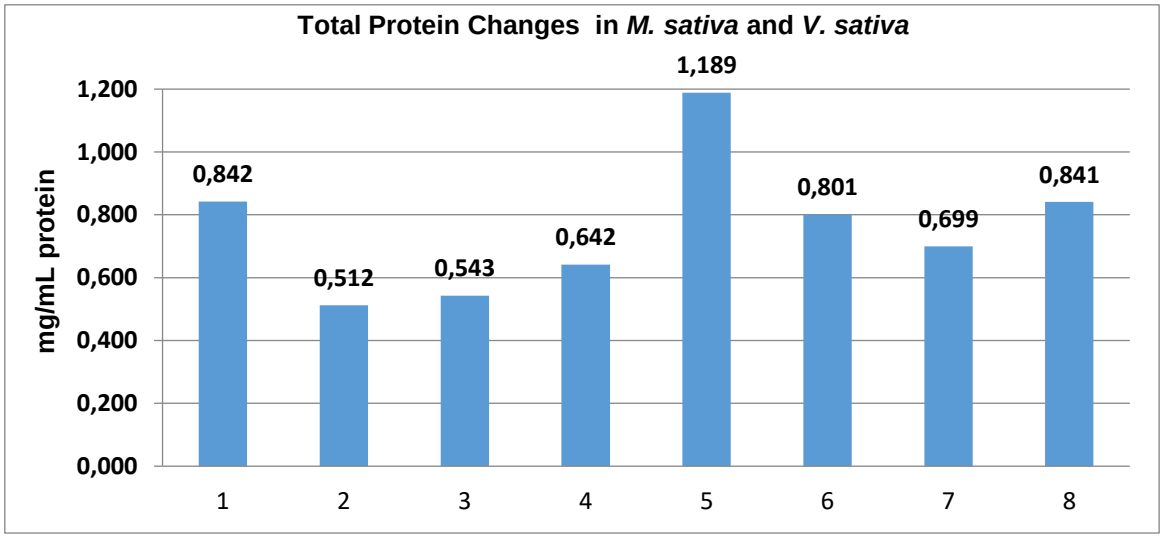
POD activity changes were performed by spectrophotometrically according to Kanner and Kinsella (1983). During the determination of the POD kinetic reaction, enzyme activity has been measured by spectrophotometer at 300nm for 120 seconds. The biggest differences between the absorbance values taken in every 10 seconds periods. The differences have been converted to mg protein level and given as mg/mL/min POD enzyme activity. All POD activity measurements were performed in three replicates.

3. RESULTS AND DISCUSSION

3.1. Total Protein Results

Changes in total protein amount in *M. sativa* (clover) and *V. sativa* (vetch) plants compared to the control groups are as follows (Fig. 1).

Fig. 1: Changes in Total Protein Amount of *M. sativa* (1-4) and *V. sativa* (5-8) Plants.

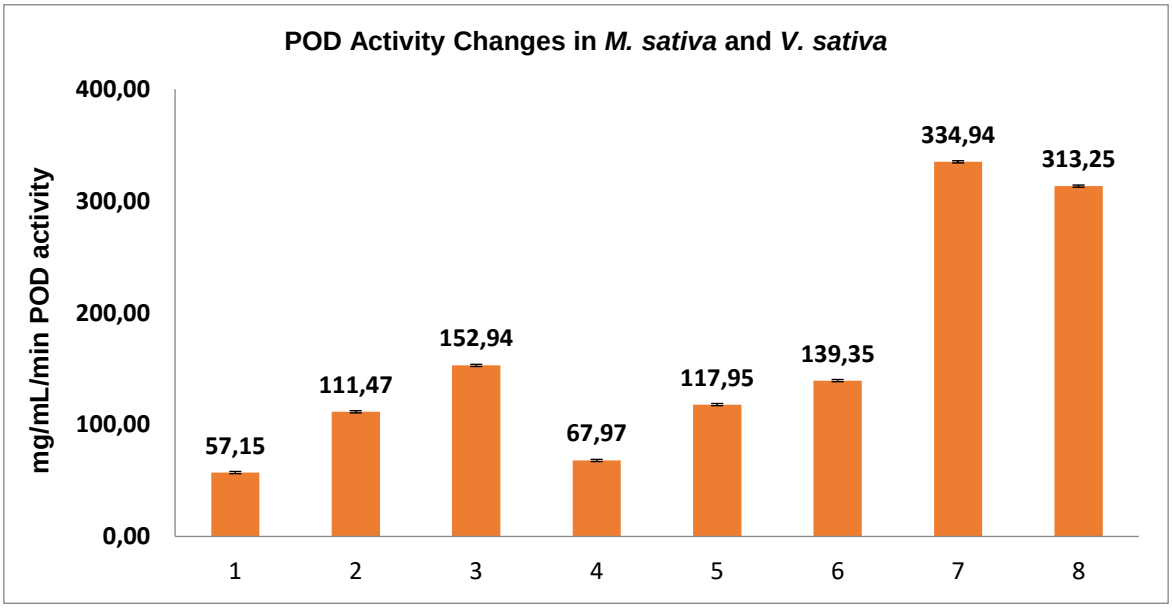


When compared to the control group in *M. sativa* plant, it was determined that the maximum decrease in terms of total protein amount occurred as a result of application of 100 ppm lead (Group 2).When compared to the control group in *V. sativa* plant, it was determined that the highest decrease in terms of total protein amount occurred after the application of 30 ppm cadmium (Group 7). According to these results, it is thought that the moderate application of lead in *M. sativa* has been inhibited protein mechanism at a level of 39%. In the *V. sativa* plant, it has been determined that the application of cadmium has been inhibited protein mechanism at a level of 41 (Fig. 1).

3.2. Peroxidase Results

Changes in peroxidase enzyme activity in *M. sativa* and *V. sativa* plants when compared with the control groups are as follows; (Fig. 2).

