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RESEARCH ARTICLES

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RESEARCH ARTICLE

DEVELOPMENT OF AN EXPERIMENT SET FOR EMBEDDED SYSTEM EDUCATION AND ANALYZING ITS CONTRIBUTION

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ABSTRACT

In this study, a teaching material developed to provide application support to the theoretical expression of the embedded systems course in undergraduate and graduate education of engineering faculties is presented. The modular experiment set consists of STM32F4 Discovery microcontroller board and digital output, digital input, analog input, relay control, DC motor control, stepper motor control, alphanumeric LCD display, seven segment display and power distribution circuit boards connected to the board. The control software of the experimental set was developed using Waijung block sets in MATLAB / Simulink environment. The Waijung block set, which can be added to the MATLAB / Simulink library, allows the card to be programmed quickly and easily. At the same time, the program codes written by the user can be included in the developed model. With this experiment set, basic and some advanced embedded system applications can be performed. To research the availability of the experiment set in education, a group of undergraduate and graduate students was given the opportunity to use this set. Students were asked several questions about the experiment set and content analysis was performed on the answers obtained. In line with the data obtained, it was concluded that the experimental set developed eliminated a significant lack of material needed in the training of embedded systems.

Keywords: *Microcontroller Board, Embedded Systems, Engineering Education*

1. INTRODUCTION

Nowadays, the theoretical knowledge taught in vocational courses of engineering faculties should be supported by using appropriate educational materials in workshops and laboratory studies. The educational context presented in this way helps students reach the expected level of understanding and plays an important role in making the program successful and permanent [1], [2].

There are three different ways to use educational materials in vocational courses. The use of simulation programs is one of them. In the absence of a laboratory or experimental set, there are qualified simulation programs that can meet the need of students to make applications. Some of them are simulation-based computer programs such as Multisim, MATLAB / Simulink, MathCad, PSpice, Tina and SimQuick. In addition to these programs, there are simulation programs for electrical

machines, computer system, signal processing, electrical circuits, microcontroller circuits which developed for special purposes. As these simulation programs do not have installation cost, system fault and system maintenance, they are quite useful. Simulation programs have some limitations as well as superior aspects [3]. Although these programs are designed close to reality, the data calculated in theory never completely correspond to the real data. This is because simulation applications are developed to estimate actual system behavior.

The other type of material used in vocational courses is the web-based experiment set. Web-based experiment sets are designed for use in distance education. Web-based training includes real-time and remotely accessible experiment sets. The remotely accessible experiment set can be controlled in real-time with the code the user sends online. There are many sets of web-based experiment in the literature. Ko et al. studied a web-based laboratory for control experiments on a coupled tank apparatus a with multiple inputs and outputs [4]. Chang et al. developed a virtual laboratory that allows PLC experiments [5]. Hurley and Lee presented a web-based power electronics laboratory [6]. Yılmaz et al. presented a real-time web platform project for robot control [7]. Stefanovic et al. developed a web-based laboratory that helps students with learning materials, science concepts and engineering principles [8–10]. Pulijala et al. presented a web based virtual laboratory for electromagnetic theory course taught at undergraduate level, which can be used as a didactic tool by teachers and as a self-learning tool by students [11]. Berm´udez-Ortega et al. presented a new approach to develop remote practices for Systems Engineering and Automatic Control laboratories based on Easy JavaScript Simulations (EJsS) Raspberry Pi and Node.js [12]. Kaçar et al. developed an internet based remote access experiment setup that could be used as a support material in engineering education for DC motor speed control with PID controller [13]. Jović and Matijević covered a topic on design and implementation of web-based laboratory for programming and use of an FPGA device [14]. Solak et al. developed, realized, and proposed a web-based virtual and remote laboratory environment for real-time control and monitoring of a mobile robot in an indoor environment [15]. Zia et al. proposed an underwater web-based testbed that evaluates the UW communication system in a controlled aquatic environment and simulates the UW channel and sound propagation models [16]. The main advantages of the web-based experiment sets are to eliminate the absence of applications in distance education and to reduce cost by using the common experiment sets by many people. Besides these advantages, the problems that arise due to multiple users trying to connect to the system at the same time and the necessity to always have a person near the experiment set to solve potential faults are the disadvantages of the web-based experiment set. Although simulation programs and web-based experiment sets are widely used in engineering faculties for electrical and electronics education, it is difficult to say that they are completely adequate for vocational applications. This is because some applications require direct student-lecturer interaction.

Interactive test sets are used as training materials in the formal education laboratory. Here, the student applies the concepts, commands and theories that exist in the context of the course in an effective and meaningful way under the guidance of the lecturer. In addition, their interest in professions increases by making different applications that improve their abilities. This is because the knowledge learned becomes permanent by seeing, hearing, and touching. There are interactive experiment sets prepared for different courses in the literature. Bay and Görgünoğlu presented an educational set specifically designed for teaching and learning the 8051 family microcontrollers[17]. Güllü and Kaplanoglu developed an experiment set for AC servo motor training [18]. Demir and Duran studied an embedded experimental set for digital signal processors [19]. Aldeyturriaga et al. presented a laboratory equipment that has been employed in experimental activities to code real-time PID control algorithms [20]. Ranjith et al. described the design of a novel do-it-yourself educational tool that is

built using low cost materials, open source software and hardware, the designs for which are freely downloadable [21]. Alfonso et al. presented the design and implementation of a laboratory experiment for first year undergraduates of Bachelor of Science or Engineering degrees [22]. Sarı proposed an application of a PLC controlled system for practical education at electronics and automation laboratory [23]. Martinez-Santos described the development of courses in the field of digital design that use Arduino boards as their main platforms [24]. Bistak focused to the usage of the Arduino Uno platform for the measurement of static and dynamic characteristics of dynamical systems [25]. Aslam et al. presented designing of a software based 16-bit PIC24F series microcontroller educational trainer by using MPLAB and Proteus Professional software packages [26]. Apaydin and Serteller designed a 16F877 microcontroller training set for use in microcontroller courses [27].

In terms of cost and functionality, the presence of experimental sets created using the preferred products in the market in laboratories provides a great advantage to the students. At the same time, using up-to-date experimental kits helps students adapt more easily to the workplace environment. In this study, the design of an embedded system experiment set is carried out using the STM32F4 microcontroller board. The STM32F4 microcontroller board has been preferred due to its affordable cost, easy-to-use and high-end technical characteristics. Basic and advanced level electronic applications can be made with this embedded system experiment set. These are led blink, digital input-output, DC motor speed and rotation control, stepper motor speed and rotation control, alphanumeric LCD display, analog-digital converter, digital-analog converter and seven segment display applications. The experiment set is also suitable to be developed with the equipment that can be added later according to the need and level. The experimental set was made available to a group of 25 person, consisting of undergraduate and graduate students of the Department of Mechatronics Engineering at Karabuk University. And it has been concluded that STM32F4 experiment set is a beneficial educational material that can be used in the embedded system education based on feedback from users. In addition, this experimental set designed for use in the embedded systems course eliminates the absence of an important material.

2. EMBEDDED SYSTEM AND STM32F407VG Microcontroller Board

2.1. Embedded System

An embedded system is a digital system with processor and peripheral units. And these peripheral units are particularly designed to be solve a problem. In the embedded systems, specific, small, and more capable systems are used instead of general-purpose systems for the applications to be made. Unlike general purpose computer systems, these systems are specialized to perform one or more predefined tasks. Therefore, they interact with the user indirectly. The core of an embedded system consists of microprocessors or microcontrollers that are programmed to perform a certain number of tasks. Software programs are semi-permanent and named firmware in the embedded systems. They have a wide range of applications in many smart systems that we use in daily life such as mobile phones, copiers, bank ATMs, microwave ovens, washing machines and medical devices.

2.2. STM32F407VG Discovery Microcontroller Board

STM32F407VG is a microcontroller board developed with ARM7 structure and manufactured by ST Microelectronics. It has 1 MB Flash, 192 KB RAM and 32-bit ARM Cortex-M4F core. An SWD (Serial Wire Debug) connector is located on it for programming and debugging processes. It works with 5V supply voltage provided via a USB data bus or an external source. There are 3.3V and 5V supply outputs to be used in applications. LIS302DL, ST MEMS motion sensor, 3 axis accelerometer, MP45DT02, ST MEMS sound sensor, digital microphone, CS43L22 integrated D class speaker driver

is also located on the card. Additionally, there are 8 LEDs, 4 of which are user- defined, and 2 buttons, one of which is user-defined and the other is reset.

One of the most important advantages of this board, which has 140 GPIO pins, is that the pin configurations can also be defined by the user. This advantage allows the user to use the pins on the board by defining them as digital input, digital output, analog input, or analog output in parallel with what user wants. Another feature of STM32F4 that makes it advantageous is "Hardware in the Loop (HIL or HWIL)" support. Hardware in the loop simulation is a technique used in the development and testing of complex real-time embedded systems. In this technique, a platform is created that will enable the embedded system to be designed effectively and easily. And mathematical models that reflect all the dynamics of the system are included in this platform. Thus, tests and designs are carried out as if a real-world system has been used and all scenarios that may occur in the real system are evaluated with this simulation. It is often used in the control of complex systems such as land vehicles, satellites, space vehicles, unmanned aerial vehicles, planes, weapon systems, submarines.

3. DESIGN OF THE EXPERIMENT SET

In this study, an experiment set is designed to ensure that embedded system is learned in a more effectively and easily. The experiment set consists of STM32F4 microcontroller board and digital input, digital output, analog input, relay control, DC motor driver, stepper motor driver, alphanumeric LCD display, seven segment display and power distribution circuit boards. In this experiment set, features of STM32F4 board such as ADC, PWM, digital input-output, analog input-output can be defined, and applications related to these features can be made. The developed experiment set is taken into consideration as hardware and software.

3.1. Hardware

Modular circuit boards that are connected to STM32F4 board are designed in a manner that they can be used with all microcontrollers. These boards that are located on the experiment set can be combined to be one or multiple on STM32F4 according to the performed applications. The designed circuit boards are given as below:

1. Digital Input Circuit Board: In this circuit board, there are 5 buttons. Meanwhile, the condition of the button (logic 1 or logic 0) can be observed with the LED that is connected to the pressed button on this circuit board. Digital input circuit board is given in Figure 1(a). Different types of control applications can be made according to the information read from the digital inputs on this card.

2. Digital Output Circuit Board: Digital data output from STM32F4 microcontroller board can be monitored through 8 different channels by using 8 units of LED on this board. Many applications like LED scroller, binary counter, BCD counter and Gray code counter can be performed with this circuit board. Digital output circuit board for LED applications is given in Figure 1(b).

3. Analog Input Circuit Board: Analog input circuit board has a potentiometer, an LM35 temperature sensor, an LDR (photoresistor) and an empty input for any analog measurement. The desired analog data is taken from the sensors on this board. It is then converted into digital data by the STM32F4 board, and some mathematical operations are performed on it. Such analog readings as humidity, temperature, wind speed and light level can be measure with this board which must be supplied by maximum 3.3V voltage. Analog input circuit board is given in Figure 1(c).

4. Relay Control Circuit Board: This designed circuit board is a digital output circuit board at the same time. Here, the purpose is to control the relays that are switching units that work at higher voltages with a voltage of 3.3V that is taken from the microcontroller board. The position of relays in the circuit can be observed with the LEDs that are connected to their outputs. With this circuit board, opening and closing control applications of a motor, a lighting unit or any electronic system can be made. Relay control circuit board is given in Figure 1(d).

5. DC Motor Driver Circuit Board: The transistors on this circuit board are H-shaped to make the "H-Bridge motor driver" logic understood and applied in an easier manner. In the H-Bridge motor driver circuit, transistor pairs are used which are conducted crosswise. The DC motor can be driven by PWM pulses. At the same time, the rotation speed of the motor can be measured with the speed sensor placed in front of the motor shaft. In Figure 1(e), DC motor driver circuit board is given.

6. Alphanumeric LCD Display Board: This is a circuit board that is created to display the desired data like alert message, and user information or measurement values such as temperature, pressure, humidity, lighting level on the screen. Circuit board with alphanumeric LCD display is given in Figure 1(f).

7. Seven Segment Display Circuit Board: This is a display circuit board with seven LED units connected to the digital output pins of the microcontroller board. Since the display has a common anode connection, the shared pins of the LEDs are connected to the circuit's (Vcc). And the circuit is controlled by connecting the cathode pins to the circuit's (Gnd). It is designed to be a seven-segment display consisting of four units to suit many applications. Seven segment display circuit board is given in Figure 1(g). With this circuit board, digital date and time applications can be made.

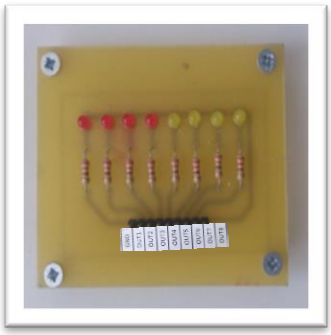
8. Stepper Motor Driver Board: An ULN2003 stepper motor driver is used on this circuit board. The ULN2003 is one of the most common motor driver ICs that houses an array of 7 Darlington transistor pairs, each capable of driving loads up to 500mA and 50V. The stepper motor can be driven by four digital output of the microcontroller board with the ULN2003 IC. Stepper motor driver circuit board is given in Figure 1(h).

9. Regulation and Power Distribution Circuit Board: Since a constant voltage value is not used in each application of the experiment set, this circuit board is used to provide the required different voltage values. 12V received from SMPS is distributed to necessary places with the switches and pins in the regulation circuit. There are 5 pins of 12V, 5 pins of 5V and 10 pins of GND outputs. The LED on the regulation circuit shows whether there is energy in the system. Regulation and power distribution circuit board is given in Figure 1(i).

These boards designed for the experiment set are modular circuit boards designed to perform basic and advanced applications with the STM32F4 microcontroller board and to teach the hardware features of the card in this way. They can be added to the STMF4 microcontroller board in different combinations depending on the application requirement and are also compatible with other microcontroller boards. The overview of the experiment set is given in Figure 1(j).


GND
IN1
IN2
IN3
IN4
IN5
+3V

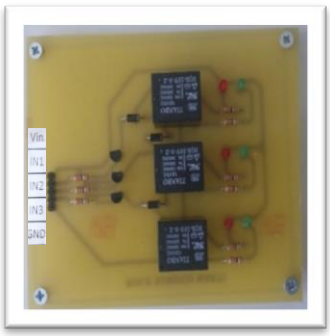
(a)



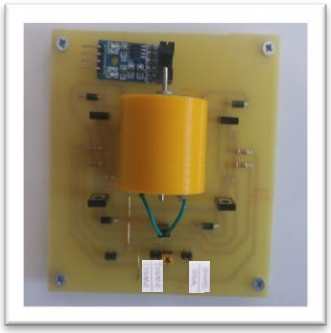
(b)


Vin
ADC1
ADC2
ADC3
ADC4
GND

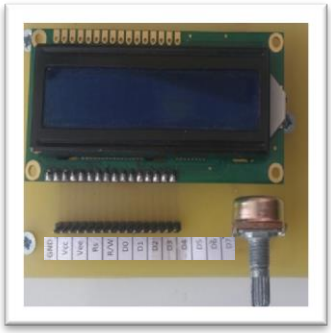
(c)


Vin
IN1
IN2
IN3
GND

(d)



(e)


GND
Vcc
Vee
RS
R/W
D0
D1
D2
D3
D4
D5
D6
D7

(f)


C1
P1
P2
C2
P3
P4
P5
P6
P7
P8
C4

(g)


12V
GND

(h)

(i)

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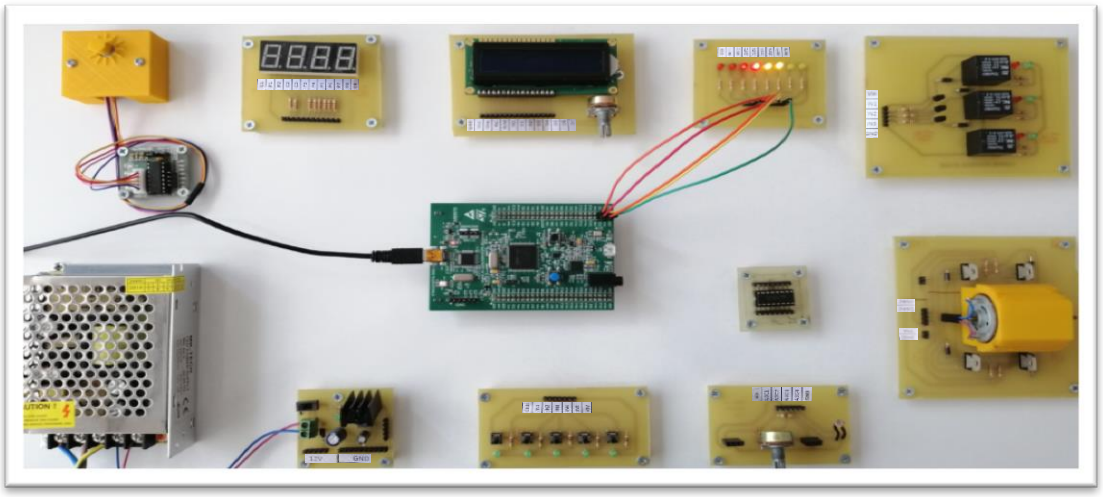

(j)

Figure 1. The developed circuit boards. (a) Digital output circuit board. (b) Digital input circuit board. (c) Analog input circuit board. (d) Relay control circuit board. (e) DC motor driver circuit board. (f) Alphanumeric LCD display circuit board. (g) Seven segment display circuit board. (h) Stepper motor driver circuit board. (i) Regulation circuit board. (j) Overview of the experimental set.

3.2. Software

In addition to being able to program the STM32F407VG microcontroller card produced by ST Microelectronics with programming languages such as C, C ++ and Assembly, it can also be provided with special package programs such as MATLAB / Simulink. MATLAB / Simulink provides a graphical environment for simulation and Model-Based Design of multidomain dynamic and embedded systems. In MATLAB / Simulink, main control applications such as system modelling, stability analysis, observation of system behaviors is carried out easily and with high accuracy. With Simulink block sets, basic concepts and theories can be taught more quickly and efficiently. Moreover, students can see the structure of a high-level system and can examine it.

With the compilation of the model developed in MATLAB / Simulink environment for STM32F4, the program files required for the embedded system are automatically created and loaded into the processor. For this purpose, the Waijung blockset is used in the study. Waijung blockset is a block set that is developed by Aimagin company that provides an option to make a Simulink-based application for its users. The "STM32F4 Target" block set included in Waijung blockSet is used to develop applications. Target Setup, Digital Output, Digital Input, Regular ADC, UART Setup, UART Tx, UART Rx, Timer IRQ, Basic PWM, Encoder Read blocks are the most used blocks.

The first step in developing applications with the Waijung blockset is to add the blockset to MATLAB/Simulink. The Waijung interface is used to compile a Simulink model created using the Waijung blockset. At the same time, the compilation process can be followed from this interface. To transfer code from the computer to STM, ST-Link Utility and ST-Link/V2 USB drives must be set up on the computer. The block diagram of code embedding process is given in Figure 2.

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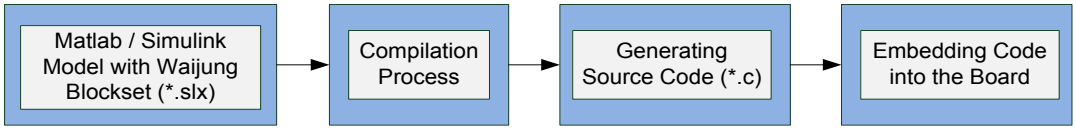

Figure 2. Block diagram of code embedding process.

It is possible to carry out many applications from basic level to advanced level in the created experimental set. The developed MATLAB / Simulink models with Waijung blocksets are given in Figure 3.

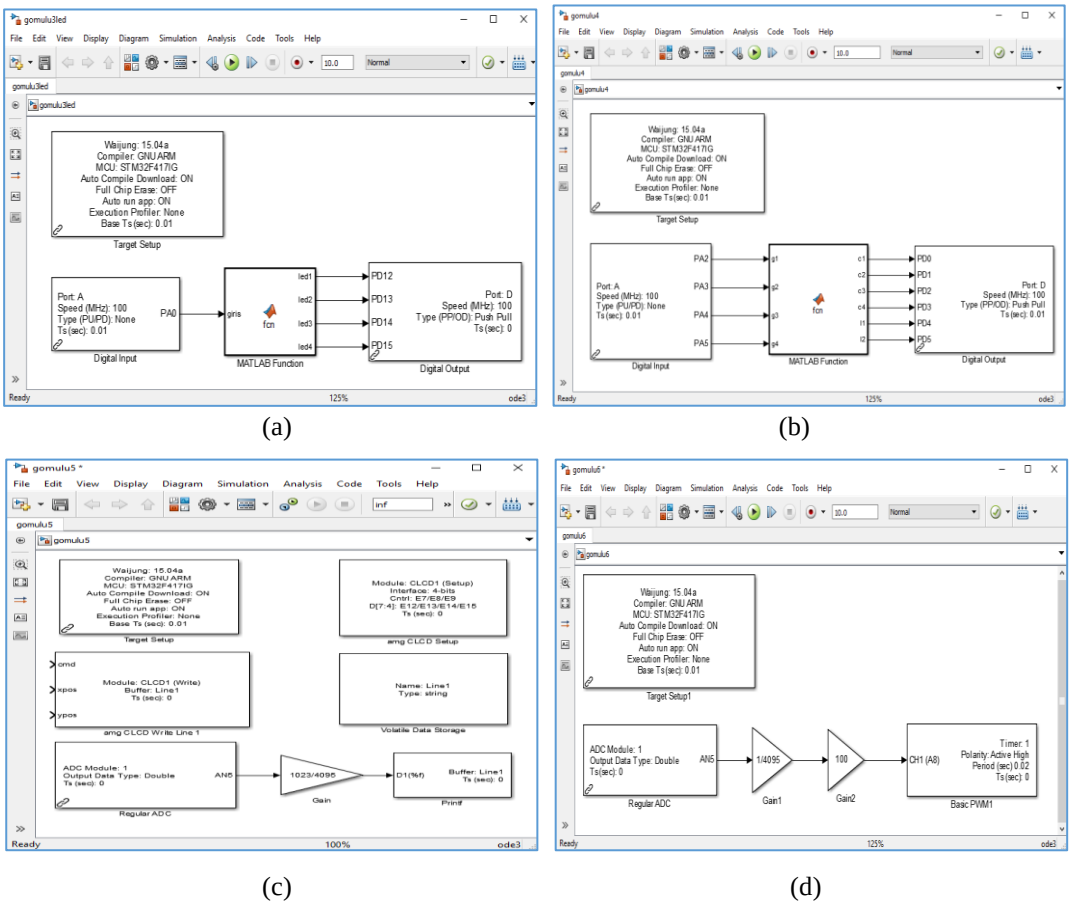

Figure 3. The developed MATLAB / Simulink models with Waijung blocksets. (a) Led - Button (Digital Input - Digital Output) application. (b) Seven segment display application. (c) Analog input and LCD application. (d) DC motor control application.