Fig. 2: Changes in Peroxidase (POD) Activity in *M. sativa* (1-4) and *V. sativa* (5-8) Plants



When compared with the control group of *M. sativa*, it was determined that the highest increase in POD activity occurred after the application of 30 ppm cadmium (Group 3). When compared with the control group of *V. sativa*, it was determined that the highest increase in

POD activity occurred after the application of 30 ppm cadmium (Group 7). According to our research results, it is thought that cadmium can stimulate the plant defense system in both plants and increase POD activities as plants perceive this heavy metal as a threat signal. Increases in POD activity against to the control group have been measured as 168% in *M. sativa* and 184% in *V. sativa* respectively (Fig. 2).

3.3. Discussion

According to our results regarding the total amount of protein and peroxidase enzyme activity in *M. sativa* and *V. sativa* plants 72 hours after heavy metal applications have been found to produce different responses in the two plants depending on the type of heavy metals. In our research, literature reviews were effective for selecting the heavy metal concentrations which applied to plants.

In the research of Kısa (2018), ascorbate peroxidase (APX), peroxidase (POD), and superoxide dismutase (SOD) activities are measured in the leaf and root of *Lycopersicon esculentum* which grown under the heavy metal conditions. All three enzyme activities showed induction after the treatment of Cd, Cu and Pb in the leaves of *L. esculentum* compared with control groups. Reduced peroxidase activity measured in all treatments of heavy metals in the roots. Cd treatment increased the SOD activity on the contrary, copper showed opposite effect in the increasing doses of copper in roots.

In another research, Cd application showed reduction in plant growth. Different doses of $CdCl_2$, added to the growth media reduced the area of the leaf, chlorophyll and carotenoid contents in the radish plant. In addition that, increasing in catalase (CAT), guaiacol peroxidase (GST) and POD enzymes have been measured (El-Beltagi et al., 2010).

Similar to our research, there are studies in which the same heavy metals are used in different plant systems. It is normal to see physiological differences in different plants as a response. In our research, analyzes were carried out from the regions belonging to the aboveground parts of vetch and clover plants. Heavy metals used in our research are currently and widely used by other researchers.

4. CONCLUSION

As a result of the widespread use of heavy metals, the load on the ecosystem is increasing day by day. As seen in our research, it was determined that Pb, Cu and Cd heavy metals applications caused different physiological responses in clover and vetch plants. The presence of heavy metals that may come from irrigation water or other sources in areas where such forage crops are grown poses a great threat. In terms of the quality of soil and source of irrigation water are very important for plant development. If we notice to this point, forage plants which economically importance can growth well in this healthy ecosystems and by this way we can prevent the damages which such plants can cause to other living things through the food chain.

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Research Article

THE EFFECTS OF PYRETHRUM EXTRACT ON *Galleria mellonella* HEMOLYMPH PHENOLOXIDASE ENZYME

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ABSTRACT

Pyrethrum is a natural neurotoxic insecticide which is obtained from the flowers of Chrysanthemum cinerariaefolium plant. Pyrethrum extract causes DNA damage, genotoxic effect, induction of autophagy and apoptosis, mitochondrial dysfunction, oxidative stress, inhibition of biochemical processes. The greater wax moth Galleria mellonella L. (Lepidoptera: Pyralidae) is gaining increasing attention in immunity studies as an invertebrate model organism. Melanization, which is the most important response of invertebrate humoral immunity, occurs when inactive prophenoloxidase turns into phenoloxidase enzyme. Changes in phenoloxidase enzyme activity are an important marker for humoral immunity. In our study, the phenoloxidase enzyme activity of hemolymph collected from G. mellonella larvae treated with different doses of pyrethrum extract was determined by reading against a certain absorbance in an ELISA microplate reader. The findings obtained from this study showed that 0.6 mg/ml pyrethrum extract increased phenoloxidase enzyme activity. Doses above and below this dose did not cause a significant change in phenoloxidase activity compared to control groups. In the evaluation made in terms of the change of enzyme activity over time, while the enzyme activity increased rapidly in the first 15 minutes, the enzyme activity rate decreased after the 20th minute. The effect of pyrethrum extract on phenoloxidase enzyme activity in G. mellonella larval hemolymph at a certain dose is consistent with the literature. The reason for this effect of the extract is closely related to its genotoxic and cytotoxic effects.

Keywords: *Pyrethrum, Galleria mellonella, phenoloxidase, hemolymph, enzyme activity*

1. INTRODUCTION

The current upward trend of the human population has brought the problem of food production. The human population shows exponential growth, but agricultural areas show an arithmetic increase. The current structure of agricultural areas and techniques are insufficient to feed the growing population. In this case, pesticides used in the fight against agricultural pests are frequently preferred in order to obtain the highest yield per unit area in existing agricultural lands (Kurutaş and Kılınç, 2003). There are pesticides specific to many species such as herbicides, insecticides, fungicides, rodenticides and acaricides.

The role of insecticides in human society is very important (Pavela, 2016). Insecticides grouped as organophosphorous, carbamates, organochlorine and pyrethroids constitute the largest and most important pesticide group. Among these groups, pyrethroids cause lower toxicity in mammals and less residues in the environment than organochlorines and organophosphates (Kojima et al., 2004; Costa, 2008; Mnif et al., 2011). Pyrethroids, along with insecticide applications, have a wide range of usage areas including agriculture, medical, veterinary, aquatic system and pest control at home. Nevertheless, this widespread use causes people to be more exposed to pesticides (Radovanović et al., 2017; Romero et al., 2017).

Natural pyrethrins are obtained from flowers of the *Chrysanthemum cinerariaefolium* type known as "pyrethrum", which contains six active ingredients (Valentine, 1990; Arslan and Yilmaz, 1993; Anadon, et al., 2009; Palmquist et al., 2012; Yang et al., 2018). This type of flower is also consumed as herbal tea in some countries. The active ingredients found in natural pyrethrins are pyrethrin I-II, synerine I-II and jasmolin I-II. Although these substances show strong activity against many different types of insects, their permanence is very low and easily degrades after contact with air and sunlight (Anadon et al., 2009; Yang et al., 2018). Long-term low-dose exposure to pyrethroids can cause chronic diseases of the nervous system, immune system, cardiovascular system, and produce toxic effects including teratogenicity, carcinogenicity and mutagenicity (Tang et al., 2018).

Pyrethrins pass through the exoskeleton of insect chitin by passive diffusion and cause depolarization by preventing the closure of the sodium channels of the cell membrane in nerve and muscle cells. Their mechanism of action is to inhibit voltage-dependent sodium channels that regulate sodium permeability in the cell membrane, which is involved in the production of neuronal action potentials of insects. In addition, sodium potassium inhibits ATPase channels and blocks reuptake, which stimulates the release of other neurotransmitters by disrupting the sodium gradient (Soderlund et al., 2002; Patel et al., 2007; Gupta et al., 2013). As a result of this change in sodium channels, either repetitive firing or neuronal depolarization is blocked, depending on the length of time the sodium channels stay open (Calderón-Segura et al., 2018).

Studies on cypermethrin (Taju et al., 2014; Huang et al., 2016), cyhalothrin (Deeba et al., 2016) and alletrin (Madhubabu and Yenugu 2014) have revealed that many synthetic pyrethrin types cause oxidative damage.

Insect and mammalian humoral responses include processes such as melanization, coagulation, and secretion of antimicrobial peptides (Sheehan et al., 2018). Among the humoral immune responses in insects, the most effective response is melanization (Lee and Ansstee, 1995). The formation of the black pigment called melanin, is catalyzed by the phenoloxidase (PO) enzyme, which is converted into its active form by the serine protease cascade (Vilmos and Kurucz, 1998). Hemocytes in insects are the only source of phenoloxidase (Ashida and Brey, 1998). The inactive phenoloxidase (PPO) that is synthesized in hemocytes, accumulates by cell breakdown in scar tissue or around the encapsulated invader (Vilmos and Kurucz, 1998). The layer formed around the foreign body as a result of melanization completely abstracts the

object from its surroundings and cuts its contact with the outside. Most of the biochemical pathways that cause melanin formation are common in both mammals and insects (Nappi and Christensen, 2005).

Galleria mellonella larvae are more likely to be used in experiments for many reasons such as low production cost, rapid breeding without special equipment, survival at 37 °C, 6 weeks of life cycle, no need for large physical areas for breeding, and generally not requiring ethical permits (Ignasiak and Maxwell, 2017). At the same time, the size of *G. mellonella* last instar larvae (250-300 mm) makes it easy for intraperitoneal injection of the compounds to be tested. In addition, the possibility of adding these compounds to food and exposure through the skin makes them stand out as a suitable invertebrate model organism for experiments. In addition, the insect immune system is functionally and structurally similar to the innate immune system of mammals (Browne et al., 2013), therefore invertebrate model organisms are preferred in immunity studies.

In this study, it was aimed to determine the effect of pyrethrum extract, a natural pesticide, on phenoloxidase enzyme activity in the model organism *G. mellonella* hemolymph. Phenoloxidase is the enzyme that carries out melanization, in other words the humoral immune mechanism, so plays a key role in humoral immune responses. The effects of pyrethrum exposed in various ways on immunity have been tried to be determined through the model organism. It is thought that an idea can be obtained about the effect of pyrethrin, which is the main active ingredient of *C. cinerariaefolium* plant that is collected from nature and consumed as tea, on the natural immune mechanism in animals.

2. MATERIAL and METHODS

2.1 Insect Rearing

The *G. mellonella* larvae were grown in 25 ± 1ºC temperature, 65 ± 1% relative humidity and 12:12 h. (light:dark) photoperiod conditions in the laboratory of Biology Department of Çanakkale Onsekiz Mart University. Adult male and female moths were placed in a 1 liter glass jar with 2 grams of natural blackened honeycomb. Since the larvae hatched from the eggs, the larvae were fed with 10 g of artificial food (Sak et al., 2006) daily. Last instar larvae (0.18 ± 0.02 g) were selected and used 271ort he experiment. The samples of *G. mellonella* larva surface were sterilized before used in experiments with 70% ethanol.

2.2. Pyrethrum Injection

Preliminary studies were carried out by dissolving Pyrethrum extract (Sigma, Germany) in 10% ethanol (EtOH). The LC_{50} value for the subjects was determined as 50 mg/kg. According to this value, 2 mg/ml was prepared as a stock solution for late stage larvae. The doses were prepared by diluting the stock solution with 10% EtOH at the rates of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.6 mg/ml. Control groups were determined as untreated and 10% EtOH. From these prepared doses, each subject was injected from the last proleg with the help of 5 µl microinjectors (Hamilton, USA). It was expected to act for 24 hours for post-injection experiments. Four replicates at each dose were performed for the experiments and 16 samples were used for each group.

2.3. Phenoloxidase (PO) Enzyme Activity

For the determination of phenoloxidase enzyme activity, 20 µl hemolymph leaking from the anterior segment of the prolegs through the hole opened with a sterile needle were collected from each Pyrethrum injected sample. The collected hemolymph fluid was then placed in microcentrifuge tubes containing 180 µl phosphate buffer solution ice-cold and immediately frozen at -20 °C without allowing it to darken. This hemolymph-phosphate buffer mixture,

which was dissolved before the experiment, was centrifuged at 10,000 g for 5 minutes in a refrigerated centrifuge (Hettich, Germany) at +4 °C and the supernatant was collected. 40 µl of this supernatant was taken and placed in a 96-well microplate. Then, 160 µl 3,4-Dihydroxy-L-phenylalanine (L-DOPA-Sigma-Aldrich, St Louis, MO) dissolved in phosphate buffer solution at a rate of 3 mg/ml was added onto the microplate. The prepared microplate was read in ELISA microplate reader (ThermoScientific Multiscan FC) at 490 nm (A_{490}) absorbance at intervals of 5 minutes from 0 to 30 minutes. The data obtained for each subject was determined as U/mg protein/min (Brookman et al., 1989).