For example, if it is desired to create a LED scroller application, a new Simulink model screen opens in the MATLAB environment. While creating application model, the Target Setup block must not be forgotten, and the version used while making the arrangements must be selected. After configuring the

Waijung blockset, Digital Input and Digital Output blocks are placed on the model page. The pins that are desired to be used as digital input or output are defined. And a MATLAB Function block is added to the model to provide control between the digital input and digital output. The user can include own codes into the model with the MATLAB Function block. Similarly, many applications can be developed for STM32F4 board with the Waijung blockset.The developed model for led-button application is given in Figure 3 (a). In this application, the LEDs connected to the output according to the number of button presses are turned on respectively and then turned off. The developed model for seven segment display application is given in Figure 3 (b). In this application, in accordance with the data received from the digital input, the numbers shown from the seven segment display units are changed in the range of 0-9999. The developed model for analog input and LCD application is given in Figure 3 (c). In this application, analog data read from the potentiometer is seen on the LCD display. The developed model for DC motor control application is given in Figure 3 (d). In this application, the speed of the DC motor is controlled with PWM according to the analog value read from the potentiometer. In the developed models, Digital Input, Digital Output, Basic PWM, Regular ADC, amg GLCD and MATLAB Function blocks are used. Algorithms of applications are written to MATLAB Function block. All models and software developed are shared at https://drive.google.com/file/d/1dRbC6IHMVN62Vp2Uc2dymFMoXc1ZVe6l/view?usp=sharing

4. RESULTS AND DISCUSSION

The experiment set created in the study was tested with different MATLAB / Simulink and Waijung blocksets and it was seen that the system worked without any problems. To use the created experiment set, a group of 25 person, consisting of undergraduate and graduate students, has been formed in the Mechatronics Engineering Department. While determining the students in the group, they have a certain level of knowledge about electronics, circuit theory, system modelling, programming and the STFM2F4 microcontroller board. After the group of 25 person was created, a presentation was made explaining the use and purpose of the experiment set for all. In addition, a leaflet with explanations was given to each of them. As it is suitable for personal use, the students can make an application with the experiment set in the laboratory. In the studies conducted with the students, approximately 5 days were allocated to get the students' opinions and suggestions about the experiment set. Because these studies were planned for the times that were out of their lesson hours and each student was given 30-40 minutes to use and evaluate the experiment set. After allowing the students to use the experiment set, the following questions were asked:

1. Is it interesting to have a new (different) experiment set in the laboratory environment?
2. The use of MATLAB / Simulink and Waijung blocksets make the created application easier in terms of programming?
3. While making an application on the experiment set, do changing the parameters as you want and observe their results make any contribution to comprehend the subject that was taught in the lesson?
4. Do you think that you will use STM32F4 microcontroller card in your future projects, thesis, homework etc.?
5. Can learning STM32F4 microcontroller board's hardware and software features be considered a preparation for the working environment after graduation?

When the answers to these questions that were asked to the students were evaluated according to the content analysis, the findings were grouped under the themes of usefulness, physical suitability, cognitive suitability, development, and creativity. The themes created because of the evaluated

answers, the sub-themes created according to these themes and the frequency values calculated according to the scoring given are given in Table 1. In addition, graphical representation of experiment results also is given in Figure 4.

Table 1. The findings that were taken from the student views.

Theme	**Sub-themes**	**f**	**%**
Physical suitability	1. Raising interest and curiosity 2. Providing a usable environment	22 20	88 80
Productivity	3. Creating his/her own applications 4. Producing solutions	18 20	72 80
Cognitive suitability	5. Comprehension 6. Clarity	25 21	100 84
Utility	7. Usefulness 8. Raising awareness 9. Sense of success	25 22 24	100 88 96
Revenue	10. Success, happiness and increase in self-confidence 11. Contribution to the projects that they will take in the future	25 25	100 100
Creativity	12. Application development, production 13. Mental development, producing an idea	22 18	88 72

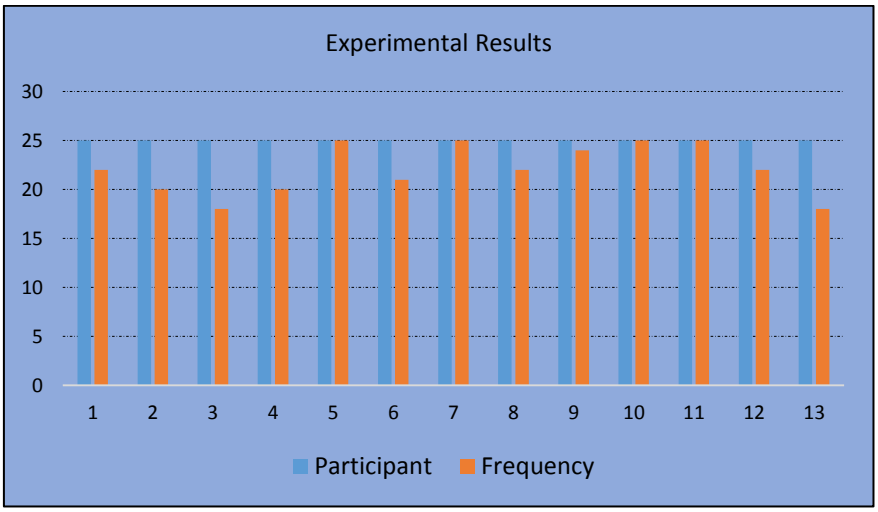

Figure 4. Graphical representation of experimental results.

According to the results obtained from the Table 1, it is concluded that the proposed material achieved an average success of 88,3% in these themes. Students stated that the existing experimental sets in the laboratory environment are out of date and the presence of an experiment set that allows the use of the

STM32F4 board raised interest and curiosity. It is understood from the given answers that this experiment set brings the characteristics of problem solving, establishing a cause-and-effect relation, analytical thinking and concreting of thoughts. Because it provides the opportunity for the students to create their own applications. They stated their views that no matter how good the theoretical teaching of the lesson is reinforcing the learned knowledge with application can create more persistent knowledge. Although the experiment set was seen for the first time, they stated that the applications that were needed to be made on the experiment set were understood quite clearly thanks to the preliminary information and distributed introductory brochures. They emphasized that making an experiment by changing as many parameters as they want during the experiment is quite instructive and allows them to observe the effect of each parameter change on the system. It was revealed that the experimental set was useful in achieving the intended outcomes and created awareness of modeling an embedded system. Most students stated that they do not prefer to use the STM32F4 microcontroller because they think it is more difficult and complex, but they can use it in applications for project-based lessons in the future. When students were told that the STM32F4 microcontroller board was the step to move to higher level processors, it was observed that students' concerns and prejudices about learning embedded systems decreased and their motivation to develop applications increased.

The created experiment set was designed to provide applied education support to the theoretical teaching of the embedded systems lesson that is in the curriculum of bachelor's and master's degrees. Thus, it is thought that it can be easier and more effective to learn the lesson and the absence of an important material that is needed in this field will be resolved by this means. In addition, STM32F4 microcontroller board, which is proposed to be used, will provide great convenience in the project-based lessons that they will make and the studies of their dissertations. As STM32F4 is a board that is preferred in the market, it will allow them to adapt easily to the working environment, when they graduate. Meanwhile, STM32F4 microcontroller board creates a basis for the high-level application developing boards such as DSP, Raspberry Pi and BeagleBone and makes it easier to move to these boards.

The experiment set has a modular structure and the applications that can be created are at the basic and advanced level. According to the students' knowledge level, the difficulty level of the applications, to be made, can be increased by diversifying the additional circuit boards that are added into the experiment set. The experiment set that is created in the study was planned to be used in the formal education. However, it can be made convenient for distance education by adding remote control modules to the study in parallel with the needs of today's education system. It was taken into consideration that the students interviewed were equivalent in terms of education and readiness levels. At the next stage of the study, it is planned to make the pretest-posttest application by benefiting from these data and a larger student group.

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RESEARCH ARTICLE

THE EFFECT OF THE DATA TYPE ON ANFIS RESULTS, CASE STUDY TEMPERATURE AND RELATIVE HUMIDITY

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ABSTRACT

In this study, the Adaptive Neuro-Fuzzy Inference System (ANFIS) was used to create models to predict mean relative humidity and temperature with the most suitable inputs. To find the most appropriate data type for these meteorological parameters both hourly-daily and raw-normalized data sets were used, and results were compared. The models were trained with 2014-2017 data observed at Kirkuk city station in Iraq, were checked with both 2018 data of Kirkuk and Sanliurfa city station in Turkey to investigate whether a model set with the data of a country could be used for another country data set. The execution of models was evaluated by using root mean square error (RMSE), mean absolute error (MAE), and determination coefficient R^2. Among the two parameters, the temperature achieved the best performance using relative humidity and dew point as input variables. According to the results, daily normalized data had lower error values and higher R^2 than hourly un-normalized data. Additionally, the results showed that the model performed successfully at the Sanliurfa city station on the temperature parameter because of similar climate conditions to Kirkuk city.

Keywords: *ANFIS, Normalization, Relative humidity, Temperature*

1. INTRODUCTION

Many academics have suggested many different methods or models to overcome the forecasting problems [1]. ANFIS is one of the most popular approaches to create prediction models. Generally, the results of this approach have been compared with the results of the other prediction models such as Artificial Neural Network (ANN), Multiple Linear Regression (MLR), and Multiple Nonlinear Regression (MNLR) [2] and ANFIS has gotten acceptable results for the prediction of parameters such as dew point temperature [3], evaporation [4-7], evapotranspiration [8, 9], rainfall [10-12], rainfall-runoff [13], groundwater level [14, 15], wind speed [16, 17], water usage [18], soil variable [19].

Scientists are trying to find the most suitable input combinations and the type of the method to generate fis to set the most accurate models. Additionally, various time scales such as minute [20, 21], hourly [22], daily [23-25], and monthly [26-29] are chosen to forecast different variables [30].

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Because of the major differences among values of the data used in the model can negatively affect the performance of the models, researchers generally use normalization procedure on data to improve the training speed and the accuracy of models. D_Min_Max [31], Z-score [32], sigmoid [33], linear, logarithmic, and square root methods [34] were used to investigate the importance of normalization in the forecasting by ANFIS.In this study, the most suitable input version is tried to be found by trying different input combinations for the prediction of temperature and relative humidity, which are the main meteorological parameters. Also, the results were compared using both hourly and daily data to determine which time series is more suitable for these two parameters. Besides, to investigate the effect of the normalization process on the outcome of the ANFIS method, the ANFIS method was applied to both raw data and data normalized with the max-min normalization method which is the most common normalization method.It is not easy to create models for each city, sometimes it is very difficult to obtain specific city data due to policy interference or the economic aspect or the lack of provision of the equipment and tools required to observe the weather condition. So, to investigate whether the model that is created based on a city data can be used for other city with similar climatic conditions, the model set with the data of Kirkuk station was checked with data of Sanliurfa station. May). The city is located at 35.4666° N latitude and 44.3799° E longitude and it is situated at elevation 346 meters above sea level in north-central of Iraq with a 9,679 km^2area of the city.

2. MATERIAL AND METHOD

2.1. Case Study

In this study, 5 years climate data of dew point (Dp), solar radiation (Sr), relative humidity (H), temperature (T), wind speed (Ws) and pressure (P) which their units Centigrade, Watt/m2, %, C°, m/sec, mm, and bar respectively have been used. Hourly data of parameters that were recorded by a meteorological station in Kirkuk city (Figure 1) were transformed into daily data by calculating their averages. Data of (2014-2017) have been used as training data and the data of 2018 used to be checking data.

Kirkuk climate is generally characterized by hot summers and rate rain in the winters, the area receives an average annual rainfall of 361.3 mm which occurs during the monsoon period (October to

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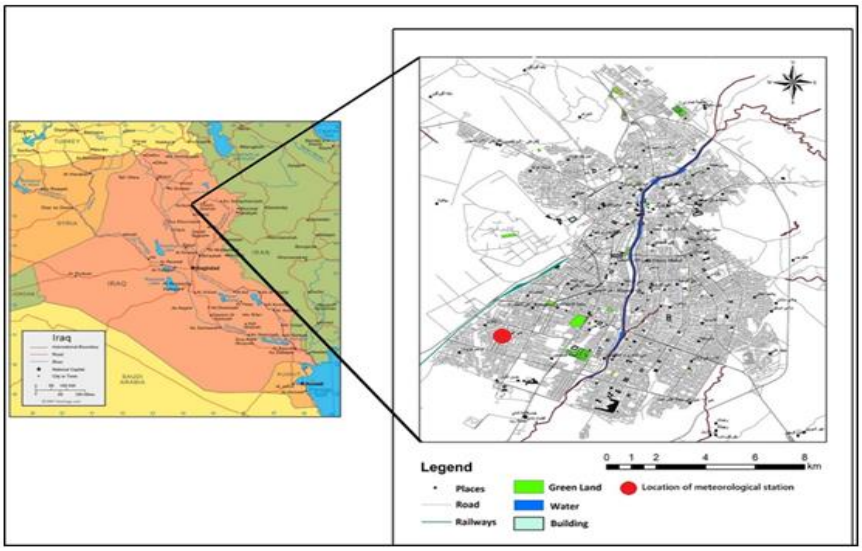

Figure 1. The location of Kirkuk station.

The results have been compared with the data of Sanliurfa city in Turkey, which have similar features with Kirkuk city. Sanliurfa city is at the southeast part of the country, the area of the city is 18,584 km^2. Its coordinates are 37°10′01″ N latitude and 38°47′38″ E longitude and it lies on 543m above sea level the climate here is mild, and generally warm and temperate. The rain in Şanliurfa falls mostly in the winter, with relatively little rain in the summer. The rainfall here is around 477 mm per year. The location of Sanliurfa station in Turkey is shown in Figure 2.

Figure 2. The location of Sanliurfa station.

Some statistical data of parameters are listed in the following tables. The tables show the mean, minimum and maximum values of parameters, standard deviation, skewness, and correlations. The tables consist of the data of Kirkuk and Sanliurfa for the years (2014-2017) and 2018 separately.

The statistical parameters and the features of the hourly dataset of 2014-2017 of Kirkuk city is presented in Table 1. The best value of correlation is between temperature and relative humidity 0.862, which means that they have a stronger linear relationship comparing with the other values of

correlation coefficients. Pressure has the lowest skewness value -0.042 and it can be called symmetrical. Wind speed has the highest value of skewness 1.837 which is highly skewed.

Table 1. The hourly statistical parameters of the climatic data of Kirkuk (2014-2017).

Data	**Units**	X_{mean}	X_{min}	X_{max}	**Standard Deviation**	**Skewness**	**Correlation with relative humidity**
Relative Humidity	%	40.75	4	99	24.20	0.605	1
Temperature	C°	24.04	-1.7	49.3	11.24	0.119	-0.862
Pressure	hpa	760.299	745.1	776.1	6.49	-0.042	0.687
Solar radiation	Watt/m^2	178.675	0	917	244.55	1.129	-0.383
Wind speed	m/s	0.676	0	6.7	0.81	1.837	-0.151
							Correlation with temperature
Relative humidity	%	40.75	4	99	24.20	0.605	-0.862
Dew point	Centigrade	6.687	-26.9	20.4	4.26	-1.022	0.309

Table 2 shows the statistical parameters of each variable for the dataset of 2018 for Kirkuk city. Among the data of 2018 of Kirkuk city, the best linear relationship is between temperature and relative humidity -0.836. Additionally, pressure has the less skewness that is 0.172.

Table 2. The hourly statistical parameters of the climatic data of Kirkuk (2018).

Data	**Units**	X_{mean}	X_{min}	X_{max}	**Standard Deviation**	**Skewness**	Correlation with relative humidity
Relative Humidity	%	37.436	8	97	20.59	0.961	1
Temperature	C°	27.059	4.6	49.7	9.84	-0.188	-0.836
Pressure	hpa	757.971	746.3	773	5.86	0.172	0.546
Solar radiation	Watt/m^2	194.488	0	880	259.36	1.06	-0.371
Wind speed	m/s	0.895	0	5.8	0.81	1.391	-0.124
							Correlation with temperature
Relative humidity	%	37.436	8	97	20.59	0.961	-0.836
Dew point	Centigrade	8.901	-9.6	19.2	4.11	-0.746	0.393

Sanliurfa city data gives a weak positive linear relationship by giving 0.105 as a correlation coefficient between relative humidity and dew point, the temperature has the less skewness -0.126, it indicates as symmetrical. The highest value of the standard deviation is 21.799 the result of relative humidity. The

highest correlation value is -0.798 and its the relationship between relative humidity and temperature (Table 3).

Table 3. The hourly statistical parameters of the climatic data of Sanliurfa (2018).

Parameters	Units	X_{mean}	X_{min}	X_{max}	Standard Deviation	Skewness	Correlation with	
							H	T
Temperature	C°	22.292	2.3	43	9.432	-0.126	-0.769	1
Relative humidity	%	45.02	9	98	21.799	0.637	1	-0.769
Wind speed	m/s	1.451	0	5.5	0.839	0.903	-0.349	0.38
Pressure	hap	947.61	933.7	964.2	5.578	0.224	0.203	-0.581
Dew point	C°	7.533	-10.2	22.7	5.443	-0.206	0.105	0.496

Among the daily dataset of Kirkuk relative humidity and temperature have the most influential correlation coefficient -0.871 which makes temperature the most effective parameter in that model. A symmetrical skewness is temperature, and it is 0.031 (Table 4).

Table 4. The daily statistical parameters of the climatic data of Kirkuk (2014-2017).

Data	Units	X_{mean}	X_{min}	X_{max}	Standard Deviation	Skewness	Correlation with Relative humidity
Relative Humidity	%	41.253	8.875	97.083	22.54	0.515	1
Temperature	C°	23.824	1.647	43.308	10.58	0.031	-0.871
Pressure	hpa	760.385	746.212	775.045	6.42	-0.056	0.728
Solar radiation	Watt/m^2	171.038	0	382.4	74.77	-0.132	-0.733
Wind speed	m/s	0.636	0	4.708	0.53	2.222	-0.189
							Correlation with temperature
Relative humidity	%	41.253	8.857	97.983	22.54	0.515	-0.871
Dew point	Centigrade	6.666	-21.962	17.808	3.96	-1.186	0.354

Despite the similarities in the characteristics of the two cities, there are some differences between their features. It is discovered that Kirkuk's average daily data can give better results than hourly data because taking average or arithmetic means give us a rough estimate about the common values in that set so that the calculations on all the values will be more or less the same. The average is a good measure of the dataset when a dataset contains values that are relatively evenly spread with no exceptionally high or low values (Table 5).

Table 5. The daily statistical parameters of the climatic data of Kirkuk (2018).

Data	Units	X_{mean}	X_{min}	X_{max}	Standard Deviation	Skewness	Correlation with Relative humidity
Relative Humidity	%	38.266	11.958	92.146	19.58	0.887	1
Temperature	C°	26.726	8.512	43.062	9.23	-0.356	-0.848
Pressure	hpa	758.076	747.237	771.862	5.81	0.136	0.606
Solar radiation	Watt/m^2	187.331	14.458	308.521	77.12	-0.413	-0.687
Wind speed	m/s	0.855	0	2.545	0.49	0.991	-0.183
							Correlation with temperature
Relative humidity	%	38.266	11.958	92.146	19.58	0.887	-0.849
Dew point	Centigrade	8.887	-5.883	17.4	3.84	-0.842	0.427

Sanliurfa daily dataset has almost the same results as hourly data there is not an obvious difference between the two performs of the datasets (Table 6).

Table 6. The daily statistical parameters of the climatic data of Sanliurfa (2018).

Parameters	Units	X_{mean}	X_{min}	X_{max}	Standard Deviation	Skewness	Correlation with	
							H	T
Temperature	C°	22.093	5.575	34.861	8.544	-0.305	-0.754	1
Humidity	%	45.671	18.21	96.166	18.967	0.805	1	-0.754
Wind speed	m/s	1.433	0.3	3.5	0.533	0.633	-0.481	0.546
Pressure	hpa	947.87	935.204	962.22	5.607	0.268	0.204	-0.623
Dew point	C°	7.556	19.487	-7.179	5.072	-0.327	0.046	0.594

2.2 Adaptive Neuro Fuzzy Inference System

The adaptive network-based fuzzy inference system (ANFIS) that was proposed by Roger Jang [35] is one of the systems that most widely used among fuzzy inference systems. ANFIS returns a Takagi–Sugeno FIS and has a network of five layers of feed forwards. The first hidden layer is used for fuzzification the input variables and in the second hidden layer T-norm operators are used to define the preceding part of the rule.

The third hidden layer normalizes the strength of the rule and the fourth hidden layer follows where the relevant rule parameters are calculated. The output layer measures all the inputs as the number of all incoming signals. ANFIS uses the learning of back propagation to test hypothesis parameters (to know about membership function parameters) and least square estimation to evaluate the correct parameters. There are two sections of a method of learning: In the first step, the patterns of input are propagated, and optimal consequent parameters are determined by the least mean square method,

although the parameters of the assumptions are believed to be defined for the current period through the training set. Within the second section, the patterns are transmitted. During this epoch, back propagation is used to change the parameters of the assumptions, although the related parameters remain fixed [14].

This system is a fuzzy Sugeno network configuration. Usually, this form of model is built and put within the context of a neural network model to facilitate adaptation. Figure 3 displays an ANFIS framework of two inputs, one output and two input rules. This system has two inputs x and y and one output, where its rule is shown in equations 1 and 2:

If x is A_1 and y is B_1 then $f = p_1x + q_1y + r_1$ (1)

If x is A_2 and y is B_2 then $f = p_2x + q_2y + r_2$ (2)

A_i and B_i are fuzzy sets, fi is the output of the fuzzy area defined by the fuzzy rule. p_i, $q_i$and r_i layout criteria that are determined during the training phase. Figure 3 indicates that each node in this layer is a fuzzy set and the output of any node in this layer belongs to the membership level of the input variable in this fuzzy set. Within this layer, the form parameters decide the structure of the fuzzy set membership function [2].

ANFIS has five layers and each layer contains several node function and node features. Nodes are classified into two groups: adaptive nodes and fixed nodes. The layers are defined as follows:

Layer 1: The nodes that exist in this layer are adaptive nodes.

$$Q_{i.1=}\mu A_i(x) \quad (3)$$
$$Q_{i.1=}\mu B_i(x) \quad (4)$$

Layer 2: The nodes are set and represented by a circle and identified by $\prod$. While W_i denotes the force of the rules. The output is determined on the basis on equation 5:

$$Q_{2.i} = w_i = \mu A_i(y)\pi B_i(y) \quad with\ i = 1.2 \quad (5)$$

Layer 3: All nodes are set and represented by a circle that called N. The name of the output of this layer is normalized firing strength. The output is determined by the i-th firing strength of the rule by summing up all of them.

$$Q_{3.i} = \bar{w}_i = \frac{w_i}{w_1+w_2} \quad with\ \ i = 1.2 \quad (6)$$

Layer 4: The nodes are adaptive nodes which are shown as follows: Consecutive criteria are p_i, q_i, r_i.

$$Q_{4.i} = \bar{w}_i f_i = \bar{w}_i(p_i x + q_i y + r_i) \quad (7)$$

Layer 5: The last layer in which the node is single and marked by $\sum$ and seen by the circle [36].

$$Q_{5.i} = \sum_i \bar{w}_i f_i = \frac{\sum_i w_i f_i}{\sum w_i} \quad (8)$$

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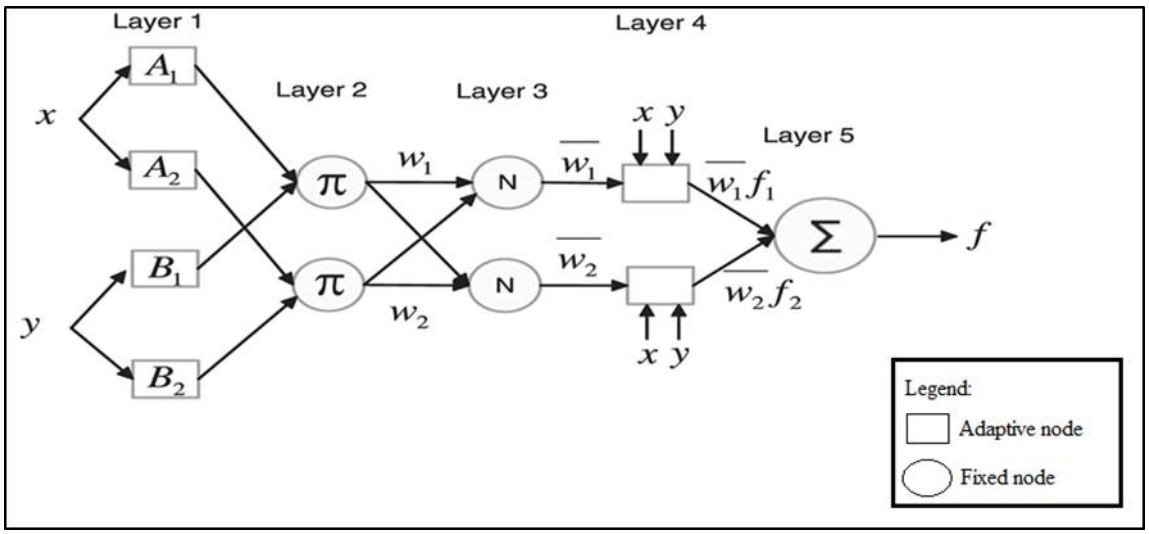

Figure 3. ANFIS structure with two inputs, one output and two rules.