2.4. Total Protein (TP)

TP determination in the study was carried out using the method of Bradford (1976). For TP determination in each subject, 5 µl of the collected supernatant was taken and placed in a 96-well microplate. 40 µl Bradford reagent (Sigma, Germany) and 155 µl deionized water were added into the supernatant. The prepared microplate was read at 595 nm (A_{595}) in an ELISA microplate reader (Thermo Scientific Multiskan FC). The data obtained were calculated as mg protein/ml.

2.5. Statistics

The data obtained after the experiments were evaluated with Tukey HSD by performing one-way-ANOVA with the SPSS v.20 program, in terms of differences between both duration and doses.

3. RESULTS

The changes in the total amount of protein according to the doses applied at the end of our study are presented in Table 1. According to the data obtained, the total amount of protein was determined the highest in the 0.2 mg/ml injection group and the lowest in the 0.6 mg/ml injection group. The difference between the groups was found to be statistically insignificant (F=1.405; Sig=0.191>0.05).

Table 1. Total protein values of *G. mellonella* hemolymph which injected by pyrethrum extract (mg protein/ml).

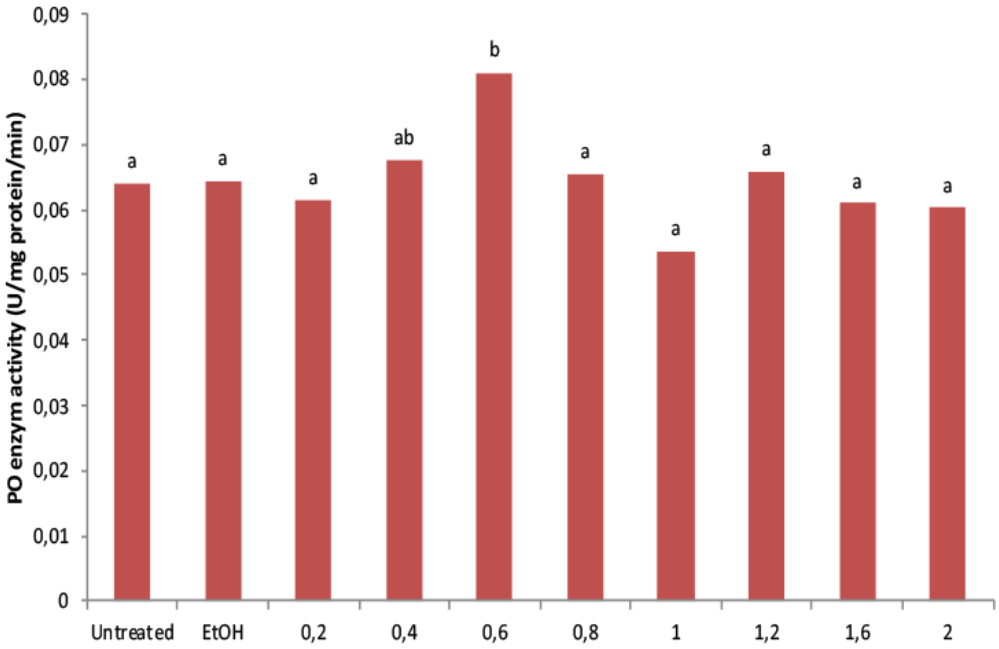
Doses	TP (protein mg/ml) ± SE*
Untreated	0,923070 ± 0,014
10% EtOH	0,919344 ± 0,018
0.2 mg/ml	0,962546 ± 0,013
0.4 mg/ml	0,928285 ± 0,011
0.6 mg/ml	0,909868 ± 0,016
0.8 mg/ml	0,931693 ± 0,009
1 mg/ml	0,937254 ± 0,012
1.2 mg/ml	0,929527 ± 0,014
1.6 mg/ml	0,912887 ± 0,008
2 mg/ml	0,914693 ± 0,011

*SE is Standart Error

The data obtained as a result of the studies on determining the phenoloxidase enzyme activity are presented in Figure 1. Accordingly, the highest enzyme activity was determined as 0.081 U/mg protein/min at a dose of 0.6 mg/ml, and the lowest as 0.054 U/mg protein/min at a dose of 1 mg/ml. The mean of the untreated group was determined as 0.064 U/mg protein/min. According to the statistical evaluation, the difference between the 0.6 mg/ml injection group

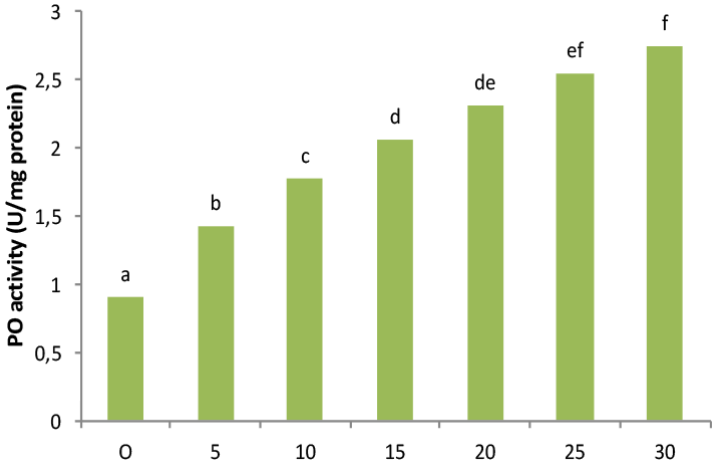
and the 0.4 mg/ml injection group is statistically insignificant, but the difference between the other groups is significant (F:4.553; df: 9; sig:000<0.05).

Figure 1. The effects of Pyrethrum extract on *G. mellonella* hemolymph phenoloxidase enzyme activity (U/mg protein/min). The difference between groups bearing the different letters in the figure is significant (p<0.05).



The change in phenoloxidase enzyme activity over time is presented in Figure 2. Accordingly, the enzyme activity increased linearly between the beginning and the 15th minute. Within this interval, the measurement at every fifth minute revealed a significant difference with the measurements before and after it. In addition, the difference between 15th to 20th, 20th to 25th, and 25th to 30th minutes was still insignificant, while the difference between 15th with 25th, and 20th with 30th minutes measurements was significant (F:108.510; df: 6; sig:000<0.05).