3. APPLICATION AND RESULTS

3.1. Normalization and Evaluation

Equation (9) was used to normalize data before the procedure was implemented. Normalization is a database design technique that organizes data in a manner that reduces redundancy and dependency of data. Normalization divides larger data into smaller data and links them using relationships. The purpose of normalization is to eliminate redundant data and ensure data is stored logically and comparing the result of the un-normalized data to discover how much does the normalization effect on reducing the error value of the program. Here, X_{norm}, X_{min} and X_{max} signify the normalized, minimum, and maximum values of the data set, respectively.

$$x_{norm} = \frac{x_i - x_{min}}{x_{max} - x_{min}} \tag{9}$$

Various variations of meteorological variables were used as inputs of the ANFIS model to analyze the magnitude of each parameter's impact on the prediction of relative humidity and temperature. As assessment criteria, Root mean square errors (RMSE) (Equation 10), mean absolute relative error (MAE) (Equation 11) and determination coefficient (R^2) (Equation 12) statistics were chosen.

$$\text{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(f_{i(\text{observed})} - f_{i(\text{predicted})}\right)^2} \tag{10}$$

$$\text{MAE} = \frac{1}{n}\sum_{i=1}^{n}\left|f_{i\ (\text{observed})} - f_{i\ (\text{predicted})}\right| \tag{11}$$

$$R^2=\frac{(f_i observed-f_i predicted)^2}{(f_{i\,observed}-average\ of\ dataset)^2} \quad (12)$$

Where; n, $f_{iobserved}$, $\overline{fi}_{observed}$; $f_{ipredicted}$ and $\overline{fi}_{predicted}$ predicted symbolizes number of data, observed, average of observed, predicted and average of predicted values, respectively. To achive the minimum error and max R^2 values between observed and predicted values ideal model parameters were chosen. Sugeno type was used to set the ANFIS model. The input membership functions were 'gaussmf' and the output membership functions were 'linear', defuzzification method was 'wtaver', generate fis type was 'subtractive clustering' method and optimization method was 'Hybrid Optimization Method'.

ANFIS model has been built many times with different inputs each time to find the best effect of each parameter.

The first model is forecasting relative humidity depending on 15 various inputs that include:
(T), (P), (Sr), (Ws),
(T and P), (T and Sr), (T and Ws), (P and Sr), (P and Ws), (Sr and Ws),
(T, P and Sr), (T, P and Ws), (T, Sr, Ws), (P, Sr and Ws) and
(T, P, Sr and Ws) respectively.

The second model predicts temperature based on 2 different input:
(H), (Dp),
(H and Dp) respectively.

Figure 4 illustrates a gaussian membership function and the rules of a model which has two inputs.

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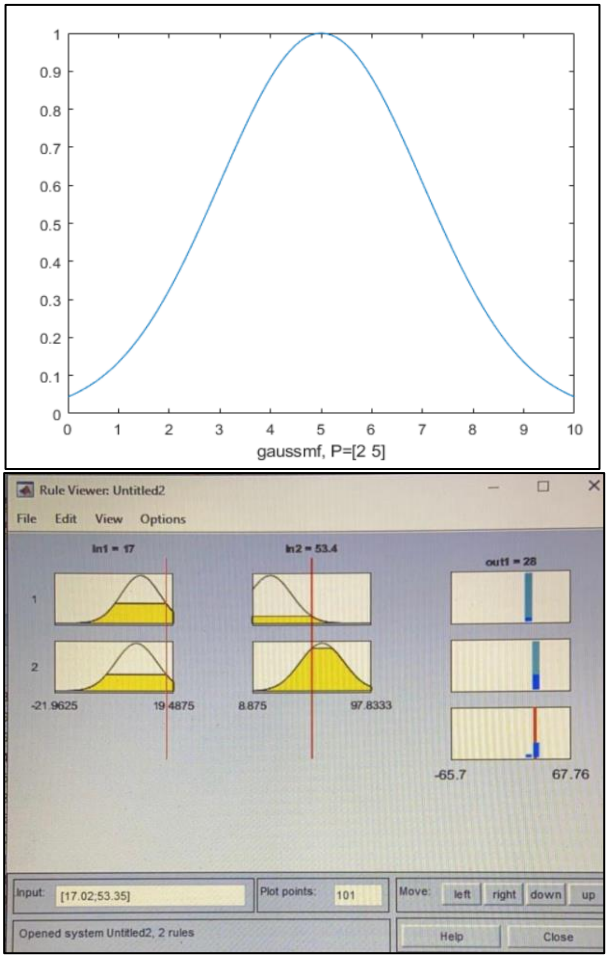

Figure 4. Agaussian membership function and rule view of a model with two inputs.

ANFIS has been applied for each of these models on daily and hourly data plus normalized and un-normalized data. The relative humidity and temperature parameters are defined to be the outputs of the models, these model's inputs have chosen according to the correlation between parameters. All data converted to normalized data to reduce the dependency and make them more accurate, so the second comparison is between normalized and un-normalized data, using the same two models. Average daily and hourly data of (2014-2017) used for training, and daily and hourly data of 2018 of Kirkuk and Sanliurfa were for checking. These comparisons were done by calculating RMSE, MAE and R^2.

3.2. The Results of Relative Humidity Model

Table 7 represents the result of daily data of the relative humidity model. Although results were so close to each other, the best result was obtained using un-normalized T, P, and Ws parameters as inputs. As well as the models that depended on one and two inputs, (T) and (T, P) had the best results with normalized data.

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Table 7. RMSE, MAE and R^2 statistics of daily relative humidity model.

Output: Relative humidity	**Un-normalized**			**Normalized**		
Inputs	**RMSE**	**MAE**	R^2	**RMSE**	**MAE**	R^2
T	10.804	7.631	0.695	10.525	7.292	0.711
P	15.936	11.533	0.337	15.567	11.164	0.367
Sr	14.752	11.106	0.432	14.422	11.004	0.457
Ws	18.535	15.711	0.103	18.865	16.045	0.071
T, P	9.852	6.931	0.746	9.631	6.573	0.757
T, Sr	10.991	7.758	0.684	10.531	7.240	0.711
T, Ws	10.984	7.611	0.685	10.526	7.178	0.711
P, Sr	14.520	10.977	0.229	13.934	10.187	0.493
P, Ws	16.171	11.692	0.317	15.487	11.278	0.374
Sr, Ws	14.441	10.770	0.455	14.163	10.723	0.476
T, P and Sr	9.966	7.199	0.741	9.789	6.725	0.749
T, P and Ws	9.455	6.551	0.766	9.479	6.430	0.765
T, Sr and Ws	10.528	7.539	0.711	10.884	7.528	0.691
P, Sr and Ws	14.509	10.717	0.451	15.875	11.329	0.342
T, P, Sr and Ws	10.023	7.031	0.737	9.745	6.737	0.752

Figure 5 shows the scattering of the ANFIS model of daily un-normalized relative humidity model for three inputs which had the highest R^2 value. In addition, the comparison of the observed and predicted data is presented in Figure 6.

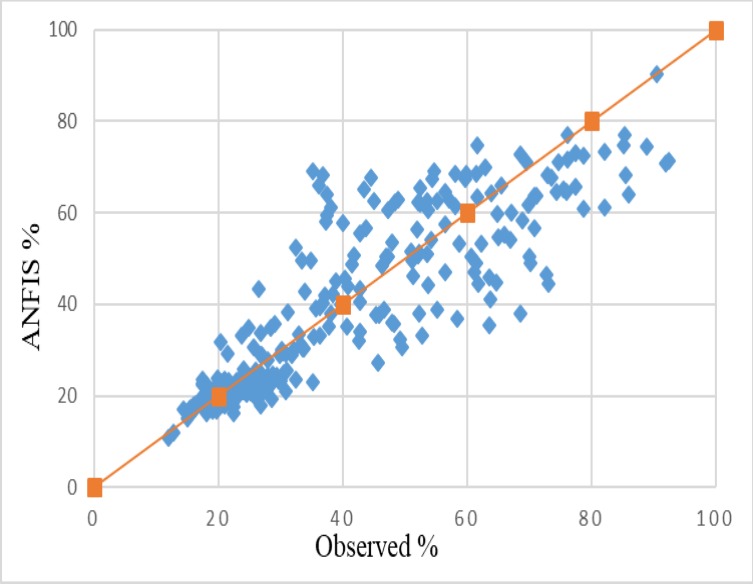

Figure 5. The scattering of ANFIS of daily un-normalized relative humidity model for three inputs.

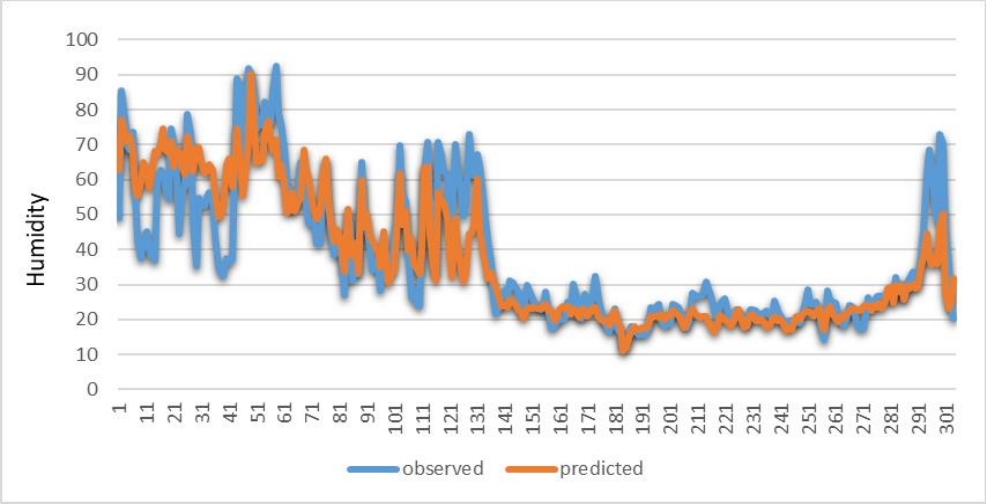

Figure 6. The observed and predicted data of ANFIS method for daily un-normalized humidity model.

Table 8 shows that hourly data got the best results depending on normalized T, P, Sr, and Ws as inputs. RMSE, MAE, and R^2 were 10.185, 7.025 and 0.756, respectively. The daily data of the relative humidity model had better results than hourly data, this means that the central tendency of a dataset may have better performance than a single data. (T) and (T, P) got the best results as one and two input models using normalized data.

Table 8. RMSE, MAE and R^2 statistics of hourly relative humidity model.

Output: Relative humidity	**Un-normalized**			**Normalized**		
Inputs	**RMSE**	**MAE**	R^2	**RMSE**	**MAE**	R^2
T	11.664	8.289	0.679	11.237	7.771	0.702
P	17.437	12.885	0.282	17.084	12.760	0.311
Sr	19.421	16.184	0.110	19.665	16.665	0.087
Ws	20.587	17.392	0.0001	20.844	17.916	-0.025
T, P	10.691	7.565	0.730	10.326	7.175	0.748
T, Sr	11.565	8.214	0.684	11.123	7.710	0.708
T, Ws	11.569	8.248	0.684	11.164	7.758	0.705
P, Sr	16.379	12.078	0.367	16.012	11.837	0.395
P, Ws	17.314	12.761	0.292	17.154	11.558	0.291
Sr, Ws	19.291	16.019	0.122	19.471	16.409	0.105
T, P and Sr	10.763	7.612	0.726	10.328	7.132	0.748
T, P and Ws	10.597	7.486	0.735	10.277	7.139	0.751
T, Sr and Ws	11.456	8.144	0.690	11.111	7.726	0.708
P, Sr and Ws	16.324	12.073	0.371	15.951	11.836	0.399

T, P, Sr and Ws	10.563	7.512	0.736	10.185	7.025	0.755

Figure 7 shows the scattering chart of the ANFIS model of hourly normalized relative humidity model for four inputs which had the highest R^2 value.

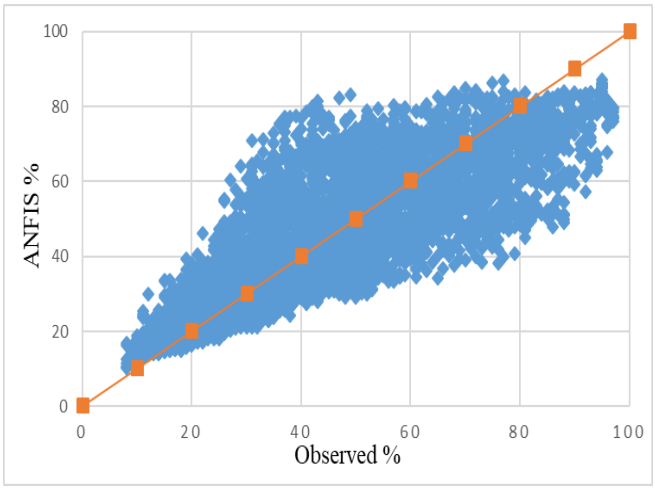

Figure 7. The scattering of ANFIS of hourly normalized relative humidity model for four inputs.

3.3. Results of Temperature Model

During this study and using different inputs and outputs, it was found out that temperature could have the best performance depending on H and Dp as input parameters. Un-normalized model got the lowest error values with RMSE= 0.629 and MAE= 0.727 also highest R^2 value. As one input parameter, relative humidity had better performance using normalized data (Table 9).

Table 9. RMSE, MAE and R^2statistics of daily dataset of temperature model.

Output: Temperature	**Un-normalized**			**Normalized**		
Inputs	**RMSE**	**MAE**	R^2	**RMSE**	**MAE**	R^2
H	4.596	3.757	0.752	4.369	3.195	0.775
Dp	8.188	7.159	0.219	8.224	7.069	0.205
H and Dp	0.629	0.727	0.993	3.184	2.803	0.881

Figure 8 and Figure 9 represents the scattering and distribution of the ANFIS model of daily un-normalized temperature model for two inputs which had the highest R^2 value, respectively.

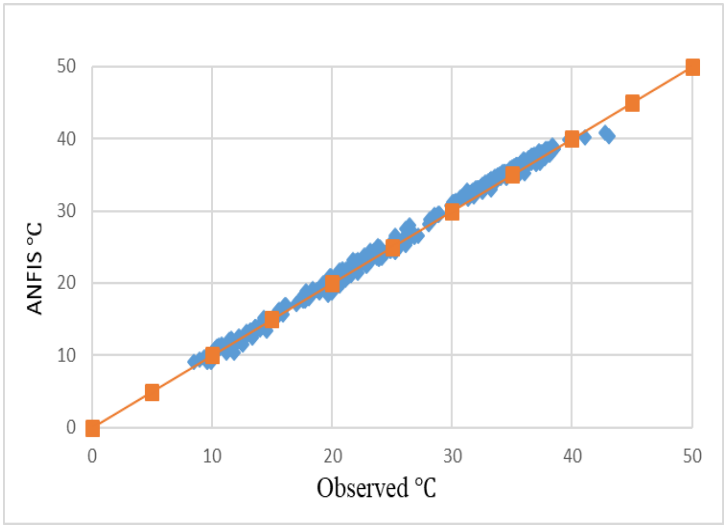

Figure 8. The scattering of ANFIS of daily un-normalized temperature model for two inputs.

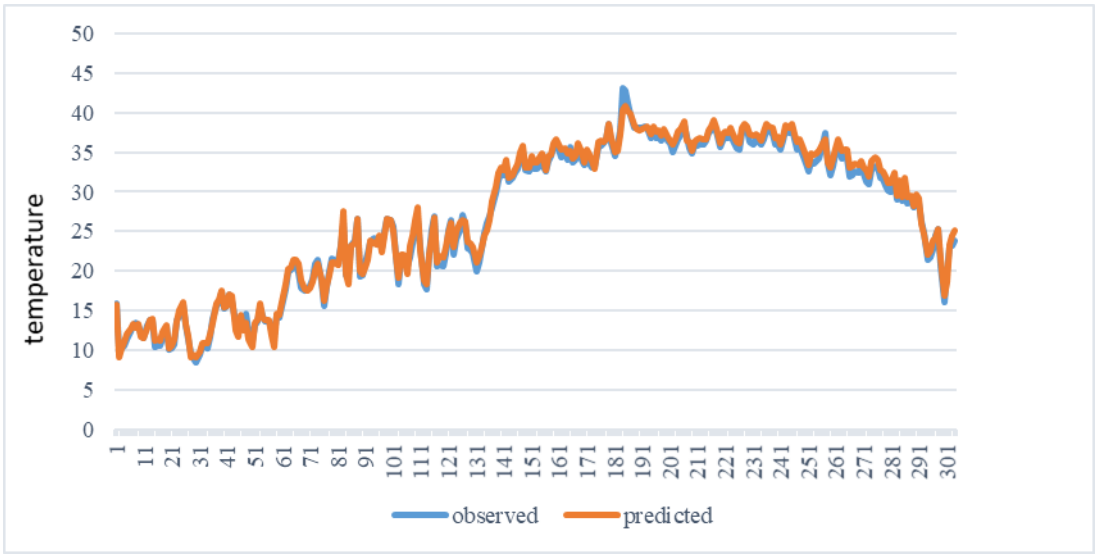

Figure 9. The observed and predicted data of ANFIS method for daily un-normalized temperature model.

Table 10 represents that there is not a big difference between R^2 of daily and hourly datasets but according to the other statistics it was clear that daily data had better performance.

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Table 10. RMSE, MAE and R^2 statistics of hourly temperature model.

Output: Temperature	**Un-normalized**			**Normalized**		
Inputs	**RMSE**	**MAE**	R^2	**RMSE**	**MAE**	R^2
H	5.086	4.224	0.732	17.087	14.831	0.708
Dp	9.022	7.616	0.159	11.463	9.631	-0.356
H and Dp	0.927	0.788	0.991	18.699	16.067	0.843

Figure 10 represents the scattering chart of the ANFIS model of hourly un-normalized temperature model for two inputs which had the highest R^2 value.

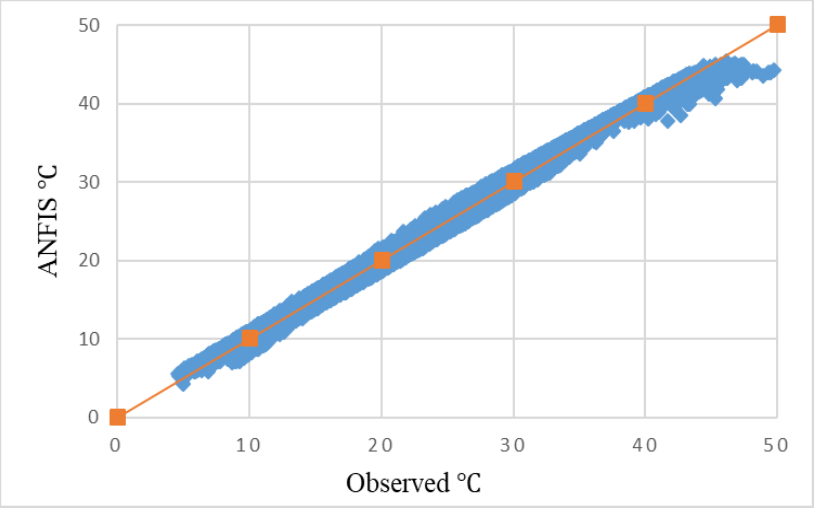

Figure 10. The scattering of ANFIS of hourly un-normalized temperature model for two inputs.

3.4. The Results of Sanliurfa Data Set

Best models of the relative humidity and temperature parameters were chosen to check with data that were observed in Sanliurfa station. Table 11 shows the results of this checking.

The relative humidity model had 14.453 as RMSE, 11.076 as MAE and 0.432 as R^2 depending on T, P and Ws as inputs for daily normalized data. But R^2 value is not sufficient. The temperature model had the best performance for a daily normalized dataset using both inputs H and Dp with R^2=0.876. Also, the error values were quite reasonable for this model.

Table 11. Results of RMSE, MAE and R^2 statistics of Sanliurfa daily dataset.

Type of data	**Inputs**	**Output**	**RMSE**	**MAE**	R^2
Un-normalized	T, P and Ws	H	17.244	13.926	0.173
Normalized	T, P and Ws	H	14.290	11.076	0.432
Un-normalized	H and Dp	T	5.548	5.044	0.578
Normalized	H and Dp	T	3.005	2.389	0.876

Figure 11 and 12 shows the result of temperature model using ANFIS analysis for prediction, depending on the daily normalized dataset.

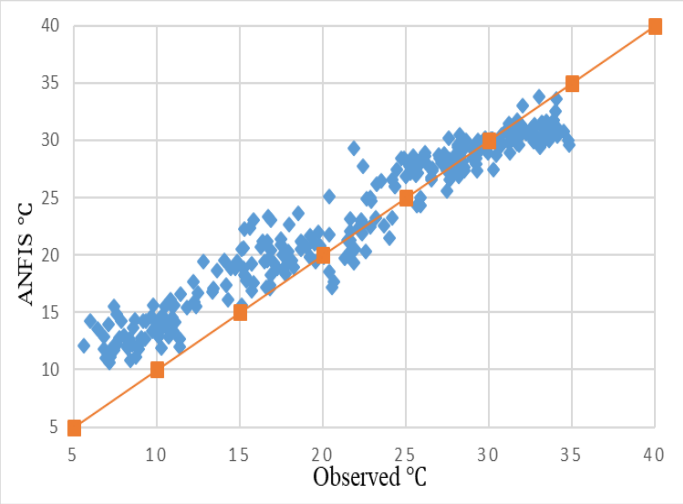

Figure 11. The scattering of ANFIS of daily normalized temperature model for two inputs at Sanliurfa station.

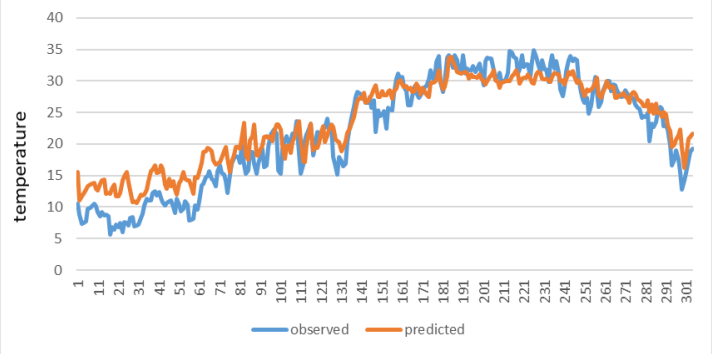

Figure 12. The observed and predicted data of ANFIS method for temperature model of Sanliurfa station.

4. CONCLUSION

In this study, the max-min normalization procedure was applied to the dataset to investigate the effect of the normalization on the results of the ANFIS method. Additionally, the results of the models which were set with hourly and daily data were compared to see the impact of the data type on the results of the models. The results of temperature and relative humidity were compared between Sanliurfa that have similar weather conditions with Kirkuk.

It was obtained that the calculation of the average daily parameters from the original dataset could affect positively the results and get much better outcomes than hourly data. Although normalized data got better results than the un-normalized data for almost all models, there were no big differences between the results. This situation indicates that the normalization process cannot help to increase the accuracy of the model without choosing the most appropriate input parameters. A better correlation between the input and output is the reason for the temperature had a perfect performance and got the highest value of R^2 and the most acceptable error values of RMSE and MAE among the two parameters. According to the Sanliurfa results, the temperature model got acceptable results due to the similar weather conditions with Kirkuks's climate. Therefore, it can be said that if it is necessary for some reason, the model obtained using a different data set may be used in another country with a similar climate.

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RESEARCH ARTICLE

INVESTIGATION OF ATTACK TYPES IN ANDROID OPERATING SYSTEM

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ABSTRACT

With the widespread use of mobile technologies, the importance of cybersecurity is increasing in our country as well as all over the world. Android operating system-based smartphones and tablets used in mobile technologies are frequently in use for communication, social networking, banking, and payment transactions and become an important part of developing technology. Although the Android operating system is among the most popular operating systems, one of the biggest challenges faced by android users and developers is to ensure the security of the operating system. In this research, the security mechanism was examined with the android operating system architecture, and the exploitation of android vulnerabilities scenarios was created. These scenarios were carried out on various examples using the Smart Pentester Framework (SPF) tool. Also, by examining the sources in the literature, exploitations of android vulnerabilities are classified into categories. Based on the created classification and the exploitation methods scenarios taking place in the virtual environment built on Kali Linux, it is aimed to raise awareness of android operating system users and developers against possible risks.