Figure 2. The change of *G. mellonella* hemolymph phenoloxidase enzyme activity avarage by time (U/mg protein/min). The difference between groups bearing the different letters in the figure is significant (p<0.05).



4. DISCUSSION

The biocidal products are preferred more than synthetic pesticides. With the increasing importance of ecological agriculture, natural insecticides are also increasingly met with interest. The primary toxic effects of pyrethrins, one of the natural insects, are related to their direct effects on the nervous system (Yang et al., 2017).

Deltamethrin, a pyrethrin synthesis, reduced the total hemocyte count in *G. mellonella* and showed genotoxic effect by inducing the formation of micronuclei (Kurt and Kayış, 2015). Deltamethrin even at a very low dose displays harmful effects by disrupting hepatic and renal function and causing DNA damages in pubescent female rats (Chargui et al., 2012). Rats treated with pyrethrin in the early period experience serious heart problems when they become adults. This situation is related to the damage in DNA in the early period (Vadhana et al., 2011). Organophosphorus insecticides cause metabolic and synaptic dysfunction as well as oxidative stress in *G. mellonella* (İçen et al., 2005; Alp and Coşkun 2018).

Studies on cypermethrin, a synthetic pyrethrinoid type insecticide, have shown that cypermethrin causes a decrease in protein, glycogen and lipid levels on Pimpla turionellae (Sak et al., 2006). Cypermethrin also increases life expectancy of female P. turionellae (Sak et al., 2009). It has been determined that as the dose of cypermethrin increases, it delays larval development and pupation time, decreases the pupation percentage and increases the mortality rate at *G. mellonella* (Sak and Uçkan, 2009). Ergin et al. (2007) in their work shown that sublethal doses of Cypermethrin could limit the development, survival, and growth of Apanteles galleriae due to possible metabolic, hormonal, and nutritional deficiencies.

The pyrethrin has a genotoxic effects and lowers the mitotic index (Azab et al., 2017). Yang et al. (2017), using the human liver cancer cell line (HepG2), found out that pyrethrins induce apoptosis, cause mitochondrial dysfunction, cytotoxic effect and DNA damage, induce autophagy, and cause oxidative stress in cells. Natural pyrethrins induce autophagy of HepG2 cells, so activation of the AMPK / mTOR signalling pathway may pose a potential risk to human health (Yang et al., 2018). It has been determined that prophenoloxidase activation is an integral component of the insect defence system, which includes a large number of enzymes (e.g. proteinases, oxidases, and dopachrome conversion enzyme) that immobilize and kill invading microorganisms (Zhao et al., 2007). During melanization, the conversion of inactive PPO to the active form of PO is provided by oxidative processes (Nakhleh et al., 2017); and this causes an increase in oxidative stress in the organism.

According to Chen et al. (2017), fenpropathrin, a type of pyrethrin, also causes an increase in total PO activity in honey bee (Apis mellifera). This increase is due to the moderate inhibition of fenpropatrin on the diphenolase activity of tyrosinase (Tang et al., 2009). Our results shown that the pyrethrum extract is effective on phenoloxidase activity at 0.6 mg/ml level (Figure 1). The data obtained from our study confirms the results of Chen et al. (2017). The injection of pyrethrum extract at the level of 0.6 mg/ml is inducing PO formation. The author suggest which is should be because of the try to deal with toxic effects in *G. mellonella* at that dose of pyrethrum. The upper doses of extract must have inhibited the biochemical process of tyrosine by increasing the oxidative stress of the organism as a result of higher exposure. In this way, PO activity decreased at high concentrations of pyrethrum.

5.CONCLUSION

It is understood from the literature that pyrethrum has negative effects on living organisms. These negative effects are such as the decrease in total hemocyte count, DNA damage, genotoxic effect, induction of autophagy and apoptosis, mitochondrial dysfunction, oxidative stress, inhibition of biochemical processes (Yang et al., 2017). The results of our

study are related to changes in hemocyte count and oxidative stress factors. Because hemocytes are the only source of phenoloxidase (Ashida and Brey, 1998) and activation processes of phenoloxidase are closely related to oxidative stress factors. Since the increasing oxidative stress interrupts the biochemical processes in the organism, decreases in PO activity are observed. The effects of pyrethrum on antioxidant enzyme activity will be determined by further studies. This studies; will be clarify changing in oxidative stress by pyrethrum applying. Determination of changes in hemocyte count will also help explain phenoloxidase enzyme activity. As a result of this study, the pyrethrum extract increased activity at a certain döşe

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Research Article

AN ENDEMIC PLANT GROWING IN KAZDAĞI IMPORTANCE AND USAGE AREAS OF Euphorbia anacampseros Boiss. var. anacampseros TAXON

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ABSTRACT

Morphological and anatomical characteristics of Euphorbia anacampseros Boiss. var. anacampseros taxa which is an endemic member of Euphorbiaceae family were investigated. Morphological characteristics and dimentions of these specimens were collected from Kazdagı National Park. Anatomical features (like cross-sections of the root and stem, cross and superficial sections of the leaf) of taxon were investigated.

Keywords: *Euphorbia anacampseros, Boiss. var., anacampseros, endemic, morphology, anatomy, laticifer, usage areas, cancer*

1. INTRODUCTION

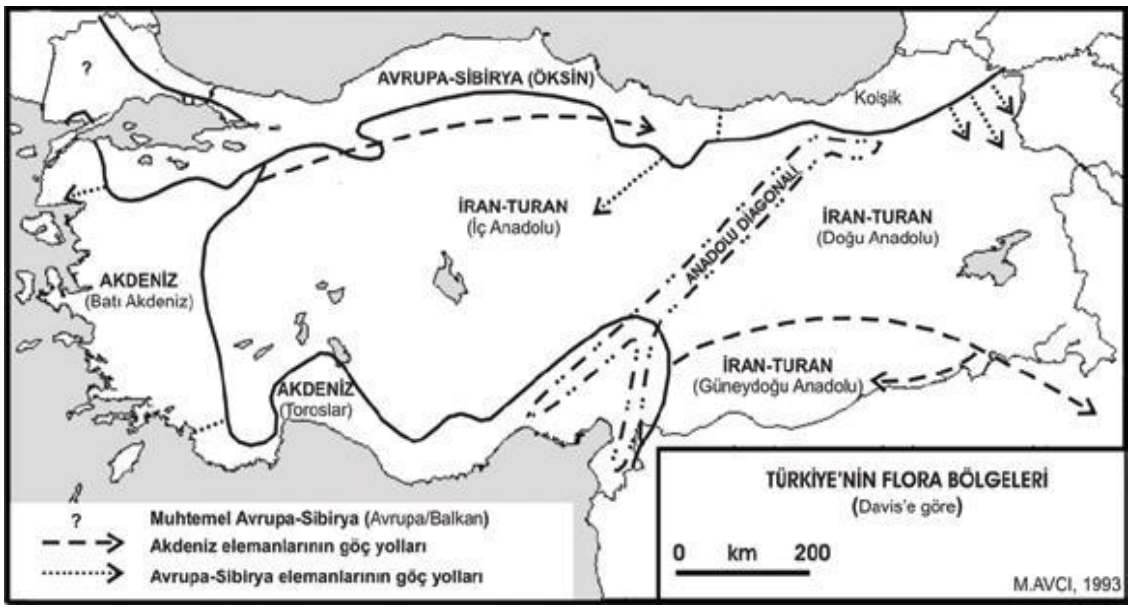
There is biological diversity on earth in the food chain. Many living species are connected each other in terms of the continuity of life and need to exist in the same process. Nowadays, Euphorbiaceae family which is increasingly importance and popularity in researches for cancer treatment, consists of succulent milky plants with mostly herbaceous forms and single or perennial species.

It is known that the Euphorbiaceae family is represented by approximately 8910 species in the world (Bercu and Popoviciu, 2015). The cosmopolitan of this family includes 300 genera and 102 species (Seçmen et al., 2004), but endemic ones are kept its importance, too. It has been recorded in our country are in the LC category (widely distributed and abundant species are placed in this category (Eken et al., 2006), according to the International Union for Conservation of Nature (IUCN) (Özgişi et al., 2017; Çalışkan, 2010; Yeşilyurt and Akaydın, 2017).

1.1. Study Areas

It constitues the border between the Euxine Region of the European-Sibirian Region and the Eastern Mediterranean Region (Ayaşlıgil, 2006), when Kazdağı is examined phytogeographically. The fact that Kazdağı is where the climatic characteristics and the three floristic regions (Özhatay et al., 2005; Gemici and Özel, 2001) meet increases the biodiversity in the region and its importance accordingly.

Fig 1: Floristic regions of Turkey (Avcı, 1993)



1.2. Usage Areas

It is known that some species of the *Euphorbia anacampseros* taxon are used for hunting by poisoning fish in rivers and lakes, and also causes poisoning by being given directly against some species such as malaria and jaundice and the risks posed by warts in terms of human health. In addition, it has been reported that the unconscious use of family members in medicine, raw materials in industry, and among the public causes an increae in cancer due to the risk factor of diterpenester (a chemical compounds) (Hecker, 1986). When the recent sources are examined (Luz et al., 2015; Mali and Jadhav, 2015; Erbay et al., 2018; Schippmann, 2018; Aylward et al., 2016; Avcı, 1993) in citotoxicity studies, especially in cancer, would healing, dental and acne treatment, industry and pharmaceutical industry, rubber extraction from latex

material, dye raw material, cultivation as ornamental plant and wax making, again, it seems that the latex material has a positive effect on the application of the chemical 'Ingenol angelate' used to suppress the growth of melanoma cells.

2. MATERIAL AND METHODS

In our study, *E. anacampseros* Boiss. var. *anacampseros* taxon has been chosen as the research subject. As a method, in the areas where the study taxon naturally grows in the Kazdağı National Park (from a weight of 600-700 m on 30.05.2019, its locality was determined on 10.07.2019), considering that the taxon is endemic, a sufficient number of herbarium name, morphological measurements and anatomical cross-sectional studies, and alcohol materials of the specimens were prepared.

Plant specimens don't have locality and herbarium number. were brought to the laboratory under suitable conditions and morphological measurements such as plant height and width, leaf height and width were taken and comparisons were made with the size details defined in the work named 'Flora of Turkey and East Aegean Islands' (Davis, 1982) (Table 1).

Table 1. Comparison of the morphological measurements of *Euphorbia* anacampseros Boiss. var. anacampseros

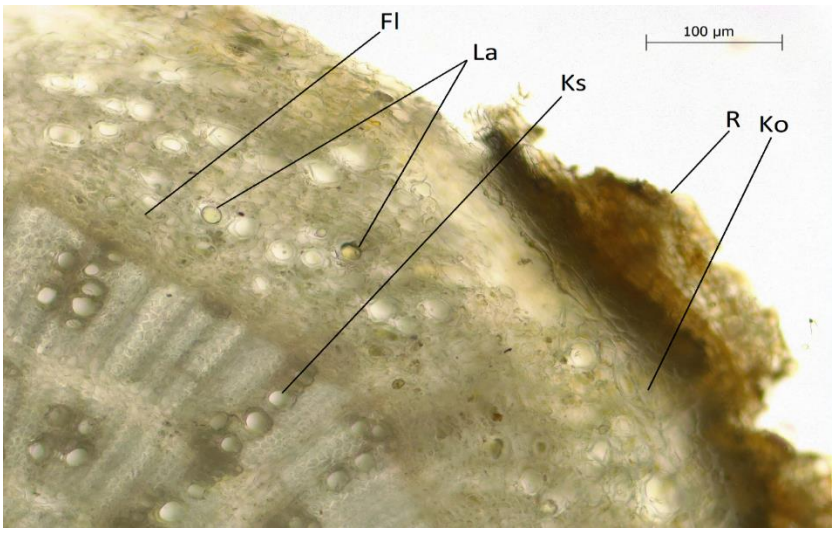
Davis (1982)	Genç (1989)	Tutgun (2020)
Root; decumbent and rarely exceed 20 mm.	Root; branched out.	Root; branched out and 1-5,5 cm.
It has been defined as perennial plants that are glabrous, drooping and rising from below at family level.	The umbella structure at the top of the body is rarely 3-5 beams, rays 2-2.5 cm.	Stem length 16.5-25 cm., width 0.4 cm.
Cauline leaves 20 mm., raylet leaves; 17 mm. and its dimentions (5-)10-35(-40) x (3-) 10-35(-40) mm.	Cauline leaves; leathery, fleshy, sesile, frequently alternating (0,9-1,6 cm.), ovate-rhombic, obovate, mukronate at the top, acute-acuminate, very fine toothed edges, side veins indistinct, leaf color mostly reddish pink, branching dichotomous up to 1 or 2 times.	Leaves; length 2.2, 2.4 and between 2.5 cm., width 1.3, 1.4 and between 1.5 cm., shape suborbiculate, margin entire, apex large and mucronate, color generally purplish.