Keywords: *Android Operating System, Cyber Security, Mobile Attack Methods, Smartphone Pentester Framework (SPF)*

1. INTRODUCTION

The android operating system is one of the most popular operating systems that are actively used in many areas such as banking, payment, transactions, and social networks. The active and widespread use of android operating systems makes the security of working on the android operating system an important and up-to-date subject [1]. Information security covers all efforts to prevent unauthorized or unauthorized access and use of information, modification, destruction, and acquisition by third parties [2]. One of the biggest problems in ensuring the security of the android operating system is that the services offered are provided by smartphone vendors of different brands and that they are released into the market with non-standard features. Hackers can attack the android operating system in multiple ways [3 - 4]. When these attacks were examined, it was determined that viruses, worms, SMS, and MMS exploits, cross-service attacks mostly target the android operating system application layer [5]. In this study, android operating system architecture and security mechanisms are examined. The mobile attacks are classified through performed various mobile attacks in the virtual environment

created using the android emulators installed on the Kali Linux operating system and the smart pentester framework (SPF) tool. There is aimed to raise awareness of android users and developers against possible attacks by examining mobile attack types and mobile security vulnerabilities.

Moore et al. in [6], to compare the security interfaces of Android 5 and Android 6, they conducted an online study with participants recruited through Amozon Mechanical Turk. While Android 5 informs the user about all the permissions requested while downloading an application, Android 6 informs the user only during the initial download phase of the application. In the study, each interface condition included a simulation of the google play store and a download instruction was given to the participants. Afterwards, each participant was asked for application permissions. The Android 5 interface performed better in informing users what permissions have accessed their device, while the Android 6 interface performed better in presenting the functionality of permissions. Kumar and Shulka [7] discussed the permission-based security mechanism and hardware vulnerabilities in the Android operating system. They examined the static and dynamic techniques used in malware detection in the Android operating system. In the study, a holistic application analysis was proposed instead of a single application analysis. It has been stated that dynamic code that does not come with an application (native code, mixed code, code written in java Kotlin, etc.) should be analyzed. Li et al. [8], they used more effective machine learning models and methods instead of the classical signature-based security systems used in the Andorid operating system. They propose a new and highly reliable classifier for Android Malware detection based on a Factorization Machine architecture and extraction of Android application features from manifest files and source code. The results showed that an application's numerical feature representation typically results in a long and rather sparse vector, and interactions between different features are critical to revealing malicious behavior patterns. After performing a comprehensive performance evaluation, the proposed method achieved a precision score of 100.00% in the DREBIN dataset and a false-positive rate of only 1.10% in the AMD dataset, with a precision score of 99.22%.

Martin et al. [9], presented an OmniDroid dataset containing 22,000 real and malicious software that can be used in malware detection studies using machine learning methods in the Android operating system. The dataset is released under the Creative Commons Attribution-NonCommercial ShareAlike 4.0 International License and is built using AndroPyTool, an automated framework for dynamic and static analysis of Android applications. A number of community classifiers have been tested on this dataset and a malware detection approach based on combining static and dynamic features through the combination of community classifiers is proposed. Experimental results demonstrate the feasibility and potential usability (for machine learning, soft computing, and cybersecurity communities) of the automated framework and publicly available dataset. Garg and Baliyan [10] discussed the role of machine learning algorithms in cyber security for malware detection on Android mobile operating system. The statistical analysis in the study identifies the different vulnerabilities affecting Android and the trend of these vulnerabilities between 2009-2019. Trend analysis can help evaluate the impact of each vulnerability. Yildirim and Varol [11], examined the mobile banking application, which has increased with the spread of mobile and internet banking, has addressed the cyber security problem. In the study, various security solutions for mobile and online banking are presented and security threats and precautions are examined.

Nilsson [12] addresses the security issues that arise as a result of the growing market of Android applications. Google has a bug bounty program where people can submit a vulnerability report on their most downloaded popular apps. The aim of Nilsson’s study was to evaluate the security of applications through the Google Play security-reward program by performing penetration tests on

applications. For this purpose, a threat model of Android applications, in which possible threats are detected, has been created. During his work he focused on the Spotify application for Android. Penetration tests were performed where the test depth was determined by the ratings associated with the attacks. The results of the tests showed that the Spotify App is safe. The biggest potential exploit found was a Denial-of-Service attack, which could be done via a malicious app interacting with the Spotify app. Sheluhin et al. [13], to automate the traffic monitoring process of traffic classification algorithms in mobile applications, they implemented a software package that allows to automatically collect network traffic packets from mobile devices and save them in a database. This work is an application that uses the application programming interface to create virtual private networks, collects network traffic packets, identifies the source application, and sends it to the server software via HTTP to collect traffic from mobile devices running Android operating system. The database created using client and server software is populated with traffic from 18 main applications.

This study is organized as follows: In Section 2 the virtual laboratory architecture and security mechanisms are examined. The SPF tool and android operating system attack experiments are explained in Sections 3. Finally, Android operating system security recommendations and suggestions for the SPF tool were presented.

2. ANDROID OPERATING SYSTEM ARCHITECTURE AND SECURITY MECHANISM

The android operating system was originally developed by the company Android (Inc.), which it took its name from, later it was purchased by Google as a mobile operating system in 2015 and the development of the android operating system based on the Linux operating system is still being continuing by Google [14]. Today, the android operating system has become a widely used software group and operating system that includes not only the operating system but also middleware and basic applications. As seen in Figure 1, android operating system architecture consists of 4 main layers: core layer, middle layer, framework layer, and application layer [15].

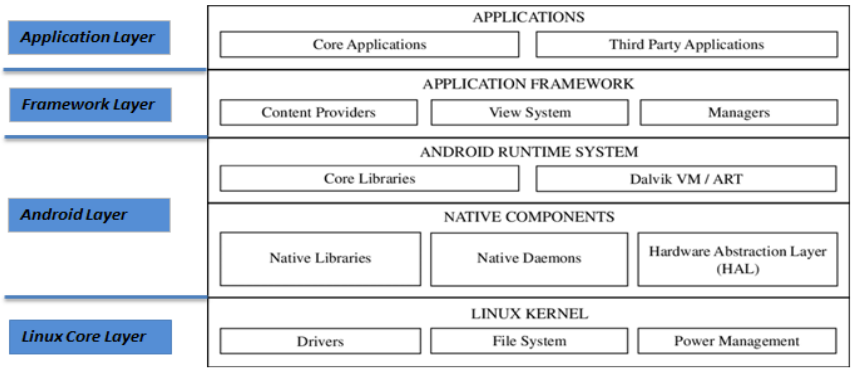

Figure 1. Android operating system architecture.

Linux core layer: Core drivers is the bottom layer of the android platform that enables the operating system's basic functions such as power management and file system to be realized.

Android layer: Android layer is located above the core layer. The android layer contains the basic elements of the android system. It consists of two layers; android run time and local components. Android hardware virtualization layer (HAL) in the local components layer is the layer where most special hardware applications such as audio devices, camera APIs are offered [8]. Two other important components in the local components layer are local libraries and backgrounds written in C++ / C languages. Local daemons (ghost programs) that figure on the same layer handle the interaction with the system at the local level. By using libraries such as SQLLite, Webkit, SSL, and OpenGL in order to improve the functionality and compatibility of the android library, android library enriches. The android runtime system layer in the android layer contains kernel libraries and runtime environments. While a java process virtual machine called Dalvik as the only runtime environment was in use until the android 4.4 version, the android run time (art) used a new working scheme in later versions. According to the just in time (jit) compilation provided by the Dalvik virtual runtime environment, the ahead of time (aot) compilation provided by art paved the way for significant improvements both in performance and in energy consumption [16].

Framework layer: Framework Layer is the layer that performs many functions of android applications and is most used by application developers. A rich and developable collection of UI components are provided by this layer. The android operating system has two main security mechanisms; an android permission-based mechanism at the application layer and a Linux user-based privilege mechanism [17]. The necessary permissions must be given by the android operating system to enable an external source to access an application. This permission-based security mechanism is implemented at the level of intercomponent communication (ICC). ICC assigns a predefined permission label to each application and components. Thus, permission tags not previously defined are rejected by the android operating system. These permissions are set at 4 levels [18];

Normal Layer: Normal layer is the lowest permission level. The developer's internet access can be allowed as long as it is specified in an application's notification file, just like the use of near field communication (NFC).

Dangerous: A level of permission that is higher than the normal level of permission. Permits at this level can only be granted during application.

The other two permission levels defined for the risky permission level are signature and system permissions systems. A signature permit is a permit system that covers only applications signed by a trusted party. Applications signed by Google and phone vendors are within the scope of this application. A security-enhanced Linux (SELinux) model has been implemented to upgrade optional access control (DAC) to the latest access control (MAC) since the android 4.3 version. The SELinux model operates at the minimum concession level regulated by the SELinux security policy for each transaction.

3. ANDROID OPERATING SYSTEM ATTACKS AND SMART PENTESTER FRAMEWORK (SPF)

In this study, the android 4.3 emulator built on the kali Linux operating system was determined as offensive and the android 4.1 emulator was determined as the target system. Among these emulators, various mobile attacks were carried out by using the smart pentester framework (SPF) tool. The smart pentester framework is an active penetration testing tool that continues to be regularly developed and

feature sets are regularly changing [19]. In general terms, the virtual laboratory environment used in this study is as shown in Figure 2.

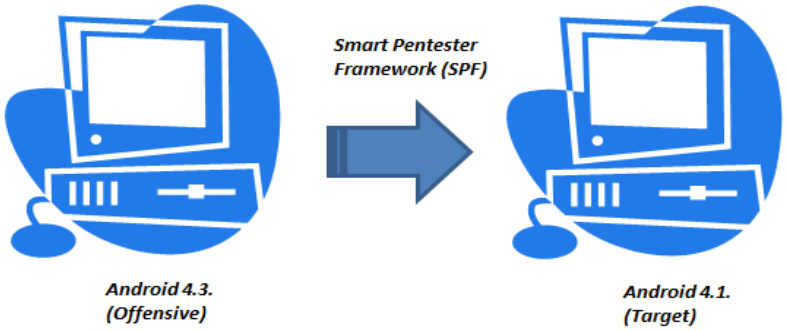

Figure 2. Virtual laboratory.

Mobile attacks can be carried out by taking advantage of the vulnerabilities of the android operating system, mobile browsers, and various social Engineering attack methods. The most used mobile attack vectors when performing these attacks are text messages, near field communications (NFC), and QR codes [20].

In attacks intending to cheat the user, such as Phishing attacks, sending a text message is preferred more than sending an email. Today, even free e-mail security programs make emails more secure. This situation led to the fact that text messages were preferred more in social engineering attacks [21]

NFC is a technology that enables devices to share data by touching each other or communicating with other devices in their nearby areas. The automatic use of these technologies allows users to share data without letting the user know. This makes NFC an important social engineering attack vector [22].

QR codes consisted of matrix barcodes were originally developed to be used in automated manufacturing. Today, QR codes can embed some URL extensions and send data to any application on mobile devices. When users scan any QR code, it causes an unwanted malicious application to be opened [23].

Android operating system mobile attacks can be carried out in more than one way [24]. These different methods enable the classification of mobile attacks. In this study, as a consequence of the exploitation of vulnerabilities carried out through SPF, classification was carried out as seen in Figure 3.

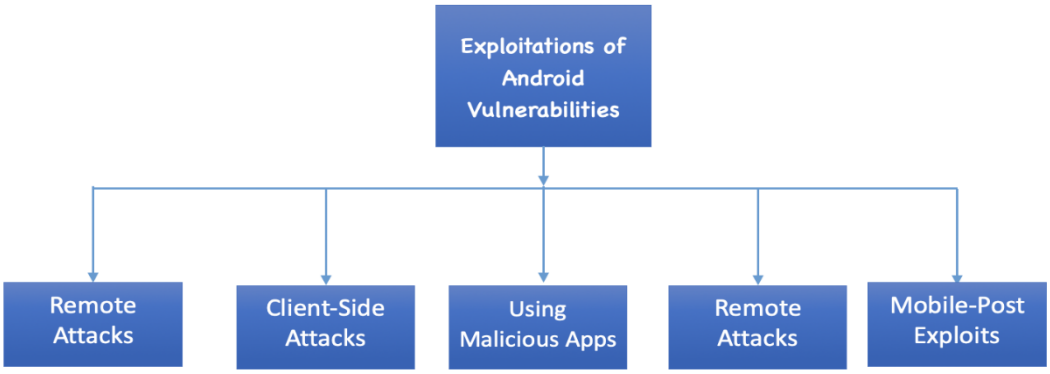

Figure 3. Exploitations of vulnerabilities.

Classified types of mobile attacks use the most up-to-date android OS security vulnerabilities for 2020, 2021, as seen in Table 1.

Table 1. Android vulnerabilities in 2020, 2021 [25].

Year-android vulnerabilities	Android Vulnerabilities					
Year	DOS	Code Execution	Overflow	Bypass	Gain information	Gain Privileges
2020	1351	3248	1618	966	1345	310
2021	872	1812	746	366	442	129

The number of security vulnerabilities for 2020, 2021 is given in Table 1. The weaknesses and reasons presented above are briefly explained below.

DOS (Denial of Service): DOS is a type of attack that prevents the target system from providing services and preventing users from accessing the system. These attacks overload the services owned by the system and disrupt network traffic. These vulnerabilities of android services are addressed as DOS vulnerabilities [26].

Code Execution: Code Execution is a type of vulnerability that occurs when user input is injected into a string or a file and the related input is applied to any programming language used with a parser. The attacker can inject his/her own malicious code into the functions of the software written [27].

Overflow: Buffer is a block of memory that stores a sequential type of data (such as int, char) in memory. Buffer overflow is called a crash of the program when the variables in a program consisting of incorrect functions (strcpy, strcmp etc.) store more data than their storage capacity. Since the capacity is exceeded, the flow of the program can be changed with the codes that are not in the normal flow called shellcode [28].

Gain information: Gain information is the collection of useful information on the target system during the attack phase. This situation usually carried out with an information-gathering tool. If the system information is public, this information can be obtained easily [29].

Gain privileges: If the session is acquired on the target system if this session has inadequate authority, the vulnerabilities existing on the system are scanned with a tool and these vulnerabilities are exploited and accessed to a useful authority that can be useful [30].

Based on the attack types classified in Figure 3 and the vulnerabilities table used in Table 1, which vulnerability types are frequently used in which attack type can be shown as in Table 2.

Table 2. Android vulnerabilities and attack types.

Attack type/ using vulnerability	Android Vulnerabilities					
Attack types	DOS	Code Execution	Overflow	Bypass	Gain information	Gain privileges
Remote Attacks	✓				✓	✓
Client-Side Attacks		✓			✓	✓
Attacks Using Malicious Apps	✓	✓	✓	✓	✓	✓
Mobile Post Exploits	✓	✓	✓	✓	✓	✓

3.1. Remote Attacks

As the security position increases on mobile devices as well as computers, the number of remote attackers decreases. When users install more software on their phones, it increases the number of potential services listening to a port. Remote attacks can be performed on services that express listening points, such as TCP ports or on mobile devices that do not have a password change, without any service vulnerability. In this study, an example of an SSH attack was realized as an example of remote attacks. SSH was originally designed as a replacement for insecure remote login procedures such as rlogin and telnet. It has since become a general-purpose tool for securing Internet traffic [31]. Version 2 of SSH is standardized by the IETF in a series of RFCs. Although many different implementations of SSH are available, the OpenSSH implementation [32] dominates, with OpenSSH and its derivatives accounting for more than 80% of SSH implementations on the Internet. The algorithm of the SSH remote attack scenario is as shown in Figure 4.

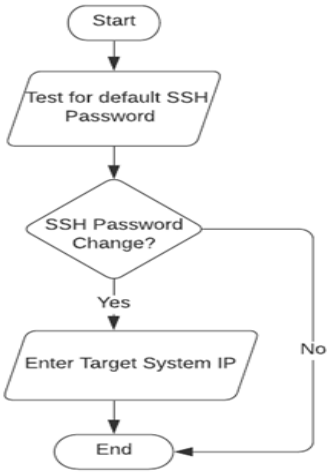

Figure 4. Algorithm of SSH remote attack (Scenario 1).

The operations performed in the order of the algorithm seen in Figure 4 are as seen in Figure 5.

Figure 5. SSH remote attack.

Option 5, shown in Figure 5 is the remote attack option in the SPF vehicle. To check if the mobile device password has been changed, option 1 is selected, the default test SSH password is selected. If the password has not been changed, the target system IP is entered to enter the target system.

3.2. Client-Side Attack (Social Engineering)

Using client-side (social engineering) attacks are more preferred in mobile devices than remote attacks. Client-side attacks are not limited to mobile browsers only. In addition to the default applications on the device, remote attacks can be organized on third-party applications. In this study, an attack was made to the Webkit package on the mobile browser to obtain a session on an android device. Vulnerabilities on the mobile browser were exploited after the user was deceived to open a malicious page. In a credential-stealing attack, an adversary tries to fraudulently gather user credentials either directly by invading insufficiently protected client systems, or indirectly by tricking users into voluntarily revealing their credentials, using, for example, phishing or other social engineering techniques. These attack strategies aren't mutually exclusive and can be combined. For example, some attacks, such as pharming and visual spoofing, affect the client but are mounted to make subsequent user-level attacks more powerful and effective. Because the attacker uses the fraudulently gathered credentials at a later time to spoof the user's identity, these are called offline attacks [33]. The algorithm scheme of the scenario where these transactions take place is as shown in Figure 6.

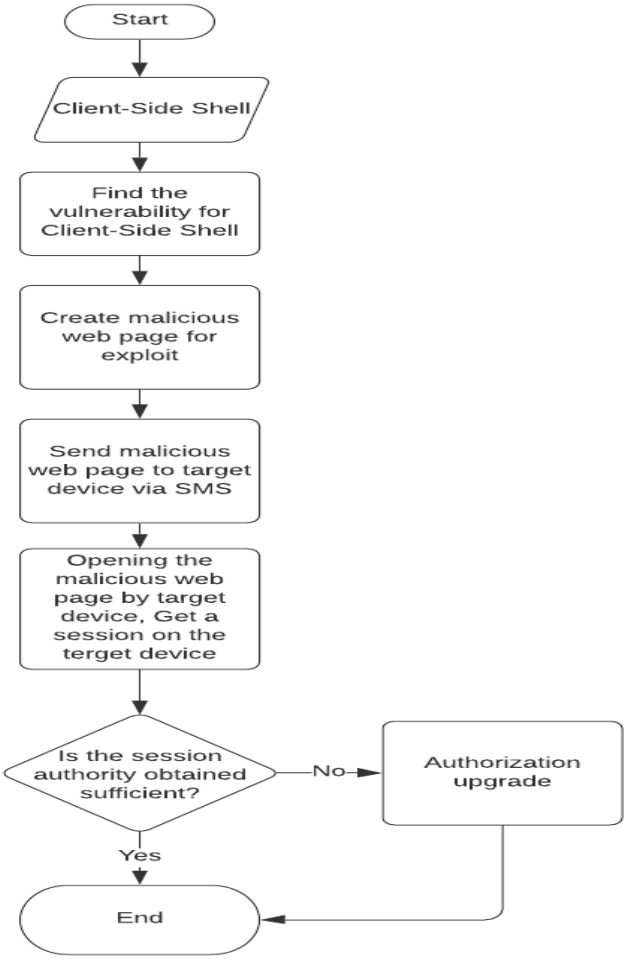

Figure 6. Algorithm of client-side attack (Scenario 2).

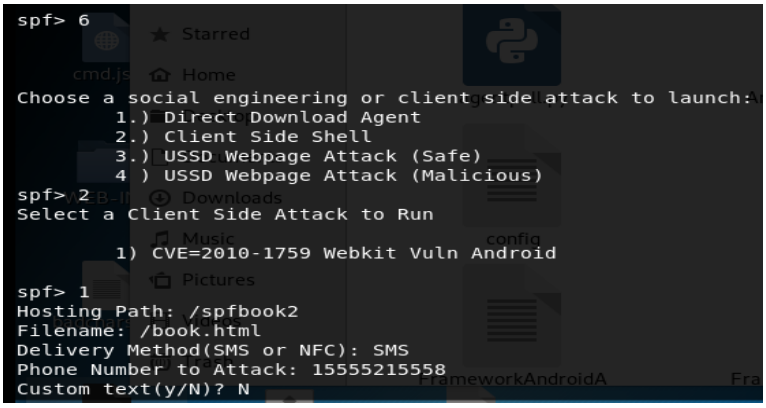

Figure 7. Client-side attack.

In Figure 7, the option of social engineering attacks, which is the 6th option of the SPF tool is selected. Then, option 2 the social engineering attack type option, the client-side attack option was chosen. The SPF tool has identified the vulnerability CVE=2010-1759 WebKit vulnerability which was available for this attack option and created a malicious page to exploit this vulnerability.

This malicious page is /book.html page under the /spfbook2 extension. The malicious page was sent to the target system android 4.1 emulator with an SMS link. The phone number shown in Figure 7 is the number given to the android 4.1 emulator. The malicious web page can be sent to the target system with a special message (such as a campaign, a news link of interest). During this process, the SPF tool ensures that the attacking android 4.3 emulator is connected and a private message is sent to the target system android 4.1 emulator. When the malicious link is opened by the target system, the mobile browser has tried to open the malicious page for 30 seconds. In this process, a session was obtained on the target system. The session information tested with the command "whoami". If the obtained session authorization is not sufficient, the authorization should be upgraded. The attacker can operate she/he wants with a session with sufficient authority on the target system.

USSD remote control attack is another client-side attack method. USSD is a service that enables mobile devices to communicate with the mobile network. The android operating system automatically performs certain operations with certain numbers. So, when the USSD codes enter the converter, the android operating system automatically searches for the processes corresponding to these numbers. USSD service is a service frequently used by attackers for remote control. Attackers can send malicious USSD codes into a web page as call and end numbers. When the USSD code which shows itself to android as a phone number on a malicious web page is opened in the phone dialer, it erases all the data of the user and performs a factory restore.

The algorithm scheme of the scenario where these transactions take place is as shown in Figure 8.

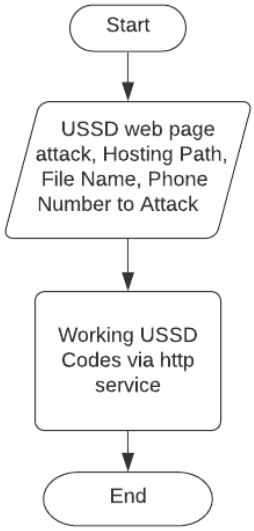

Figure 8. Algorithm of USSD client-side attack processing (Scenario 3).

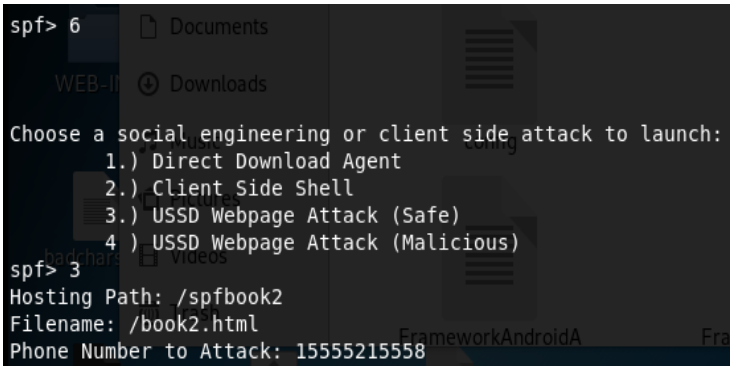

Figure 9. USSD client-side attack.

The transactions performed in Figure 9 are largely the same as those performed in Figure 7. Here the 3rd option USSD web page attack option is used. When the target system named /book2.html page under the /spfbook2 extension was turned on by android 4.1, instead of locking the mobile device for 30 seconds, it put the USSD codes directly into the dialer.

3.3. Attacks Using Malicious Apps

When mobile devices advertise with the new software they produced, the possibility of downloading malicious software on devices increases. Mobile antivirus programs often require excessive permission and authority from the user to run applications. Installing antivirus applications on a mobile device requires installing more applications for it to run the applications. Malicious software is installed by the attackers under the extensions of these applications. As a result of downloading this software with the applications, the attacker can operate such as stealing data, controlling the mobile device remotely, attacking other devices in the mobile device directory, and using the applications on the mobile device as he/she desired [34]. Malware hidden under mobile applications cannot be installed on a mobile device without user permission. However, users generally accept the permissions asked while installing an application without reading. In this study, malicious applications with various functionality were created by using the SPF tool. The created SPF agents have taken control of the SPF-controlled mobile modem by logging into the webserver via the HTTP service. The SPF application created to perform these operations is shown as a reliable or interesting application. If the SPF agent can access the source code, it can be placed under a compiled legitimate application. The algorithm of the scenario where these operations are performed is as shown in Figure 10.

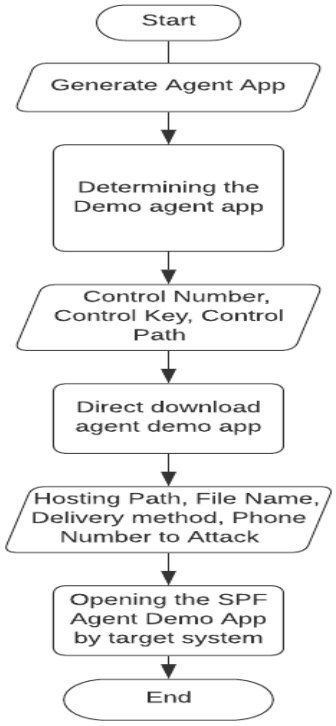

Figure 10. Algorithm of attacks using malicious apps (Scenario 3).

In Figure 11, an SPF agent that looks like a Google Maps application has been created.

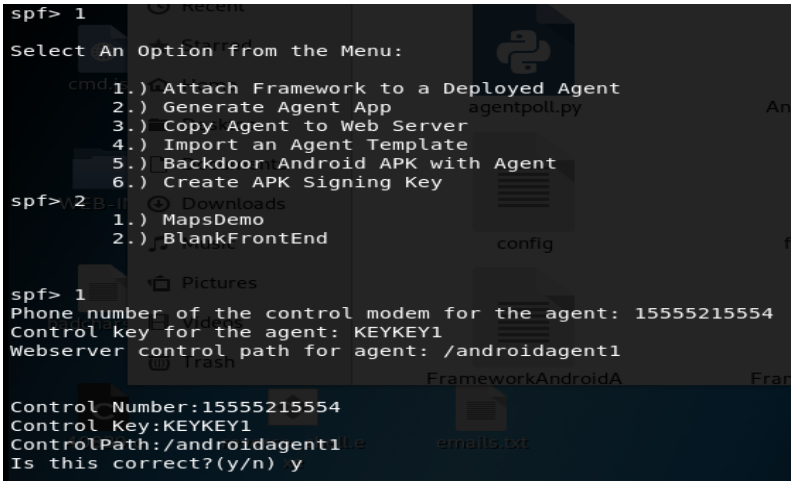

Figure 11. Creating SPF agent.

An attacker phone number, control key and control extension information were provided to send SMS commands when requested and to check that if the SMS was sent. After the agent was created, the social engineering attack was carried out as shown in Figure 12 for the user to download this application.

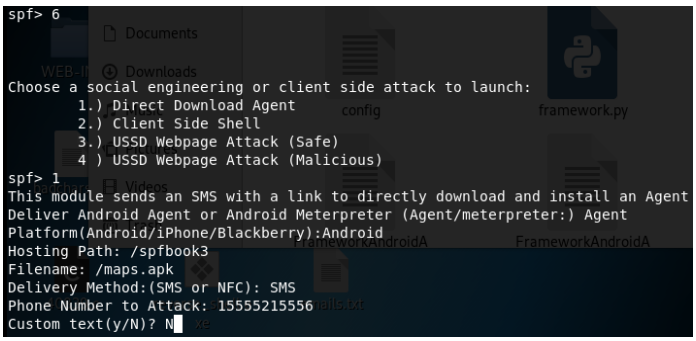

Figure 12. Login to the user to install the agent.

In Figure 12, option 1 which is the option to install the agent directly from the SPF social attacks option is selected. In order to perform an operation, the platform, extension path, agent, application name, sending method, and target system phone number information of the agent requested by the SPF tool was automatically entered. When these operations are performed, when the SPF agent that is sent via SMS through the target system android 4.1 emulator is turned on, the agent looked like the google maps application as shown in Figure 13.

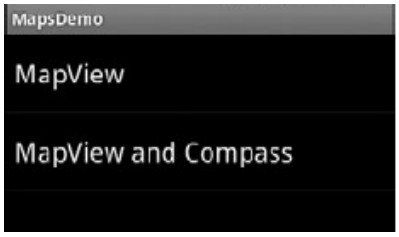

Figure 13. SPF agent appearing on the target device.

When the user uses the application shown in Figure 13, malicious software is also running in the background.

Malware attacks can also be carried out by embedding malicious software without the need to create a fake application under the installation files of legal applications. This attack type is called Backdooring APKs. In this scenario backdooring apks exploits the android master key vulnerability of the android operating system. The algorithm of the backdooring apks scenario is as shown in figure 14.

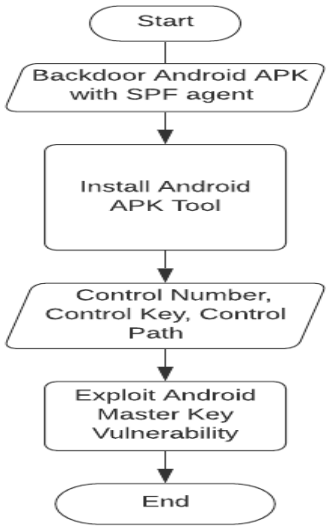

Figure 14. Algorithm of backdoor android APK with agent (Scenario 4).

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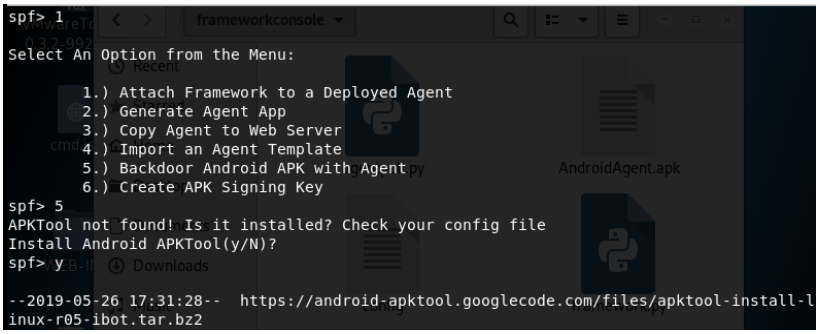

Figure 15. Backdooring APKs.

In Figure 15 it was not deemed necessary to create a mock application for the SPF agent. In order to manage the SPF agent installed in the applications here, the attacker's phone number, control key, and control path option settings were made as seen in Figure 16.

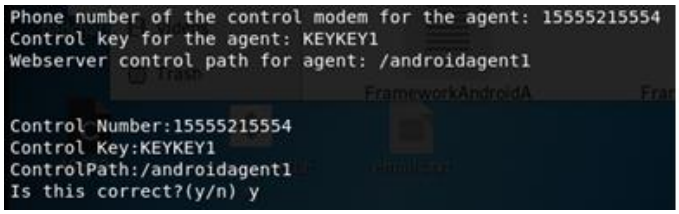

Figure 16. Setting options.

Android security mechanism signs google play apps with a developer key registered in google play [35]. The control of these signatures is performed and the installation of applications without signatures is rejected by the android operating system. The signature check phase does not take place because the application codes are recompiled with the default android keys, the android SDKs that auto-sign when entering the APK files secretly. The recompilation of the existing android APK is shown as the update process of the related application and the android master key vulnerability is exploited.

Android APK structure is as shown in Figure 17. Android apk structure is almost no different from zip files. When an apk file is opened, the structure is seen in Figure 17.

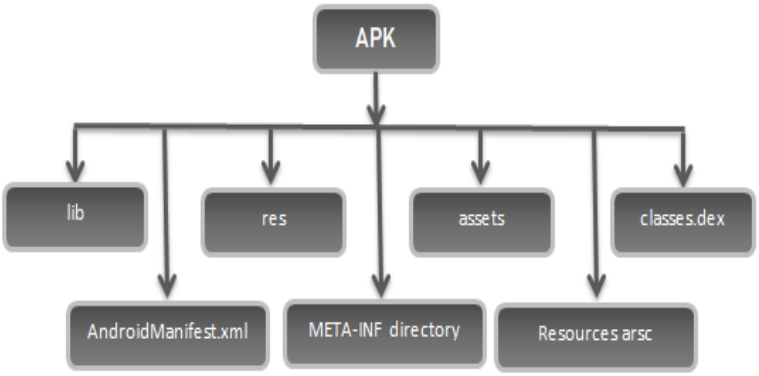

Figure 17. Android apk structure.

Android apk structure is almost no different from zip files. When an apk file is opened, the structure is seen in Figure 17. Below, the attributes in each structure content are given respectively.

META-INF directory: This is the region where developer signatures and developer keys are located.

Lib: Lib is the region where local libraries are compiled according to the processor architecture.

Res: Res contains compiled audio, image, uni (xml), some files such as sequences.

Assets: Assets are the field containing files such as font, picture, video. Malware is installed on mobile devices within this area.

Classes.dex: Classes.dex contains class files compiled for DVM (Dalvik Virtual Machine). In a sense, the android operating system can also be called exe.

Resources.arsc: The region where the compiled resources are located.

3.4. Mobile Post Exploits

Mobile post exploits are carried out after a type of attack has been achieved on the target system. Information gathering processes are carried out on the target device from the guide on the mobile device from which the session is obtained or with the vectors such as SMS, mail that comes to this device. If the session obtained on the target device is insufficient for authorization, the process of upgrading the session authority to admin privileges is carried out in the type of mobile post exploitation attack. In this attack type, other devices in the network on the mobile device exploited are detected and their exploitation is carried out. Mobile post exploits provide more successful results if the attack accessed mobile device is using a VPN or connected directly to corporate networks [36]. The stages of the mobile post exploits attack type are as seen in Figure 18.

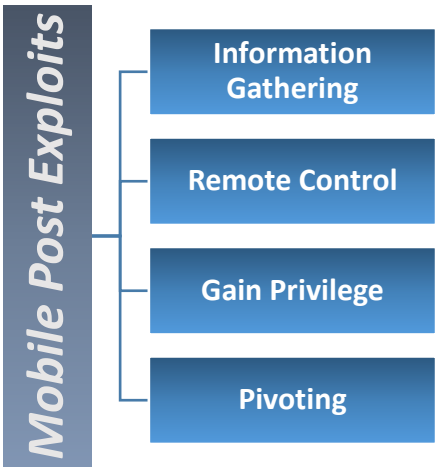

Figure 18. Mobile post-exploitation stages.

Information gathering was achieved by reaching the list of applications built on the infected target devices logged in by the attacker. While performing the information gathering, first the second option which serves to command agent, then the agent in question which was created with the SPF to be commanded was selected from the SPF tool menu. An agent can communicate via means such as HTTP service, SMS, and receive commands. A command to list installed applications on the device was sent to the agent on the target device with HTTP service. This process is as shown in Figure 19.

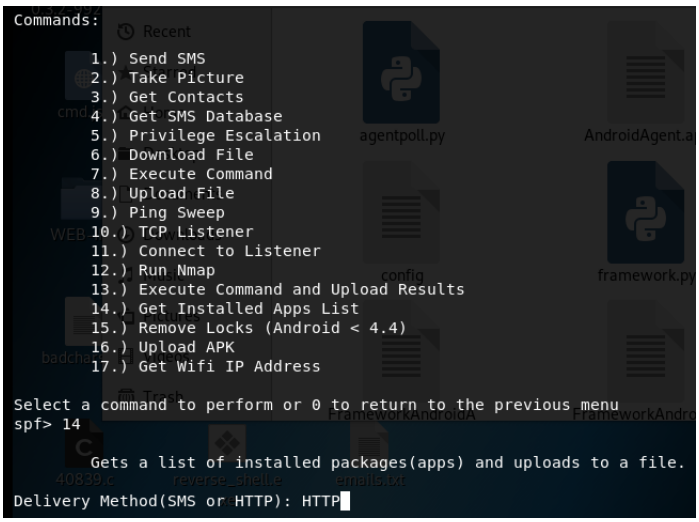

Figure 19. To command the SPF agent via HTTP.

After sending the list of installed applications command to the agent as shown in Figure 19, 0 should be entered to return to display the results of the agent and return to the SPF main menu. The main menu displayed is as shown in Figure 20.

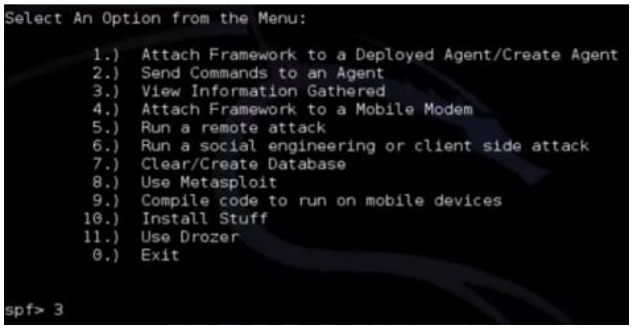

Figure 20. Listing information that gathered by agent.

From the menu shown in Figure 20, the third option, "view the information gathered" was selected, and information gathering was completed. The information gathered can be used in attack operations. The remote control was carried out by directing the SPF agent. The agent was commanded to send a confidential message to the persons in the device guide obtained during the information-gathering phase. This message cannot be displayed by the user in SMS records. So, the user will not have any information that this message has been sent. The message content is to encourage the recipients to download an application created by the SPF agent. Victims who receive messages from someone they know are more likely to download this app. After the application is downloaded, the devices registered in the guide can also be controlled remotely by the agent. Related operations were carried out with the SPF tool as seen in Figure 21.

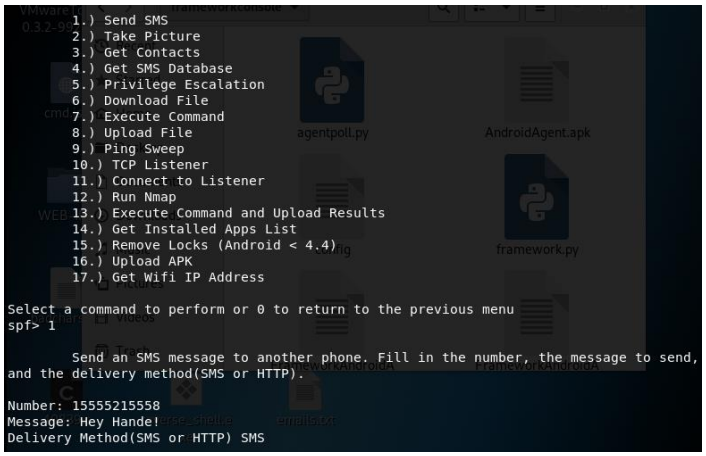

Figure 21. Remote control via SPF agent.

In Figure 21, the option to send SMS which is the 1st option was given to the agent from the SPF tool. The message to be sent is in the type of SMS and the phone number of the device to be sent is given to the SPF Tool. The title of the relevant message content can be adjusted with the SPF tool. Here, the phone number of the android 2.2 emulator in the android 4.1 target device guide, which was accessed from the android 4.3 emulator was used. The gain privilege was carried out by exploiting gain

privilege vulnerabilities based on the Linux operating system kernel. These vulnerabilities can be determined automatically with the SPF tool.

Android 2.2 is vulnerable to vulnerability called "Rage Against the Cage". The rage against the cage vulnerability is found automatically when a vulnerability scan is performed with the SPF tool on the android 2.2 emulator which was accessed from the android 4.1 emulator guide. The automatic detection of this vulnerability by the SPF is as seen in Figure 22.

```
Commands:
--snip--
Select a command to perform or 0 to return to the previous menu
spf> 5
1.) Choose a Root Exploit
2.) Let SPF AutoSelect
Select an option or 0 to return to the previous menu
spf> 2
Try a privilege escalation exploit.
Chosen Exploit: rageagainstthecage
Delivery Method(SMS or HTTP): HTTP
```

Figure 22. Detection of "Rage against The Cage" vulnerability via SPF.

In Figure 22, the vulnerability detected by the SPF was exploited through the HTTP service. As a result of this process, the result of "Rooted: Rage against The Cage" was reached and the session was upgraded to root authority. Thus, many processes requiring root authorization on the android 2.2 emulator have been realized.

Pivoting is the process of using the mobile device obtained as a pivot to access other devices. The fact that many employees in the corporate companies know the wireless network password and connect to the company network makes corporate companies vulnerable to external attacks. Weak passwords, missing patches, outdated software cause vulnerabilities in the local network. If any mobile device connected to the company network has access to one of these vulnerable systems, the mobile device can be exploited and accessed to these systems. The pivoting process is as seen in Figure 23.

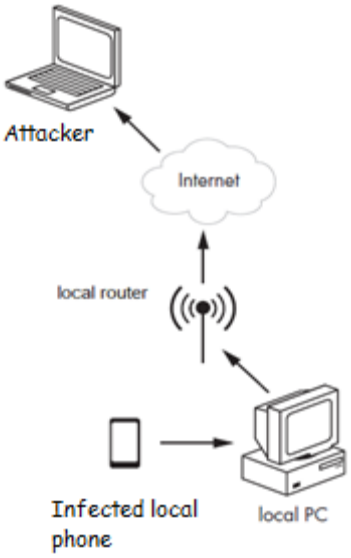

Figure 23. Pivoting process [37].

In order to perform the operations shown in Figure 23, port scanning was performed with the Nmap tool installed in the android SPF tool. If the Nmap tool is not installed on the SPF, the Nmap tool installation should be performed. The service and target device IP information that the Nmap tool will use to scan on the target device should be provided.

```
Select a command to perform or 0 to return to the previous menu
spf> 12
Download Nmap and port scan a host of range. Use any accepted format for
target specification in Nmap
Nmap Target: 192.168.92.136
Delivery Method(SMS or HTTP) HTTP
```

Figure 24. Usage of Nmap.

As shown in Figure 24, the command of the Nmap tool to perform port scanning on the device with IP address 192.168.92.136 using HTTP service. This IP address is the IP address of a computer on the network to which the mobile device is connected. As a result of the relevant device scan, it was found that TCP port 21 on the computer was open. After this process, the computer can be attacked over the TCP port. Android devices cannot detect scripting languages such as Python, Perl. For this reason, it may be necessary to compile the C code in order to run on the android device after the relevant payload is determined.” windows/meterpreter/reversetcp” is a vulnerability exploitation code that is used to obtain sessions belonging to windows operating system. The C code of payload was compiled with the SPF tool as shown in Figure 25 to work on android.

```
spf> 9
Compile code to run on mobile devices
1.) Compile C code for ARM Android
spf> 1
Compiles C code to run on ARM based Android devices. Supply the C code file and the output
filename
File to Compile: /root/Smartphone-Pentest-Framework/exploits/Windows/warftpmeterpreter.c
Output File: /root/Smartphone-Pentest-Framework/exploits/Windows/warftpmeterpreter
```

Figure 25. Compile the C code via SPF.

After compiling the C code, the download of the related vulnerability is as shown in Figure 26.

```
Select a command to perform or 0 to return to the previous menu
spf> 6
Downloads a file to the phone. Fill in the file and the delivery method(SMS or HTTP).
File to download: /root/Smartphone-Pentest-Framework/exploits/Windows/warftpmeterpreter
Delivery Method(SMS or HTTP): HTTP
```

Figure 26. Downloading vulnerability.

Before exploiting the vulnerability downloaded in Figure 26, the multi/handler module was opened on the msfconsole tool, and option settings were made. Multi handler module enables the payload command ("windows/meterpreter/reversetcp"), which is used to connect to the target system with the windows operating system on the network to which the android device is connected via TCP service. The process of providing the system with the TCP port is as shown in Figure 27.

```
msf > use multi/handler
msf exploit(handler) > set payload windows/meterpreter/reverse_tcp
payload => windows/meterpreter/reverse_tcp
msf exploit(handler) > set LHOST 192.168.92.132
LHOST => 192.168.92.132
msf exploit(handler) > exploit
[*] Started reverse handler on 192.168.92.132:4444
[*] Starting the payload handler...
```

Figure 27. Usage of multi handler module.

In Figure 27, the LHOST value refers to the IP address of the android device and payload refers to the command used to obtain a reverse TCP connection on the target system. After the reverse TCP connection is obtained on the target windows system, the exploitation of the downloaded vulnerability is as seen in Figure 28.

```
Select a command to perform or 0 to return to the previous menu
spf> 7
Run a command in the terminal. Fill in the command and the delivery
method(SMS or HTTP).
Command: warftpmeterpreter 192.168.92.136 21
Downloaded?: yes
Delivery Method(SMS or HTTP): HTTP
```

Figure 28. Exploiting of downloading vulnerability.

In Figure 28, the command information and transmission method information are given to the SPF tool for its operation. The IP information used in the full command is the IP information of the windows device. The 21 used after the IP address is the TCP port number of the target device. After the downloaded vulnerability was exploited, a user session was obtained on the target windows operating system which was previously reversed.

4. CONCLUSION

In this study, the architecture and security mechanism of the android operating system are examined and different attack scenarios are created. For this purpose, classifications were made in four different categories; mobile attacks, remote attacks, client-side attacks, attacks using malicious applications, and mobile post exploits. In the remote attacks category, SSH attack scenarios were performed, while in the client-side attacks category, Webkit package attack and USSD attack scenarios were realized. Attacks using malicious applications were carried out with the scenario of embedding malicious software in legitimate APKs (backdooring apks) and creating fake applications directly with the SPF tool. Gathering information, remote control, gain privileges, and pivoting scenarios were used in the category of mobile post exploits. As a result of attack scenarios applied, the following suggestions are presented;

Passwords for remote attacks category should be changed regularly and kept up to date. In order to prevent client-side attacks, users should pay attention to fake links sent via SMS, mail, QR codes and should not keep NFC-based applications in an automatic setting. As a precaution against attacks caused by malicious applications, there is recommended to pay attention to the installation permissions required during the installation process of APKs, and to keep patches and versions up-to-date. As a precaution against mobile post-exploitation, the data which can be obtained by information gathering tool should be kept secret and the configuration of the wireless network and database should be taken care of.

To ensure mobile security, code developers can create more secure applications with “code scrambling” tools such as “proguard” and “dexguard”. It necessary to pay attention to the code gaps that will occur after the APK applications are converted to source codes with the help of decompiling tools. Authorization and code blocks can be checked and measures can be taken with mobile application analysis programs such as MobSF, Drozer, and AndroBugs.

In parallel with the updated technologies, cyber attacks are also updated. Current security vulnerabilities should be followed for future studies. Depending on these vulnerabilities, the penetration testing tools (msfconsole, metasploit framework v.b.) to be used should also be updated. During virtual installation, it should be noted that all operating systems are connected to the same

Domain on VMWare Workstation. The Kali Linux operating system on which the attack was carried out can be installed on a separate machine. Thus, the realism of penetration tests is increased.