Speciments were taken into a 70% ethyl alcohol solution prepared on the same day and fixed for 24 hours. The fixed specimens were protected at +4 ºC in the refrigerator. The cross and superficial section were taken for stem, body and leaf tissue examinations.

2.1. Anatomical Features of the Root

In cross-sectional examinations, there is suberinized and ligninized rhizoderm tissue on the outermost part and cannot be observed since the epiderma cells are crushed. Just below is the is the parenchymal cortex tissue consisting of an average of 10 cell lines. In the cortex tissue, there are secretory cells that carry latex. Under the cortex tissue, there is a conduction tissue showing a collateral structure. Phloem tissue occupies less space in general than xylem tissue cells, and cambium is not very prominent. There is a sclerenchymal pith under the conduction issue and in the inner most region (Fig 2).

Fig 2. Root cross-section of *Euphorbia anacampseros* Boiss. var. *anacampseros:* R:Rhizoderm, Ko: Kortex, Fl: Phloem, Ks: Xylem, La: Laticifer



2.2. Anatomical Features of the Stem

There are papillose structures in the cuticular tissue from the outside to the inside. Collencymatic tissue is located just below the single-row epidermic cells. The cortex tissue is composed of 12-16 rows of parenchymal cells in places and contains a number of latex-bearing cells that can be evaluated densely. In addition, milk pipers are located in the cortex tissue in places. From the end of the cortex cells, phloem tissue and xylem tissue just below respectively, form the collateral bundles. Cambium is not obvious. There are pith rays between the vascular bundle. Under the vascular tissue and in the innermost region there is the parenchymal pith. There are milk tubes containing latex between the pith tissue and the parenchyma cells. In cross-sections taken from well developed roots, it was observed that the pith regions were fragmented (Fig 3, 4).

Fig 3: Stem cross-section of *Euphorbia anacampseros* Boiss. var. *anacampseros:* P: Papillose E: Epidermis Ko: Cortex, La: Laticifer, Fl: Phloem, Ks: Xylem Ö: Pith region

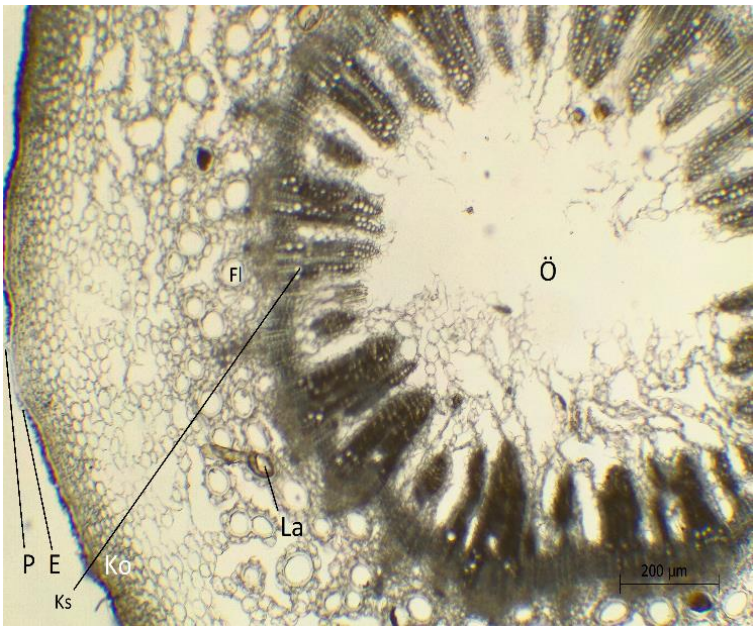
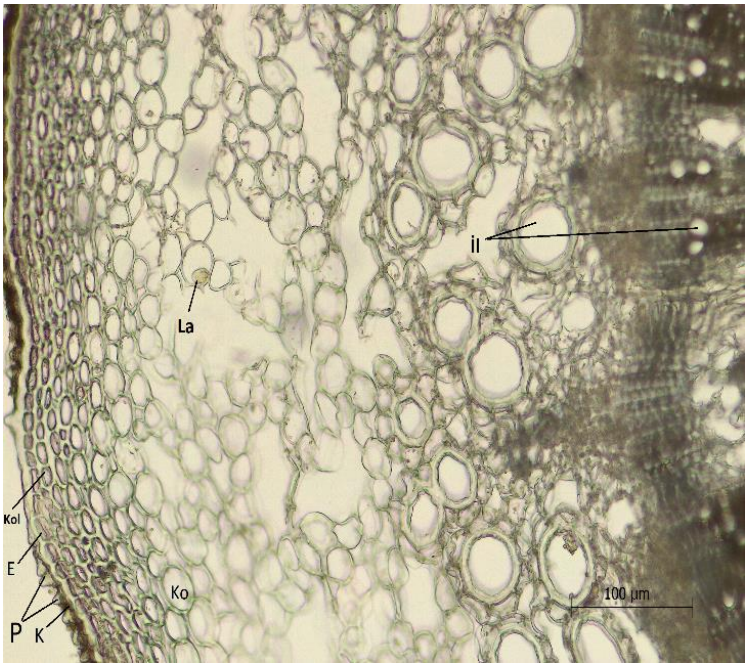