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RESEARCH ARTICLE

ALIEN VERTEBRATES AND VERTEBRATE PESTS IN TURKEY WITH AN OVERVIEW OF RODENT MANAGEMENT

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ABSTRACT

Considering alien and invasive species are major threat to indigenous species, the recent status of these type of vertebrates were reviewed along with the current rodent management application in Turkey. More than twenty-five alien, potentially invasive freshwater fish species have been reported in Turkey. Of these, seven were recorded from inland waters as alien and two as translocated. Eighteen marine fishes from the Mediterranean and three from the Black Sea have previously been reported as invasive or alien. In this study, of twelve fishes, eleven were determined as potential invader in our long term observations in Mediterranean Sea; puffer fish and Vanikoro sweeper (*Pempheris vanicolensis*) were evaluated as considerably successful invasive ones. Two reptile species are known as alien in Turkey: the Red-eared Slider (*Trachemys scripta*) is an imported species in south-western rivers of Anatolia; İstanbul Wall Lizard (*Podarcis siculus*) is a transported species found in the Marmara region in Turkey. Four bird species are alien and potential invaders; Laughing dove (*Spilopelia senegalensis*), Ring-necked parakeet (*Psittacula krameria*), White-spectacled bulbul (*Pycnonotus xanthopygos*) and Common myna (*Acridotheres tristis*). Mammalian species; nutria (coypu-*Myocastor coypus*) which is introduced to two rivers in Turkey, black and Norway rats (*Rattus rattus* and *Rattus norvegicus*) along with house mouse (*Mus musculus*) are alien and also synanthropic species. According to the rodent management report released in 2013 for agricultural areas, 1.202 kg zinc phosphide was used for rodent control in 73 provinces of Turkey; Konya province was most active against rodents, using 400 kg zinc phosphide in 19 956 kg poisoned baits. Only anticoagulant rodenticides are permitted to use in urban areas. Application doses of 50 mg/kg of anticoagulant rodenticides caused death three days later after poisoned baits were given to rats. In our experiments, no resistance to anticoagulant rodenticides was determined in black and Norwaybrown rats around

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Ankara provices. Control of other potential vertebrate pests such as snakes, Egyptian fruit bat and porcupine is not legally permitted in Turkey.

Keywords: alien vertebrates, rodent control, Turkey

1. INTRODUCTION

As a country connecting Asia to Europe, Turkey has high biological diversity due to the faunal migration routes through Europe, The Caucasus and the Arabian Peninsula. The Dardanelle and Bosporus Straits play a major role in these routes. Recent colonisation of terrestrial vertebrate fauna in Turkey took place during the Pleistocene after the withdrawal of an internal sea in Central Anatolia, and Anatolia has also many endemic vertebrate species [1], [2], [3], [4], [5], [6], [7], [8], [9], [10]. [7] suggested that glacial contractions and expansions in Turkey during the Pliocene and Pleistocene created an area of convergence of many species with rich endemism. Recent vertebrate inventories of Turkey have reported 377 freshwater fish, 512 marine fish, 34 amphibians (17 frogs, 17 salamanders), 131 reptiles (1 blind lizard, 64 common lizards, 55 snakes, 11 testudines), 478 birds and 170 mammals (15 marine mammals) with total of approximately 1707 species [9], [10], [11], [12], [13], [3], [4], [14], [15], [16], [17], [18], [19], [20], [21], [22], [23]. Invasive species have been reported to cause extinction of animals [24], [25]. The alien and translocated fishes in Turkish inland waters were reviewed in detail by "İnnal and Erk'akan (2006), İnnal (2012), [10], Tarkan et al. (2012, 2015), Saç and Özuluğ (2017), Yoğurtçuoğlu and Ekmekçi (2018), Özuluğ et al (2018) and [23]. [10] also pointed out that invader fishes can cause changes in species composition, and can replace native species in aquatic ecosystems. [17] reported that Turkey had 314 freshwater fishes with 54 endemic species and 49 under threat. [10] found that the total freshwater fish inventory was 350 species with 135 endemics.

[21] reported that total of 377 fish species live in the inland waters of Turkey. Among these species, three fishes are globally extinct, 5 fishes are extinct in Turkey, 28 of them are non-native, 340 of them are native, and 157 species are endemic to Turkey [23]. The number of the non-native fish species and distribution areas of these species have increased in recent years with new data about some species, such as *Heteropneustes fossilis*, *Pterygoplichthys disjunctivus*, *Pseudorasbora parva*, *Carassius gibelio*, *Gambusia holbrooki*, *Lepomis gibbosus*, *Pygocentrus nattereri*, *Pangasius sanitwongsei* "(Tarkan, 2006; Yalçın Özdilek, 2007; Ünlü et al., 2011; Tarkan et., 2012; Şaç & Özuluğ, 2017, Yoğurtçuoğlu & Ekmekçi, 2018, Özuluğ et al., 2018)". Alien fish species are introduced into the freshwater ecosystems by different ways such as aquaculture, biological control, pet trade and fisheries. Within these species, *G. holbrooki* is the first species deliberately vaccinated in freshwater systems for biological control to fight against malaria [10]. "Copp et al (2005)" and "Kennard et al. (2015)" mentioned that the detrimental impacts of these fishes on ecosystems were recognized mainly with predation, food and habitat competition, hybridization, habitat degradations, and disease transfer "(Özuluğ et al., 2018)". [10] also pointed out that invasive fishes can capable to change species compositions, and can replace the native species in aquatic ecosystems.

"Otero et al. (2013)" listed 18 marine fish species in a black list of Mediterranean invaders while drawing attention to factors causing invasions, such as the Suez Canal, Gibraltar Strait, rising salinity, warming sea temperatures and ballast water dumping. The marine fish of Turkey comprise 512 species and are reviewed in detail by [18]. The majority of fishes, including the black list of Mediterranean invaders (Otero et al., 2013), have also been recorded on Turkish coasts "(Artüz, 1999;

Bilecenoğlu et al., 2002a; Dobrovolov et al., 2003; Çınar et al., 2005; Çiçek, 2006; Aleksandrov et al., 2007; Engin et al., 2007; Vasil'eva, 2007; Tuncer et al., 2008; Bilecenoğlu, 2010; [18])".

Although the exact numbers of Turkish amphibians and reptiles is not certain, according to recent reports, the Turkish herpetofauna comprises approximately 34 amphibians and 132 reptilian species "(Baran et al., 2012; [21]; the reptile database, 2015)". Until now, no amphibian species have been reported as introduced or invasive, but two reptilian species — the Red-eared Slider and İstanbul Wall Lizard — have been reported as invasive species "(http://www.europe-aliens.org/pdf, Hür et al., 2008; Mollov, 2009; Ficetola et al., 2012; Ilgaz et al., 2013; Silva-Rocha et al., 2014; Tok et al., 2015; [21], The reptil database, 2015)".

The avifauna of Turkey includes nearly 483 bird species. These are native, migrant, and rare or transit birds. IUCN Invasive Species Specialist Group (ISSG-2017) reported that eleven bird species are invasive in Turkey; *Acridotheres tristis*, *Oxyura jamaicensis*, *Psittacula krameri*, *Alectoris chukar*, *Anas platyrhynchos*, *Anser anser*, *Bubulcus ibis*, *Columba livia*, *Cygnus olor*, *Porphyrio porphyrio* and *Streptopelia decaocto* (http://www.iucngisd.org/gisd/). Turkey consists of two major geographic parts, Turkish Thrace and Anatolia (Asia Minor). Due to its geographical isolation and its location at the crossroads of three migration routes, the Turkish mammalian inventory comprises species mixtures of arboreal mammals from Europe, steppe mammals from The Caucasus and desert mammals from the Arabian Peninsula. With recent records, the number of mammalian species has reached 170 [3], [4], [12], [15], [16]. Nutria (*Myocastor coypus*) is known as alien among these species (Mursaloğlu, 1973; Özkan, 1999; İliker et al., 2009)". "Khlyap et al. (2010)" stated that 62 mammalian species are alien to Russian territory grouping such as (1) Intentional introduction, (2) Reintroduction, (3) Self-dissemination and (4) Accidental introduction. Some rodent species especially Synanthropic and Agrophilic have been considered as alien species "(Khlyap and Warshavsky 2010)". In consistent with these, "Kosoy et al. (2015)" reported that the term of invasive species indicates that Black (*Rattus rattus*) and Brown (Norway) (*Rattus norvegicus*) rats are characterized as historically introduced species; cosmopolitan in their distribution, and alien where they did not inhabit previously, and they mostly lives inside houses or using other man-made feature.

Biocides, including rodenticides, are used worldwide to manage pests in both agricultural and urban areas. Rodents are the one of the largest mammalian orders, with more than 2000 species, and cause economic losses, damage infrastructure, eat and contaminate large quantities of food, and transmit diseases to humans (e.g. bubonic plague, typhus, Weil's disease, toxoplasmosis, trichinosis, hantaviruses, babesiosis, Lyme disease, tularaemia, cutaneous leishmaniosis, etc.). Rodent management and related topics are explained in detail by "Buckle and Smith (1994), Atkinson (2000), Gratz (2006), and WHO (2006)". In Turkey, rodent pest management is regulated and supervised in agricultural areas by the Ministry of Food, Agriculture and Livestock, and in urban areas by the Ministry of Health. In present study, the alien or invasive status of non-native vertebrate in Turkey were discussed, and also the current pest management on Turkish rodents was evaluated.

2. MATERIALS AND METHODS

The findings is based on data obtained from the entire Turkey between 2010-2019, comprising data from sampling, observations from both scuba diving and searching inland ecosystems, and previous studies. The vertebrate species (fishes, reptile and birds) listed in this study were mainly classified according to terms for ecological invasion and its corresponding stages defined by "Colautti and MacIsaac (2004)", and mammalian species were classified by the definitions of "Khlyap and

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Warshavsky (2010)". Fish samples were collected from various rivers, dams and lakes in Turkey (Table 1, Figure 1). Equipment such as electro shocker, gill nets and scoop nets were used to sample fish. The samples were fixed in 4–5% formaldehyde solution and were deposited in the Biology Department of Ankara University. Our data on marine fishes was mainly obtained from three locations: at Kaş (m1), Alanya (m2) and Gazipaşa (m3) (see Figure 1). The data from Alanya is based on long term observation between 2010-2019. The data on reptiles, birds and mammals was obtained from field observations and published literature. The studies on rodent pest management were performed on both wild rats (n= 5 in each groups) (Black and Brown Rats from Ankara provinces) and laboratory (Wistar) rats using first and second-generation anticoagulant rodenticides (warfarin, brodifacoum, bromadiaolone, chloropacinone, difenacoum, difethialone) readily available for commercial sale in Turkey. The active ingredients of rodenticides were analysed with HPCL before being given to rodents. Five samples were used in each experiment with application doses of 50 mg/kg.

Table 1. Record locations of alien inland fishes in the present study.

Record locations of inland fishes in the present study	Numbers on the map
Işıklı Lake (Denizli)	r1
Büyük Menderes, Eber Lake (Dinar, Çay, Afyon)	r2
Yamula Dam Lake (Kayseri)	r3
Su Çatı, Menzelet (Göksun,Kahramanmaraş)	r4
Eşen Çayı (Fethiye, Muğla)	r5
Alara river (Alanya, Antalya)	r6
Köyceğiz Lagoon (Muğla)	r7
Langır Creek (Dalaman-Muğla)	r8
Kızılırmak River (Yozgat)	r9
Kızılırmak River (Sivas)	r10
Büyük Menderes (Denizli)	r11
Çine Stream (Aydın)	r12
Topçam Lam Lake (Aydın)	r13
İznik Lake (İznik, Bursa)	r14
Gölmarmara (Manisa)	r15
Hirfanlı Dam Lake (Ankara)	r16
Kesikköprü Dam Lake (Kırıkkale)	r17
Mogan Lake (Ankara)	r18
Sakarya River and Sarıyar Dam Lake (Ankara)	r19
Uluabat Lake (Bursa)	r20
Bafa Lake (Muğla)	r21
Beyşehir Lake (Konya)	r22
Upper Ceyhan River (Ekinözü, Kahramanmaraş)	r23
Spring water around Burdur Lake (Burdur)	r24
Cuma Creek (Orhaneli, Bursa)	r25
Eğirdir Lake (Isparta)	r26

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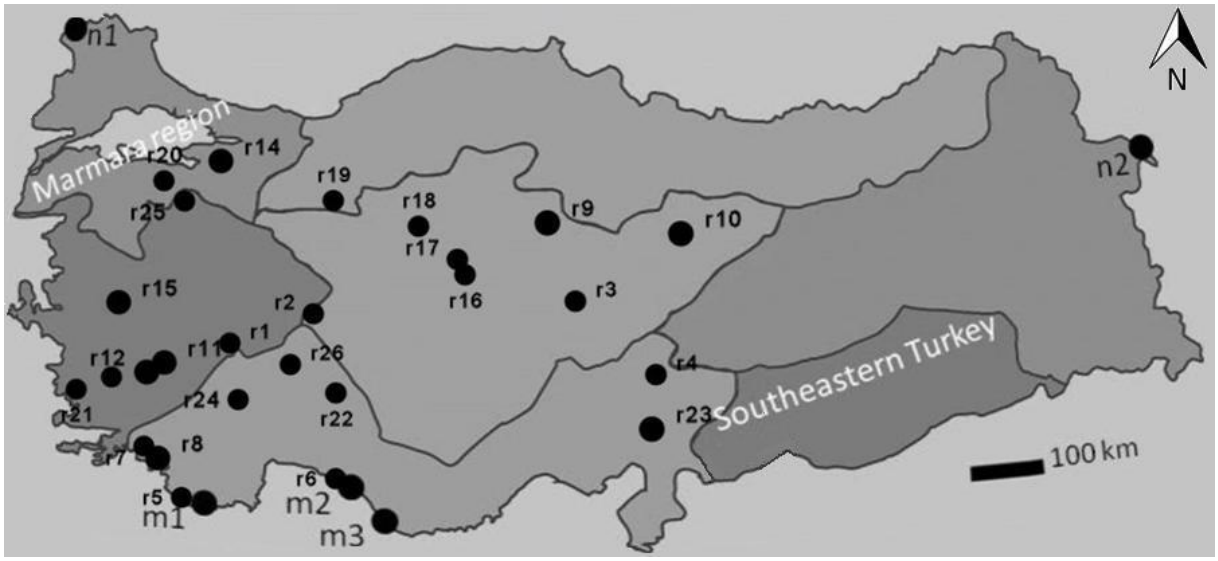

Figure 1. Record locations of alien vertebrates of Turkey; m (marine locations), n (locations of Nutria), r (rivers), see in Table 1 for abbreviations.

3. RESULTS

3.1. Alien Vertebrates in Turkey

The invasive status of alien vertebrate species recorded in Turkey was evaluated using our distribution records (Tables 2, 3, 4). A non-indigenous species may arrive in a country by means of travelling, or being transferred, transported, introduced or imported (terms described by "Colautti and MacIsaac, 2004"). Some of indigenous and non-indigenous species may be intentionaly introduced from one parts of Turkey to another. Some species can be defined as synanthropic and agrophilic "(Khlyap and Warshavsky 2010)". The statuses of Turkey's vertebrate species were evaluated under this framework.

3.2. Freshwater Fishes

The numbers of fresh water fishes are controversial and have variously been reported to be 213 [14], 236 [13],, 314 [17], 310 [10] and 377 [22], [23]. Thirty fishes were introduced from other countries (exotic) and sixteen translocated to to Turkish inland waters by "İnnal and Erk'akan (2006). Later, İnnal (2012)" listed 30 fish species as alien and 24 species as translocated, including some marine genera such as *Liza*, *Mugil* and *Sparus*. In this study, nine fishes were reported to be alien and two translocated in 26 river systems in Turkey (Table 2), invasive ones; Gibel carps, Topmouth gudgeon, Rainbow trout, Eastern Mosquitofish, Zander, Piranha, Nile Tilapia, Pumpkinseed sunfish, Giant Pangasius. Pirahna was only reported in Uluabat-Sapanca Lake, and Giant Pangasius in only Sakarya River. Other invasive fishes were recorded from more than one aquatic system, and translocated fishes are Tench and Sand smelt which are also invasive and occupy in many rivers and lakes (Figure 1, Table 2). However it is unknown whether these species have extended their range or have established natural populations in inland waters, and also taxonomic status of some of these fishes is uncertain. "İnnal (2012)" also reported that 12 species have established natural populations in inland waters. Within the invasive fish species, some "*Alburnus chalcoides*, *Cyprinus carpio*, *Sander lucioperca*, *Perca fluviatilis*, *Silurus glanis*" are vaccinated to the reservoir for fishery and production purpose,

some "*Acipener baeri, Captogon zilli, Oreochromis aureus, Oreochromis mossambicus, Oreochromis niloticus, Salmo salar, Salvelinus alpinus, Salvelinus fontinalis*" are accidentally released to the freshwater systems during research carried out in universities, some "*G. holbrooki, Ctenopharyngodon idella, Hypophatalmichthys malitrix* " vaccinated to the freshwater systems for biological control, and some of the aquarium species "*P. sanitwongsei, P. nattereri*" are intentionally entered into the freshwater system by human [10], Yoğurtçuoğlu and Ekmekçi, 2018)". Fishes alien to Turkish inland waters such as *C. gibelio*, *P. parva*, *Oncorhynchus mykiss*, *G. holbrooki*, *Sander lucioperca*, *Oreochromis niloticus* and *L. gibbosus* were introduced for commercial or pest management purposes, and are reported to be invasive species "(Wildekamp et al., 1997; Şaşı and Balık, 2003; Özuluğ et al., 2004, 2007; Uğurlu and Polat, 2007, Pyke, 2008; Ekmekçi et al., 2010; Przybylski and Zieba, 2011; Aydın et al., 2011; Tarkan et al., 2012; [10]; Karakuş et al., 2013; Tarkan et al., 2015)". *C. gibelio,* one of the first invaders, has been recorded in eight rivers and lakes. It is important for commercial fishing, especially in the eutrophic and polluted Eber Lake (Table 2, see Figure 1), and it is said to be exported to Iraq. Alien fishes such as *Oreochromis* sp., *Captodon zillii*, and *L. gibbosus* are known to have spread to costal reservoirs and creeks in the west and south-west of the country [11], Çalışkan and Yerli, 2000; İnnal and Erk'akan, 2006; Özcan, 2007; Mert and Çiçek, 2010, Aydın et al., 2011)". Our records for *L. gibbosus* from Büyük Menderes River in Denizli and Cuma Creek in Bursa provinces (Table 1; r11, r25 and see Figure 1) prove the expansion of the distribution of the species. *Ctenopharyngodon idella* is other alien species that has been introduced into many aquatic systems, but has not established natural populations in most of these systems "(İnnal and Erk'akan, 2006; Tarkan et al., 2015)". In consistent with these reports, this species was not caught in our survey of inland waters. Apart from this, Janitor fish (*P. disjunctivus*), Stinging catfish (*H. fossilis*) and Piranha (*P. nattereri*) are also alien to Turkish inland waters and are reported from the rivers of south-eastern Anatoli and Sapanca Lake "(Yalçın-Özdilek, 2007; Ünlü et al., 2011, [10], but there is no record for these species that establishing population in in the freshwater systems. *Salmo salar* and *Salvelinus fontinalis* occasionally appear in inland waters of Black Sea coast of Turkey "(İnnal and Erk'akan, 2006)". The intentional introduced species *Tinca tinca* were caught at four locations. This species is known to be introduced to many aquatic systems, and is reported to have a harmful effect on natural populations "(Ekmekçi, 2010; [10]. Another intentional introduced species *Atherina boyeri,* which is native to the Black Sea coast of Turkey and has high ecological tolerance to salinity, was recorded from eight inland aquatic systems (Table 2). This species is known to be translocated to Turkish lakes and rivers, and to be a successful invader "(Balık et al., 2005)". "Crivelli (1995)" has stated that fish introductions threaten endemic freshwater fishes in the northern Mediterranean region. Consistent with this report, the introduction of *Sander lucioperca*, *T. tinca*, *Pseudorasbora parva* and *A. boyeri* to Hirfanlı Dam Lake in Central Anatolia caused the extinction of *Alburnus* sp. "(Ekmekçi et al., 2010)". In addition, two endemic fishes, *A. akili* and *Pseudophoxinus handlirschi* were extinct after *S. lucioperca* was introduced to Lake Beyşehir "(Küçük et al., 2009; [10]. Also "Pyke (2008)" pointed out the harmful effects of mosquito fishes on invertebrates, fishes and amphibians. The recent status and detailed evaluations of alien fresh water fishes were revised by "İnnal and Erk'akan (2006), İnnal (2012), [10] and Tarkan et al. (2015), Özuluğ et al. (2018) and [23]. In the assessment of "Tarkan et al. (2015)", 30 species were reported to be introduced, 11 translocated, and 19 established self-sustaining populations, they used a term translocated as the range extension considering the criteria given by "Blackburn et al. (2011)". According to this criteria, "Tarkan et al. (2015)" stated in consistent with our findings that invasive species were listed as fully invasive (criterion E: *A. boyeri, C. gibelio, Cyprinus carpio, P. parva, G. holbrooki*), self-sustaining populations in the wild, with individuals surviving and reproducing a significant distance from the original point of introduction (criterion D2: *T. tinca, L. gibbosus, Clarias gariepinus*) and self-

sustaining populations in the wild, with individuals surviving a significant distance from the original point of introduction but not reproducing (criterion D1: *Captodon zillii*).

3.3. Marine Fishes

Apart from fresh water fishes, the potential invader and alien Mediterranean and Black Sea marine fishes were listed by "Taşkavak and Bilecenoğlu (2001), Bilecenoğlu (2010), Otero et al. (2013) and Yankova et al. (2013)". Of these species, sixteen from the Mediterranean and three from the Black Sea have been introduced due to range extension or human transportation and are evaluated along with our records in Table 3. The number of non-native fish species was 33 in 2002 "(Bilecenoğlu, 2002a)"; in 2008 that number reached 49 "(Bilecenoğlu, 2010)", including 18 fish species as a black list of Mediterranean invaders "(Otero et al., 2013)". In this study, *Alepes djedaba*, *Fistularia commersoni*, *Lagocephalus spadiceus*, *Lagocephalus sceleratus*, *Nemipterus randalli*, *Hemiramphus far*, *Pempheris vanicolensis*, *Sargocentron rubrum*, *Siganus luridus*, *Siganus rivulatus*, *Upeneus molluccensis* and *Upeneus pori* were captured and observed via scuba diving, fishing or in fish markets; *A. djedaba* was rarely and seasonally observed in fish markets. Supporting our records, "Golani et al. (2013)" reported that *A. djedaba* had expanded its westward distribution to the island of Crete. *F. commersoni* was observed in Kaş (see Figure 1; m1) as single individual at a depth of 10 m, and was also reported in Alanya Bay (see Figure 1; m2). The first puffer fishes were observed in 2005, the current density of these two species are very high and cause threat to angling in daytime, and the range expansion of *L. sceleratus* was given in detail by "Bilecenoğlu (2006)", and *L. spadiceus* by "Tuncer et al., (2018)". Although another Tetraodontid fish, *Torquigener flavimaculosus*, was reported to be very abundant in Fethiye Bay "(Bilecenoğlu, 2010)", this species is not listed on the Mediterranean black list by "Otero et al. (2013)", and not caught or observed in Alanya and Gazipaşa coasts. Randall's threadfin bream (*N. randalli*) is frequently caught in fishing boats around Alanya and Gazipaşa bays, and is a commercial species in fish markets. This fish is reported to be a successful expander by "Bilecenoğlu and Russell (2008), Bilecenoğlu (2010)". Vanikoro sweeper (*P. vanicolensis*), a successful invader, has begun to dominate in inshore caves. However, Redcoat (*S. rubrum*), which occupies the same niche as Vanikoro sweeper, has not increased in population density in the last ten years in Alanya and Gazipaşa coasts. According to "Bilecenoğlu (2010)", *U. molluccensis* is a successful coloniser with commercial importance in trawl catches. This species is frequently seen at local fish markets, and is caught from the sandy grounds of Alanya and Gazipaşa Bays along with *U. pori*. "Çiçek (2006)" suggested that *U. molluccensis* formed 3% of alien fish biomass on the Turkish Mediterranean coast. *S. luridus* was rarely caught but *S. rivulatus* was abundant in the records from scuba diving and fishing with gill nets. Apart from the fishes listed as invasive by "Otero (2103)", Black-barred halfbeak (*H. far*) was also caught in shore angling, but not considered as invasive due to the rarity in Alanya and Gazipaşa Bays. This fish is also listed as alien by being emphasized in their abundance in İskenderun Bay by "Bilecenoğlu (2010)". Three fishes in the Black Sea are reported to be alien: *Parablennius incognitus*, *Gobius cruentatus*, and *Liza haematochelia*. *L. haematochelia* was introduced to the Black Sea, but this species seemingly has not expanded to the Marmara and Aegean Seas, and no detailed information is available about its invasive status "(Tuncer et al., 2008; Bilecenoğlu, 2010)".

3.4. Reptiles

Currently there is no amphibian recorded as alien to Turkey, but one turtle species (*Trachemys scripta*, which are released to inland water by pet owners) and one species of lizard *Podarcis siculus* are known as alien species to Turkey (Table 4). The Red-eared Slider, *T. scripta*, was reported from a river in the south-west of Turkey "(Ficetola et al., 2012; [21])". This species is reported to threaten native turtles and feed on aquatic insects, fishes and amphibians "(Ficetola et al., 2012)", and its likely

distribution overlaps with two other native turtles in Turkey, *Emys orbicularis* and *Mauremys rivulata*. This species was not seen in the rivers in our surveys performed throughout south-west parts of the country, but the Red-eared Slider is considered to be a potential competitor with native turtles in the future.

P. siculus "İstanbul Wall Lizard" was first recorded from Istanbul by "Berthold (1842)" and is mostly distributed in the Marmara region of Turkey (mostly in the Asiatic part, see region in Figure 1). Even though this species is non-native, recent studies have shown that this lizard has not expanded its range extensively in Turkey "(Podnar et al., 2005; Uğurtaş et al., 2007; Mollov, 2009; Ilgaz et al., 2013; Silva-Rocha et al., 2014; Tok et al., 2015)". It is found as far to the east as Zonguldak province in the western Black Sea region of Turkey "(Ilgaz et al., 2013)". No additional records are presented in this study for this taxon and its invasive status is unknown.

3.5. Birds

Of eleven birds on the IUCN Invasive Species Specialist Group (ISSG-2017) invasive species list, three species are observed in Turkey. *A. tristis* (Common myna) is an alien species in Turkey and has been observed in İstanbul, Ankara, İzmir, Kayseri and Samsun "([19])". *O. jamaicensis* (Ruddy duck) have been recorded as alien species in Turkey but there are no distribution records for the species. *P. krameri* (Ring-necked parakeet) has colonised some parts of Turkey. This species was imported and accidentally released in Ankara in the 1980s, and is frequently observed in Ankara. They were recorded in Ankara (in this study), and they have also been observed in İstanbul as large colonies, İzmir, Denizli, Antalya, Adana, Gaziantep, Şanlıurfa "([19])". The native bird, *Pycnonotus xanthopygos* (White-spectacled bulbul), is not included in the ISSG list as an invasive species, but has been recorded as expanding its distribution and has started to colonise new localities "(Aslan and Erdoğan, 2007)". *Streptopelia senegalensis* (Laughing dove) is not a native bird in Turkey but not covered in the ISSG list. However, its distribution is expanding; they now occur in many cities but were known only in İstanbul and south-east Turkey (see the region in Figure 1) in the early 20th century "(Albayrak, 2011)". Record locations for these birds are given in Table 3.

3.6. Mammals

Among Turkish mammals, only one, *Myocastor coypus* (Nutria), was accidental introduced. It is known from only two river systems: the Tunca and Meriç Rivers in Turkish Thrace and the Aras River in eastern Turkey (Table 4, see Figure 1; n1, n2, "Mursaloğlu, 1973; Özkan, 1999; İliker et al., 2009"). We also observed nutria around the Tunca and Meriç Rivers. There is no information about its effects on ecosystems and local fauna. "Khlyap and Warshavsky (2010)" also reported that synanthropic and agrophilic rodents are a part of danger invasive alien mammals of Russia, and they also reported that Brown rat, the House mouse (*Mus musculus*), the Striped Field mouse (*Apodemus agrarius*), the East European vole (*Microtus levis*) are as synanthropic and agrophilic rodents in Russia. By the considering definitions of "Khlyap and Warshavsky (2010)", three synanthropic/alien rodent species such as Western House Mouse (*Mus domesticus*), Brown (*R. norvegicus*) and Black (*R. rattus*) rats are common in Turkey. The genus *Mus* is represented by two species: *M. domesticus* and *M. macedonicus* (Balkan House Mouse), later occupies mostly rural areas and the grain fields. Brown rat is mostly distributed in central and northern urbans of country instead of southern parts through Mediterranean cost. Black rat is also common throughout the country with various colour morphs. Turkish synanthropic rodents belong to groups 3 (Self-dissemination) and 4 (Accidental introduction) according to the groups given by "Khlyap et al. (2010)". In addition to Brown, Black rats and House mouse, *A. agrarius* and *M. levis* are distributed in Turkey but it is not possible to say anything about

their dissemination ways to Turkey, and they are not taken into account as synanthropic and agrophilic for Turkey.

3.7. Rodent Control in Agriculture and Urban Areas

In Turkey such rodents are regulated and supervised (rodent pest management) in agricultural areas by the Ministry of Food, Agriculture and Livestock, and in urban areas by the Ministry of Health. Rodents are classified simply as voles or moles in agricultural areas, according to a document on the plant health and application program released in 2014, and management procedures are explained in the related documents "(General Management of Food and Control, 2014)". Many of these rodents are not alien and their distributions are detailed given by [4]. The first group of rodents ("voles") includes mainly *Microtus* spp. (*M. hartingi, M. lydius, M. guentheri, M. socialis, M. anatolicus* and *M. dogramacii*) and *Meriones tristrami* which are distributed in grain fields, and *Rattus rattus frugivorus* in citrus gardens in Southern Turkey. However, the same fields are also occupied by non-target species such as grey dwarf hamster (*Cricetulus migratorius*), Turkish hamster (*Mesocricetus brandti*), Anatolian ground squirrel (*Spermophilus xanthoprymnus*), and European ground squirrel (*Spermophilus citellus*).

The second group, "moles", includes *Nanospalax leucodon* in Turkish Thrace and *N. nehringi* in the Asiatic part of Turkey. Poisoned grains (2–5% zinc phosphide) are traditionally used for rodent control in agricultural areas. According to a 2013 report, 1.202 kg zinc phosphide was used for rodent control in 73 provinces of Turkey in 2013. Konya province was most active against rodents, using 400 kg zinc phosphide in 19956 kg poisoned baits, followed by Afyon with 2610 kg, and Balıkesir and Elazığ with 2500 kg each. Mechanical methods are used for mole management "(General Management of Food and Control, 2014)". Apart from zinc phosphide, the fumigant aluminium phosphide is also used for rodents and is freely available on the Turkish market.

In this frame first- and second-generation anticoagulant rodenticides are permitted for use in urban areas for synanthropic rodents. In our experiments performed on wild rats (Brown rat and Black rat) and Wistar rats (laboratory race of Brown rat), 50 mg/kg doses of anticoagulant rodenticides such as warfarin, brodifacoum, bromadiolone, chlorophacinone, difenacoum and difethialone caused death three days later after the poisoned baits were laid. No resistance to the rodenticides was observed in wild rats or Wistar rats captured around Ankara province. However, our findings were obtained from the small sample group, genetic tests for anticoagulant rodenticide resistance is needed for more precise results. Apart from the rodenticides listed above, other licensed rodenticides such as coumatetralyl and flocoumafen are used for rodent control and can be purchased from shops by individuals. However, the pest controls in urban areas are permitted to perform private licensed companies. The Ministry of Health also manages cases concerning public health. For example, the Ministry of Health successfully manages tularaemia, a well-known rodent-borne disease in rural settlements. Pest control procedures are not legally permitted for any species apart from rodents, despite complaints about snakes, Egyptian fruit bats *Rousettus aegyptiacus* and porcupines *Hystrix indica*. A naphthalene + sulphur mixture is permitted for use against snakes as a repellent, but there is no robust evidence of its efficacy. Egyptian fruit bat and porcupine are mainly found on the Mediterranean coast of Turkey. Egyptian fruit bat droppings foul the walls and windows of facilities when the flight path to fruit trees is close to buildings. The porcupine is a protected rodent and is under threat, but illegal hunting for folk remedies is very common in Turkey. There have been some complaints about porcupines gnawing avocado and olive seedlings. However, the control of Egyptian fruit bat and porcupine are not legally permitted in Turkey. Not all the listed harmful species are considered alien in Turkey.

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Table 2. Alien and translocated (Tra) freshwater fishes recorded in Turkish aquatic ecosystems (Int: Introduced, Inv: Invasive, Col: Colonizing, r: record locations in the present study, see map).

Order/Family	Scientific name	Common name	Status	r
Cypriniformes/ Cyprinidae	*Carassius gibelio*	Gibel carps	Int, Inv	2, 9, 10,11, 12, 13,14,15
Cypriniformes/ Cyprinidae	*Pseudorasbora parva*	Topmouth gudgeon	Int, Inv	11,12,12,16 17,18,19,20
Cypriniformes/ Cyprinidae	*Tinca tinca*	Tench	Tra	9,10,16,17
Salmoniformes / Salmonidae	*Oncorhynchus mykiss*	Rainbow trout	Int, Col, Inv	1,2,3,4,5,6
Atheriniformes / Atherinidae	*Atherina boyeri*	Sand smelt	Tra, Col, Inv	3,14,16,17, 18, 21,22,23
Cyprinodontiformes / Poecilidae	*Gambusia holbrooki*	Eastern Mosquitofish	Int, Inv	1,14,15,16,17, 23
Perciformes / Percidae	*Sander lucioperca*	Zander	Int, Inv	16,22,26
Charciformes/ Serrasalmidae	*Pygocentrus nattereri*	Piranha	Int, Inv.	20
Perciformes / Cichlidae	*Oreochromis niloticus*	Nile Tilapia	Int, Inv	7, 8
Perciformes / Centrarchidae	*Lepomis gibbosus*	Pumpkinseed sunfish	Int, Inv	12, 13, 25
Siluriformes Pangasiidae	*Pangasius sanitwongsei*	Giant Pangasius	Int, Inv	19

Table 3. Some potential invader fishes (based on Otero et. al., 2013) in Turkish coast of Mediterranean and Black Sea (PS: present study).

Order/ Family	Scientific name	Common name	PS (see map)	References
Mediterranean				
Perciformes/ Caragidae	*Alepes djedaba*	Shrimp scad	Alanya, Gazipaşa	Akyüz, 1957; Geldiay, 1969; Gücü et al., 1994; Otero et al., 2013
Perciformes/ Apogonidae	*Apogonichthyoides pharaonis*	Pearlycheek cardinalfish	-	Otero et al., 2013; [18]
Atheriniformes/ Atherinidae	*Atherinomorus forskalii*	Red sea hardyhead silverside	-	Kosswig, 1950; Geldiay, 1969; Otero et al., 2013
Syngnathiformes/ Fistulariidae	*Fistularia commersonii*	Bluespotted cornetfish	Kaş, Alanya	Bilecenoğlu, 2002a; Otero et al., 2013
Tetradontiformes/ Tetraodontidae	*Lagocephalus spadiceus,*	Puffer fishes	Alanya, Gazipaşa	Akyol et al., 2005; Bilecenoğlu et al.,

	Lagocephalus scleratus			2002b, 2006; Tuncer et. al., 2008; Otero et al., 2013
Perciformes/ Nemipteridae	*Nemipterus randalli*	Randall's threadfin bream	Alanya, Gazipaşa	Bilecenoğlu and Russell, 2008; Gülşahin and Kara, 2013; Otero et al., 2013; Bilecenoğlu et al., 2013
Beloniformes/ Exocoetidae	*Parexocoetus mento*	African Sailfin flyingfish	-	Ben-Tuvia, 1966; Avşar and Çiçek, 2000; Otero et al., 2013
Beloniformes/ Hemiramphidae	*Hemiramphus far*	Black-barred halfbeak	Alanya	Kosswig, 1950; [18]
Perciformes/ Pemperidae	*Pempheris vanicolensis*	Vanikoro sweeper	Alanya, Gazipaşa	Papaconstantinou, 1988; Gücü et al., 1994; Otero et al., 2013
Beryciformes/ Holocentridae	*Sargocentron rubrum*	Redcoat	Alanya, Gazipaşa	Kosswig, 1950; Otero et al., 2013
Aulopiformes/ Synodontidae	*Saurida undosquamis*	Brushtooth lizardfish	-	Ben-Tuvia, 1966; 1973; Otero et al., 2013
Perciformes/ Siganidae	*Siganus luridus, Siganus rivulatus*	Spinefoot species	Kaş, Alanya	Ben-Tuvia, 1973; Fischer, 1973; Kosswig, 1950; Otero et al., 2013
Tetraodontiformes/ Monacanthidae	*Stephanolepis diaspros*	File fish	-	Ben-Tuvia, 1966; Çiçek, 2006; Otero et al., 2013; Bilecenoğlu et al., 2013
Perciformes/ Mullidae	a. *Upeneus molluccensis* b.*Upeneus pori*	Goatfishes	Alanya, Gazipaşa	Kosswig, 1950; Ben-Tuvia, 1973; Akyol et al., 2006; Otero et al., 2013
Black Sea				
Perciformes/ Blenniidae	*Parablennius incognitus*	Pygmy blenny	-	Vasil'eva, 2007; [18]
Perciformes/ Gobiidae	*Gobius cruentatus*	Red-mouthed goby	-	Artüz, 1999; Engin et al., 2007; [18]
Mugiliformes/ Mugulidae	*Liza haematocheila*	Redlip mullet	-	Kosswig, C., 1974; Dobrovolov et al., 2003; Aleksandrov et al., 2007; [18]

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Table 4. Imported (Imp), intentional (Int), invasive (Inv), transported (Tra), travelling (Trv), spreading (Spr), colonizing (Col) reptiles, birds and mammals of Turkey (Ps: present study).

Order/Family	Scientific name	Common name	Status	PS (see map)	References
Reptiles					
Testudines/ Emydidae	*Trachemys scripta*	Red-eared slider	Imp, Col	-	Daisie., 2015; Ficetola et al., 2012
Squamata/ Lacertidae	*Podarcis siculus*	İstanbul Wall Lizard	Tra, Spr	-	[21]; Berthold, 1842; Bird, 1936; Bodenheimer, 1944; Başoğlu and Baran, 1977; Çevik, 1999; Uğurtaş et al., 2000; Hür et al., 2008; Mollov, 2009; Ilgaz et al., 2013; Silva-Rocha et al., 2014.
Birds					
Columbiformes/ Columbidae	*Streptopelia senegalensis*	Laughing dove	Inv, Col	Bursa, Niğde	Albayrak, 2011; [19]
Psittaciformes/ Psittaciculidae	*Psittacula krameri*	Ring-necked Paraket	Imp, Col	Ankara, İstanbul	[19]
Passeriformes/ Pycnonotidae	*Pycnonotus xanthopygos*	White-spectacled Bulbul	Spr, Col	Mediterranean coast	Aslan and Erdoğan, 2007; [19]
Passeriformes/ Sturnidae	*Acridotheres tristis*	Common myna	Trv, Col	İstanbul	[19]
Mammals					
Rodentia/ Myocastoridae	*Myocastor coypus*	Nutria (coypu)	Int	n1: Meriç, Tunca, n2: Aras	Mursaloğlu, 1973; Özkan, 1999; İliker et al., 2009

4. CONCLUSIONS and DISCUSSION

Present research focused on the alien, invasive alien vertebrate species and pest management of synanthropic rodents in Turkey. We reported nine fresh water fishes as alien (seven potential invasive and two intentional introduced). Invasive freshwater fishes in Turkey are known to be harmful, and caused to extinction of the some native species. There are no certain data the effect of alien Marine fishes on the indigenous species in Turkish seas. No alien species was reported from amphibian classis. Two alien species from reptilian classis are thought to be potential threat for natural species, especially freshwater turtle in Turkish rivers. Mammalian alien is nutria in Turkey and there is no information about any threat on the natural population in the ecosystem occupied by nutria. There are

3 synanthropic rodents which are originally not native to the country. The pest control in urban are performed on these rodents using anticoagulant rodenticide. However, their efficiency on the target species and anticoagulant resistance are not reported up to now.

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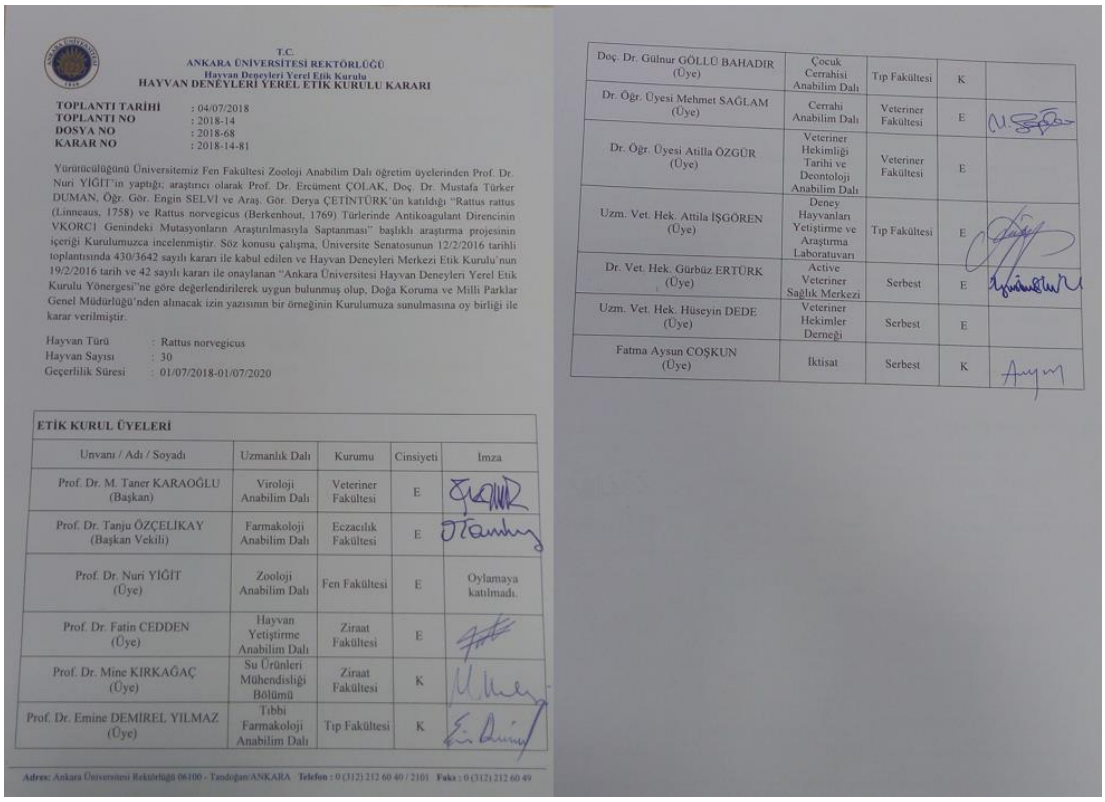
ADDITIONS

Permission was given by Ankara University Local Ethics Committee for Animal Experiments (Document no: 2018-14-81).



T.C.
ANKARA ÜNİVERSİTESİ REKTÖRLÜĞÜ
Hayvan Deneyleri Yerel Etik Kurulu
HAYVAN DENEYLERİ YEREL ETİK KURULU KARARI

TOPLANTI TARİHİ : 04/07/2018
TOPLANTI NO : 2018-14
DOSYA NO : 2018-68
KARAR NO : 2018-14-81

Yürütücülüğünü Üniversitemiz Fen Fakültesi Zooloji Anabilim Dalı öğretim üyelerinden Prof. Dr. Nuri YİĞİT'in yaptığı; araştırıcı olarak Prof. Dr. Ercüment ÇOLAK, Doç. Dr. Mustafa Türker DUMAN, Öğr. Gör. Engin SELVİ ve Araş. Gör. Derya ÇETİNTÜRK'ün katıldığı "Rattus rattus (Linneaus, 1758) ve Rattus norvegicus (Berkenhout, 1769) Türlerinde Antikoagulant Direncinin VKORC1 Genindeki Mutasyonların Araştırılmasıyla Saptanması" başlıklı araştırma projesinin içeriği Kurulumuzca incelenmiştir. Söz konusu çalışma, Üniversite Senatosunun 12/2/2016 tarihli toplantısında 430/3642 sayılı kararı ile kabul edilen ve Hayvan Deneyleri Merkezi Etik Kurulu'nun 19/2/2016 tarih ve 42 sayılı kararı ile onaylanan "Ankara Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu Yönergesi"ne göre değerlendirilerek uygun bulunmuş olup, Doğa Koruma ve Milli Parklar Genel Müdürlüğü'nden alınacak izin yazısının bir örneğinin Kurulumuza sunulmasına oy birliği ile karar verilmiştir.

Hayvan Türü : Rattus norvegicus
Hayvan Sayısı : 30
Geçerlilik Süresi : 01/07/2018-01/07/2020

ETİK KURUL ÜYELERİ

Unvanı / Adı / Soyadı	Uzmanlık Dalı	Kurumu	Cinsiyeti	İmza
Prof. Dr. M. Taner KARAOĞLU (Başkan)	Viroloji Anabilim Dalı	Veteriner Fakültesi	E	
Prof. Dr. Tanju ÖZÇELİKAY (Başkan Vekili)	Farmakoloji Anabilim Dalı	Eczacılık Fakültesi	E	
Prof. Dr. Nuri YİĞİT (Üye)	Zooloji Anabilim Dalı	Fen Fakültesi	E	Oylamaya katılmadı.
Prof. Dr. Fatin CEDDEN (Üye)	Hayvan Yetiştirme Anabilim Dalı	Ziraat Fakültesi	E	
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RESEARCH ARTICLE

THE EFFECT OF NITROGEN FIXING ASYMBIOTIC BACTERIA FERTILIZER ON NUTRIENT CONTENTS AND SOME AGRONOMIC PROPERTIES OF CORN PLANT

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ABSTRACT

This study aims to examine the effects of biological fertilizer consists of microorganisms, which bind the free nitrogen (N_2) of air in an asymbiotic way, like *Azospirillum* sp., *Azorhizobium* sp. and *Azoarcus* sp. on the properties of soil and nutrient contents of the plant and some agronomic properties of corn. The experiment was set up in a randomized block design with 3 repetitions. In the experiment, 2 doses of biological fertilizer (0–0,5 g ha^{-1}; BF_0-BF_1) and 3 doses of nitrogen fertilizer (0 – 125 – 250 kg N ha^{-1};N_0-N_1- N_2) were applied. Consequently, the effects of the applications on the electrical conductivity of soil, NO_3-N were statistically significant, and in the second period, the effects on available Mn in the soil, and iron and copper in the plant, and agronomic properties like cob diameter, height of the first cob, stem thickness were determined. The biological fertilizer enabled the corn plant to make better use of the Fe element in the soil.Compared to the control parcel (BG_0N_0), an increase of 22,3 g cob-1 (24,03%) in grain weight and 183 kg da^{-1} (24,04%) in grain yield was detected with biological fertilizer application (BG_1N_0). These results Show the effectiveness of biological fertilizer. It was concluded that for environmentally friendly and economical corn production 125 kg N ha^{-1} nitrogen may be recommended along with a 0,5 kg ha^{-1} biological fertilizer.

Keywords:*Maize, Biofertilizer, Nutrition, Yield, Soil Properties.*

1. INTRODUCTION

Population growth causes increasing nutritional requirements. Agricultural areas are gradually decreasing. For this reason, it is necessary to get more crop from per unit. This is also a degradation problem i.e. pressure on natural resources due to exceeding carrying capacity. Since using fertilizers is important to crop increase more chemical fertilizers are using by the day. Using chemical fertilizers causes soil deterioration (Salinization, heavy metal accumulation, nutrient imbalance, deterioration of microorganism activity), water eutrophication and nitrate accumulation, air pollution by nitrogen and sulfur and

greenhouse effect [1]. Human health is threatened, and environmental pollution is increasing [2]. These negative situations guided researchers to work on alternative agricultural production techniques and plant nutrition strategies and new production systems like “organic farming” and “good farming practices” have been put into practice. These of biological fertilizers in plant nutrition and protection and sustainability of the ecological balance are aimed in these systems [3].

It is recommended to use microorganisms selected from the rhizosphere in order to protect and increase soil fertility and to obtain maximum product from plants. For this purpose, plant growth-promoting rhizobacteria (PGPR) are used as biological fertilizers (BF) due to their beneficial effect on plant growth. Biofertilizers are of great importance in sustainable agricultural production [4]. PGPR are bacteria which are colonized in plant root. PGPR provide mineralization of soil organic matter, immobilization of mineral nutrients, dissolution of phosphates, balanced nitrification, nitrogen fixation and plant hormone production, acquisition of nutrients that encourage plant growth and reduction of heavy metal toxicity, and protect the plant against root pathogens. As a result, they contribute to the increase of agricultural production by promoting plant growth [5].