Fig 4: Stem cross-section of *Euphorbia anacampseros* Boiss. var. *anacampseros:* P: Papillose, K: Cuticle, E: Epidermis, Kol: Collenchyma, Ko: Cortex, La: Laticifer, İl: Vascular bundle, Ö: Pith region



2.3. Anatomical Features of the Leaf

The leaf is bifacial. From the outside to the inside, there are papillose structures in both the upper and lower tissue. Epidermis cells are in a single row under a thick cuticle layer. In the upper epidermal structure, there are 2-4 rows of palisade parenchyma cells with dense chloroplasts, sponge parenchyma cells with large intercellular areas just below. Vascular bundles are in collateral type. There are latex-bearing secretory cells throughout the mesophyll tissue. Stomas are of amphistomatic type. Stomata were observed to be anomocytic in the superficial sections taken from the leaves (Fig 5, 6).

Fig 5*:* Leaf cross-section of *Euphorbia anacampseros* Boiss. var. *anacampseros:* P: Papillose, K: Cuticle, E: Epidermis, Pa: Palisade parenchyma, Sü: Sponge parenchyma

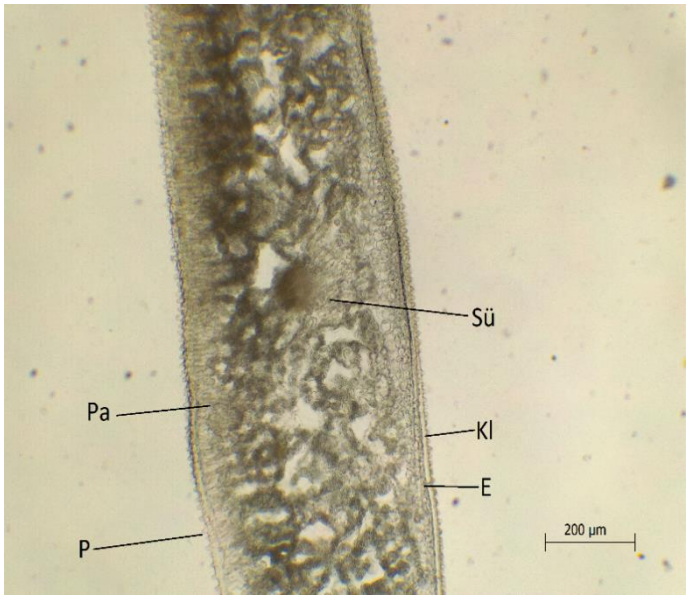
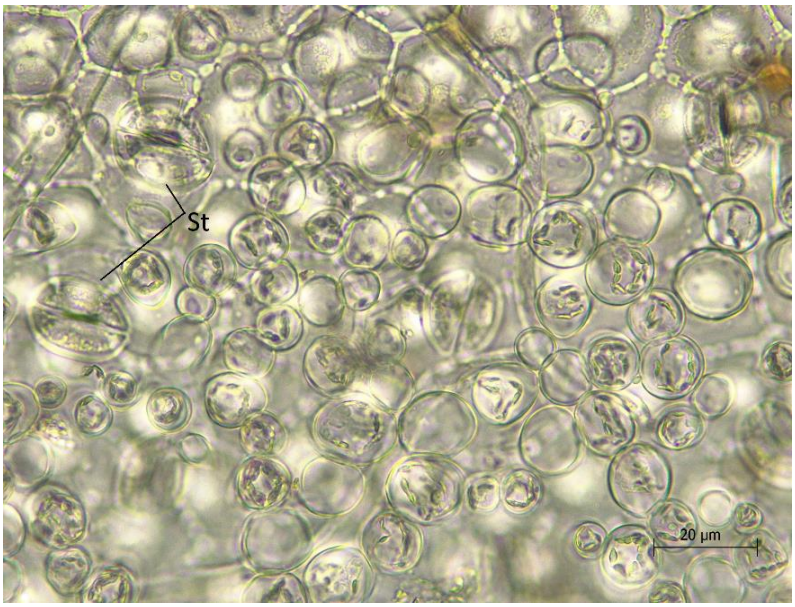


Fig 6*:* The appearance of stoma cells in leaf superficial section of *Euphorbia anacampseros* Boiss. var. *anacampseros*



3. DISCUSSION

In our study, the morphological and anatomical features of *Euphorbia anacampseros* Boiss. var. *anacampseros* taxon were examined.

The systematic of *Euphorbia anacampseros* Boiss. var. *anacampseros* taxon:
Regnum: Plantae
Phylum: Magnoliophyta
Classis: Magnoliopsida
Ordo: Malpighiales (Euphorbiales)
Family: Euphorbiaceae
Genus: *Euphorbia*
Species: *anacampseros*
Variety: *anacampseros*
Synonymous: *Euphorbia anacampseros* Boiss. var. *minor*

Euphorbia anacampseros Boiss. var. *minor* Boiss. in DC., Prodr., 15 (2): 174 (1862) was determined by Davis (1862).

As a result of limited research, Gökçen et al. (2018), in their morpho-anatomical study on *Euphorbia anacampseros* Boiss. var. *anacampseros* taxon observed anomocytic type stomata between leaf lower and upper surface epidermis cells.

4. CONCLUSION

In our study, some botanical features of *E. anacampseros* Boiss. var. *anacampseros* taxon such as morphological and anatomical structure. Besides this specimens were prepared for identification studies, especially in the herbarium symbol.

Due to the fact that *E. anacampseros* Boiss. var. *anacampseros* taxon, which has a natural distribution in the Kazdağı ecosystem, which is important in the field of biodiversity on a world scale, is included in 122 Important Plant Areas (IPA) of our country, is especially endemic, is in the LC category and is of great economic importance, negative effects of possible climate changes are in particular. It's must to take the necessary precautions within the scope of protecting all biotic factors from harmful factors by in-situ or ex-situ methods of Kazdağı National Park.

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