Corn (*Zea mays* L.) is a plant which can grow under different climatic conditions. It has high potential of yield, is called the "king of grains" and ranks third in the world in terms of production area [6]. Due to its high yield potential, it requires a high amount of nutrients and a good nutritional management strategy [7]. Corn plant has a high need for nitrogen among nutrients [8]. Nitrogen is a factor which limits productivity in corn cultivation most. The nitrogen need of plants is supplied by chemical fertilizers, mineralization of organic matter and biological fixation of atmospheric nitrogen. Nitrogen given by chemical fertilizers is lost by leaching, turning into gas and volatization, and its effectiveness decreases. It also causes environmental pollution. Plants benefit more effectively from nitrogen which is biologically bound to the soil. Especially bacteria, algae and fungi are used individually and in combination as biological fertilizers [4]. Some of the biological fertilizers include *Rhizobiums* pp. species which live in symbiosis with legumes, some include *Azospirillum* sp., *Azotobacter* sp., *Acetobakter* sp, *Azoarcus* sp. which are free-living species [10].

It has been determined that the use of chemical nitrogen fertilizers can be reduced by 50% by using biological fertilizers [2]. The use of 200 ml ha^{-1}*Azospirillum brasilense* containing biological fertilizers in corn plants has proved to be effective and has been shown as an alternative to the use of chemical fertilizers [11]. The positive effects of biological fertilizer and urea application on corn yield were determined, and it was stated that planting corn seeds after inoculating with biological fertilizers would decrease urea application rate [12]. İnoculation with *Azospirillum brasilense* has been reported to increase the efficiency of nitrogenous fertilizers and corn yield [13].

Many researches have been carried out to demonstrate the effectiveness of biological fertilizers in corn cultivation. However, biological fertilizers can be produced to contain microorganism species for single or multiple purposes. Biological fertilizers may consist of imported or native species in terms of the microorganism they contain. In the application of these fertilizers, the problem of adaptation in biological fertilizers consisting of imported species, and the competition and resistance potentials which may occur

in biological fertilizers containing native species should be taken into account. Therefore, it is important to determine the suitability of these fertilizers in different climate, soil and plant conditions.

Along with investigating the efficiency of a freeze-dried commercial preparation containing asymbiotic bacterias *Azospirillum sp., Azorhizobium sp.* And *Azoarcus sp*. in corn cultivation, this study will also help to determine whether the effectiveness of chemical nitrogen fertilizer may be increased with the use of biological fertilizers or not.

2. MATERIAL and METHOD

2.1. Material

The research was carried out on the experimental field of Manisa Celal Bayar University Alaşehir Vocational School. The experimental area is located at 38° 22 '24 "north latitude; 28° 31' 35" east longitude and is 152 meters above sea level. The climate in Alaşehir district is warm in winter, and very hot and dry in summer (Mediterranean climate). The average annual precipitation is 598 mm, and most of the precipitation occurs in the winter months. Annual average temperature is 16,3 °C, with a temperature of 25,4 °C, July is the hottest month of the year. [14].

The materials of the experiment are the field soil in which the research was conducted, chemical fertilizers used in the research, biological fertilizer (BF) and corn (*Zea mays* L.var. Indentada) cultivated as test plant. Some physicochemical properties of the soil belonging to the experimental area are given in Table 1.

Table 1. Some physicochemical properties of the soil.

Parameter 0 – 20cm	**20 – 40cm**			
pH	7,77*	(0.05)**	7,81	(0,04)
Electrical conductivity (µScm^{-1})	716	(152)	610	(66)
Lime (%)	4,17	(0.10)	4,4	(0,45)
texture	Loam		Loam	
Sand (%)	50,10	(1,11)	48,74	(1,66)
Silt (%)	20,26	(0,87)	20,76	(1,00)
Clay (%)	29,64	(0,36)	30,50	(2,18)
Organic Matter (%)	2,13	(0,31)	2,07	(0,21)
NH_4-N (mg 100g^{-1})	18,83	(2,64)	21,27	(1,47)
NO_3-N (mg 100g^{-1})	1,74	(0,59)	1,54	(0,22)
Total-N (%)	0,13	(0,01)	0,13	(0,01)
Available-P (mg kg^{-1})	37,69	(1,57)	38,21	(4,87)
Extractable-Na (mg kg^{-1})	34,23	(14,8)	28,77	(9,04)
Available-K (mg kg^{-1})	501	(76)	486	(58)
Available-Ca (mg kg^{-1})	2150	(30)	2034	(140)
Available-Mg (mg kg^{-1})	743	(64)	714	(63)
Available-Fe (mg kg^{-1})	6,07	(0,14)	6,79	(0,74)
Available-Cu (mg kg^{-1})	1,95	(0,11)	1,70	(0,07)

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Available-Zn (mg kg^{-1})	4,26	(0,22)	4,18	(0,16)
Available-Mn (mg kg^{-1})	13,35	(0,97)	8,49	(0,44)

* All values are the average of 4 repetitions and are calculated on the oven-dry weight.
**The numbers in parentheses give the standard deviation of the mean.

The experimental soil has "slightly alkaline" reaction [15] and is non-saline [16]. The soil texture is in the loamy, "medium humus" [17] and medium calcareous class [18]. According to the macro plant nutrient content in the experimental soil; nitrogen was identified as "good" [19], phosphorus as "good" [20], potassium as "very high" [21]. Calcium as "good" and magnesium as "very high" [19]. The microelements (Fe, Zn, Mn and Cu) were found to be at a "sufficient" level [22] in the experimental soil.

The experimental plots were irrigated with artesian water in the experimental area. Irrigation water quality characteristics were determined by analyzing water samples taken from this artesian at the beginning, middle and end of the irrigation season for 3 times. The results obtained are shown in Table 2. The irrigation water has mild alkaline reaction and is in the C_2S_1 irrigation water class. It does not cause any salinity and alkalinity problems under normal conditions [16]. Concentrations of Na^+, Cl^- and boron, which are toxic to plants, are suitable [23].

Table 2. Quality properties of irrigation water.

Parameter		
pH	7,71	(0,23)*
Electrical conductivity (EC) (µScm^{-1})	600	(27,09)
Na^+ (me l^{-1})	0,95	(0,05)
K^+ (me l^{-1})	0,11	(0,01)
Ca^{++}+ Mg^{++} (me l^{-1})	4,85	(0,25)
Total cation (me l^{-1})	5,91	
Cl^-(me l^{-1})	0,97	(0,16)
$CO_3^=$ (me l^{-1})	0,00	
HCO_3^-(me l^{-1})	3,86	(0,13)
$SO_4^=$ (me l^{-1})	1,16	(0,05)
Total anion (me l^{-1})	5,99	
Sodium adsorption ratio(SAR)	0,61	
B (mg l^{-1})	0,17	(0,02)
Class	C_2S_1	

* The numbers in parentheses give the standard deviation of the mean.
The biological fertilizer used in the study is a commercial preparation consisting of endophyte bacterias of *Azospirillum* sp., *Azorhizobium sp.* and *Azoarcus* sp., which have the ability to bind atmospheric nitrogen (N_2) to soils in an asymbiotic way. It is in 0.5 g packages and the number of live bacteria is reported as 10^{11}cfu / gr.

In the study, single hybrid and mid-late Helen corn variety (*Zea mays* L. var. Indendata) with a vegetation period of 130-135 days was used as a test plant. The variety has no soil selectivity and has high

adaptability. It is a variety with high hectoliter weight, low grain moisture at harvest and high yield. It was preferred in the experiment as it is a variety cultivated in the Aegean region because of these features.

2.2. Method

In the research, biological fertilizer and chemical nitrogen fertilizer were considered as independent variables. The experiment was created in a randomized block design with 3 replications. Biological fertilizers were tested at doses of 0 – 0,5 g ha^{-1} (BF_0 – BF_1), and nitrogen at 0 – 125 – 250 kg N ha^{-1} (N_0 – N_1 – N_2). 3 nitrogen doses without biological fertilizers ((BF_0N_0, BF_0N_1, BF_0N_2) and 3 nitrogen doses with biological fertilizers (BF_1N_0, BF_1N_1, BF_1N_2) constituted the application subjects. Parcel dimensions are 3,0m x 3,4m and a parcel area is 10,2 $m^{2.}$ In order to reduce the edge effects in the experiment, 2 m space was left between the blocks and 1 m between the parcels. Considering the previous studies and regional conditions, the nutrient needs of the corn plant were determined as 250 kg ha^{-1} N, 150 kg ha^{-1} P_2O_5 and 200 kg ha^{-1} K_2O in the study.

Nitrogen applications considered as independent variable are zero nitrogen dose ($N_{0)}$, half nitrogen dose ($N_{1)}$ and full nitrogen dose ($N_{2).}$ While planting all of the N_1 and half of the N_2 doses were applied with ammonium sulfate fertilizer (21%N) as an amount of 595 kg ha^{-1} on May 21, 2015. 41 days later (on June 6.2015) half of the N_2 was applied with ammonium nitrate fertilizer (33%N) as an amount of 379 kg $ha^{-1.}$ Phosphorus and potassium fertilizers were applied to all parcels with triple super phosphate (42-44% P_2O_5) in the amount of 349 kg $ha^{-1}$and with 400 kg ha^{-1} potassium sulfate ((50%K_2O). Then the fertilizers applied to the parcels were mixed with a cultivator to a depth of 10-12 cm soil.

After chemical fertilizer applications, 5 rows of 68cm x 18cm row spacing and two seeds in each row in each plot, 32 seeds in each row and 160 seeds in each parcel were planted (May 21. 2015). When the plants reached a shoot length of about 10 cm, a single plant was left with 18 cm row spacing by singling. When the plants reached a shoot length of about 14-30 cm (June 17. 2015), biological fertilizer was applied at a dose of 0,5 g ha^{-1} by following the fertilizer manufacturer's recommendations. Biological solutions of 5 liters prepared for each parcel on which biological fertilizer was used were applied homogeneously to the root of the plant rows in the evening in cool hours with a ridge sprayer. Immediately after the application, irrigation was made with the drip irrigation system, thereby enabling the microorganisms to reach the root area. The development of the test plant in the experimental field was precisely observed from seed planting to harvest. All cultural processes were carried out in a way that would not harm the microbial population in the soil. No pesticides or herbicides were used during the experiment.

In the experiment, composite soil samples were taken from each plot in two periods, before biological fertilizer application (17 June 2015) and at harvest (19 October 2015). The samples taken were made ready for analysis in accordance with the rule.Also, leaf samples were taken from the first leaves opposite and below the cob while the corn plant was in the full detasking stage, representing the plots. The samples cleaned according to the methots were dried at 65^{o}C and made ready for analysis by grinding [24]. In order to determine the yield and agronomic characteristics, the edge rows of each plot were excluded from the sampling at harvest, and randomly 10 plants from the three rows in the middle were cut from the soil surface.

pH of soil samples was analyzed according to Jackson [25], electrical conductivity [26], Rayment and Higginson, organic matter according to Rautenberg and Kremkus [27], calcium carbonate according to Schlichting and Blume [28], the texture analysis of the soil according to Bouyoucos [29], total N according to Bremner [30] , NH_4-N according to Kandeler and Gerber [31], NO_3-N according to Scharpt and Wehrmann [32], available P according to Olsen and Sommers [20], available K, Ca, Mg according to Pratt [33], available Fe, Zn, Mn, Cu according to Lindsay and Norwell [22], respectively.

Plant samples prepared for analysis were subjected to wet combustion with concentrated nitric-perchloric acid mixture. Potassium and calcium flame photometric, magnesium, iron, copper, zinc, manganese elements were determined by Atomic Absorption Spectrophotometric methods in extracts obtained by wet combustion [24]. Phosphorus contents of the samples were measured in a colorimeter using the Vanado-Molybdo phosphoric acid (yellow colour) method [34]. Total nitrogen analysis in plant samples was made via the modified Kjeldahl method [30].

10 plant samples were taken randomly from each plot during the harvest. In the samples taken, measurements were made regarding planth eight, first cob height, stem thickness in plant, cob height, cob diameter, number of rows on the cob, grain weight on the cob, single stem weight, thousand-grain weight and grain yield. The results of the measurements are given as mean values. All the data obtained in the study were evaluated using the "SPSS 20.0" software, using the randomized blocks experimental design in factorial order according to multivariate ANOVA (MANOVA) variance analysis technique. Differences between group averages were determined via Duncan test.

3. RESULTS and DISCUSSION

3.1. The Effects on Some Physicochemical Properties of Soils

The effects of biological fertilizer and nitrogen applications on properties of soil are given in Table 3. While the effects ($P<0,05$) of the applications on electrical conductivity (EC) and NO_3-Nin the 1^{st} period and on available–Mn ($P<0,01$) in the 2^{nd} period were determined to be significant, the effects of applications on other properties were found to be insignificant (Table 3). Soil reaction (pH) decreased due to nitrogenous fertilizer applied in the 1^{st} period and EC values, which are indicators of salinity, increased. The reason for these changes is that the nitrogen fertilizer (Ammonium sulphate) used is a salt with physiological acid character. In the second period, an increase in pH values and a decrease in EC values were observed in all parcels with and without biological fertilizers (Table 3). The decrease in biological activity in the second period may be interpreted as the reason for the increase in pH, and rainfall and leaching of the salts through irrigation as the reason for the decrease in EC values. These results show that BF application has no significant effect on pH and EC values. Goutami et al. [35] stated that inorganic fertilizer, BG and organic farm manure applications do not significantly affect pH and EC values. Mulyani et al. [36] found that the pH value of the soil was affected by the BF application in their study to reveal the effect of BF application on the chemical properties of the soil. Orhan et al. [37] reported that PGPR application significantly affected the pH value. In the first period, the amount of organic matter in the soil was not affected by the practices. In the second period, there was a decrease in the amount of organic matter compared to the first period (Table 3). Second period soil samples were taken approximately 4 months after the first period. This period is the time when microbiological activity is most intense. Due to

the high microorganism activity during this period, the organic matter has been mineralized and its amount has decreased.

Total-N contents of soils were not affected much by the subjects applied in both periods and were determined to be at the same levels. NH_4-N values generally increased with nitrogen application in the 1st period. In the second period, NH_4-N values were determined at approximately the same levels. In both periods, the highest NH_4-N values were reached with BF and half dose nitrogen application. NO_3-N amounts increased in the first period due to nitrogen applications. The reason for this may be the conversion of nitrogen in the form of NH_4 to NO_3 by nitrification. In the second period, there was a decrease in NO_3-N values due to leaching compared to the first period (Table 3). In a study conducted, NH_4-N and NO_3-N were found to be higher in control and bio-fertilizer applications than chemical fertilizer applications in the soil [2].

The effects of the discussed subjects on available P, K, Ca, and Mg in the soil were not found to be statistically significant (Table 4).Available P was higher in parcels where BF and nitrogen were not applied (control parcels) than all other parcels. Since BF and N applications increase microorganism activities, it is thought that the use of available P also by microorganisms causes this decrease. In the second period, the amount of available K increased in other applications compared to the control. Organic and inorganic acids which pass into the soil as a result of increased microbial activity with microorganism and nitrogen applications may be the reason for this increase. In the second period, available Mg increased in the N_1 and N_2 parcels compared to the N_0 parcel where BF was not applied. The reason for this may be the physiological acid character of the nitrogen fertilizer applied. In the parcels where BF was applied, an increase occurred only in the N_2 dose. However, in general, the amounts of these macro nutrients tended to decrease in parcels N_1 and N_2 compared to the N_0 dose in plots treated with BF. This tendency to decrease was observed more clearly in N_1 parcels (Table 4). Similar results were obtained with the amounts of available micronutrients (Fe, Zn, Mn, Cu) (Table 5.). Goutami et al. (2015) reported that inorganic fertilizer, BF and organic farm manure applications cause significant effects on available N and P among macro elements in the soil and on Fe among micro elements. In their study which was conducted by applying organic fertilizer, different N doses and BF containing *Azotobacter* and *Azospirillum*, Tiyagi et al. [38] found that the N, P, K content in plants and soil increased significantly in all BF applied combinations. Orhan et al. [37] found that PGPR application significantly affected the total-N, available P, K, Ca, Mg, Fe, Mn, Zn contents in the soil. These results show that the application of BF and chemical nitrogen fertilizer together increases the utilization rates of the plant in nutrients. A better observation of this effect at the N_1 dose compared to the N_2 dose proves that the application of BF may reduce the amount of chemical nitrogen fertilizer to be used.

Table 3. The effects of biological fertilizer application on some chemical properties of the soil, total –N, NH_4-N and NO_3N contents.

Biological fertilizer (g ha^{-1})	**Nitrogen dose (kg N ha^{-1})**	**pH**		**EC (µScm^{-1})**		**OM (%)**		**Total-N (%)**		**NH_4-N (mg N 100 g^{-1})**		**NO_3-N (mg N 100 g^{-1})**	
		I	II	I	II	I	II	I	II	I	II	I	II

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	0	7,89	8,00	522c	467	2,49	1,88	0,120	0,130	9,03	9,36	0,87b	0,47
0	**125**	7,80	8,04	712ab	533	2,67	2,25	0,128	0,120	9,34	9,17	1,44a	0,57
	250	7,82	8,07	666abc	511	2,16	1,95	0,120	0,117	8,81	9,81	1,14ab	0,44
	0	7,88	8,05	573bc	492	2,11	1,90	0,115	0,113	8,49	9,77	0,95b	0,50
0,5	**125**	7,75	8,01	796a	447	2,04	1,74	0,120	0,117	11,27	10,35	1,54a	0,49
	250	7,78	8,00	722ab	508	2,17	1,87	0,116	0,113	9,42	9,20	1,37a	0,68
Significance		0,053	0,201	<0,050	0,899	0,159	0,825	0,792	0,543	0,375	0,697	<0,050	0,597

EC = Electrical conductivity, OM = Organic matter.

Table 4. The effects of biological fertilizer application on macronutrient contents.

Biological fertilizer (g ha^{-1})	**Nitrogen dose (kg N ha^{-1})**	**Available-P (mg kg^{-1})**		**Available -K (mg kg^{-1})**		**Available-Ca (mg kg^{-1})**		**Available-Mg (mg kg^{-1})**	
		I	II	I	II	I	II	I	II
	0	41,20	32,10	304,44	265,88	2168	1811	790	802
0	**125**	50.64	25,90	330,69	375,66	2228	2291	779	820
	250	51,37	26,51	304,44	300,27	2235	2211	827	828
	0	38,47	27,16	335,93	357,14	2155	2237	799	795
0,5	**125**	31,19	26,90	288,69	292,33	2242	1891	782	752
	250	31,75	26,51	304,44	328,04	2195	2211	813	815
Significance		0,12	0,808	0,739	0,874	0,455	0,84	0,623	0,6

Table 5. The effects of biological fertilizer application on micronutrient contents.

Biological fertilizer (g ha^{-1})	**Nitrogen dose (kg N ha^{-1})**	**Available -Fe (mg kg^{-1})**		**Available-Zn (mg kg^{-1})**		**Available -Mn (mg kg^{-1})**		**Available -Cu (mg kg^{-1})**	
		I	II	I	II	I	II	I	II
	0	5,68	5,75	4,07	3,52	6,28	5,10a	1,59	1,53

0	**125**	6,13	6,50	4,34	3,91	5,98	5,09a	1,74	1,65
	250	7,10	6,85	3,98	3,24	5,52	4,44ab	1,45	1,48
0,5	**0**	6,42	6,73	3,41	4,28	5,25	3,69bc	1,55	1,40
	125	6,45	6,67	3,22	3,37	5,35	3,53c	1,47	1,32
	250	6,06	5,86	3,50	3,72	5,72	4,12bc	1,57	1,55
Significance		0,211	0,853	0,133	0,514	0,168	<0,010	0,529	0,359

3.2. The Effects of Biological Fertilizer Application on Nutrient Content of Corn Plant

The effects of biological fertilizer and nitrogen doses on the nutrient contents of the corn plant are given in Table 6. While the effects of the discussed subjects in the study on N, P, K, Ca, Mg, Zn and Mn contents in the corn plant were not found statistically significant, the effect on Fe and Cu was($P<0,05$) significant (Table 6). When the amount of nutrients determined in the corn plant in the study were compared with the limit values suggested by Jones et al. [39], the N, P, K, Mg and Mn contents of the corn grown as test plant were found to be at the limit of "deficient" level and "sufficient" level, while Ca, Fe, Zn and Cu contents were "sufficient".

Nitrogen contents of plant samples taken from BF applied parcels were found to be higher than the plant samples taken from the unapplied parcels. It was determined that BF and nitrogen applications do not affect the phosphorus content of the plant. Similar results were found in all applications. In the examination of the effect of bio-fertilizer applications on the potassium content of the plant, it was found that the plant potassium was lower in the parcels where BF was applied, except for the N_2 dose. Calcium content of the plants taken from the parcels where BF applied was found to be higher compared to the unapplied parcels. Magnesium contents of plants were higher in N_0 doses where nitrogen was not applied, compared to the nitrogen doses of N_1 and N_2, in parcels with and without BF (Table 6.).İn their study investigating the effects of inoculation of corn seeds with diazotrophic bacteria and nitrogen doses applied as sprinkling, Longhini et al. [40] reported that N application up to 120 kg N ha^{-1} increased the nutrient content of the leaves, and also that N, P, K and S increased, Ca and Mg decreased through BF application.

Iron as one of the micronutrients of the plant samples increased through BF application. This increase was 10,3% for the N_0, 14,4% for the N_1 and 21,2% for the N_2, compared to the control respectively. In the parcels where BF was not applied, there was no increase in parallel with the nitrogen doses, even a decrease in the N_2 dose occurred (Table 6). These results show that BF application significantly affects the iron uptake of the plant and consequently the iron content of the plant. İpek and Eşitken [41] stated that Some PGPR increase the Fe availability in soil by decreasing pH by releasing organic acids or synthesizing low-molecular-weight iron-chelating agents (siderophores). While the BF application had no effect on the zinc and manganese contents of the corn plants, an increase in copper content was observed. Copper content of the plants did not change depending on nitrogen doses in parcels where BF not applied.

The increase in copper content of plant samples taken from BF applied parcels was 13,3% in the N_0, 31,3% in the N_1 and 26,7% in the N_2 compared to the control (Table 6).

Table 6. Theeffect of biological fertilizer application on nutrient contents of corn plant.

Biological fertilizer (g ha^{-1})	**Nitrogen dose (kg N ha^{-1})**	**N (%)**	**P (%)**	**K (%)**	**Ca (%)**	**Mg (%)**	**Fe (mg kg^{-1})**	**Zn (mg kg^{-1})**	**Mn (mg kg^{-1})**	**Cu (mg kg^{-1})**
0	**0**	2,59	0,19	1,65	0,24	0,23	117bc	26	21	15c
	125	2,43	0,19	1,55	0,27	0,21	118abc	29	19	16bc
	250	2,54	0,18	1,49	0,24	0,20	113c	25	19	15c
0,5	**0**	2,63	0,20	1,60	0,30	0,23	129abc	27	16	17bc
	125	2,58	0,18	1,44	0,29	0,19	135ab	24	18	21a
	250	2,57	0,19	1,60	0,30	0,21	137a	28	18	19ab
Significance		0,722	0,302	0,182	0,056	0,200	<0,050	0,623	0,814	<0,050

3.3. The Effects of Biological Fertilizer Application on Some Agronomic Properties of Corn Plant

Effects of BF application and different nitrogen doses on height of the first cob ($p<0,01$), stem thickness ($p<0,05$) and cob diameter ($p<0,05$) were found to be important. Although the effects on other agronomic properties were not statistically significant, efficiency of BF application was observed (Table 7).

The average plant height measured in the study varied between 246 cm and 261 cm (Table 7). Plant height decreased in N_1 and N_2 parcels where biological fertilizer was not applied, compared to control (N_0). In the parcels where biological fertilizer was applied, plant height increased by 1,6% in N_2 parcels and 5,2% in N_1parcels, compared to N_0 First cob binding height amounts varied between 98 cm and 107 cm (Table 7). The highest cob binding height was achieved with N_0 (BF not applied) with height of 107 cm. The next highest cob forming was achieved with N_1(BF applied) with a height of 105 cm. Both of them were statistically in different groups. While nitrogen application generally decreased the first cob forming height, an increase occurred with only half dose of nitrogen and BF application. Stem thickness amounts in the plants varied between 16mm and 18 mm (Table 7). The highest stem thickness (18 mm) was achieved with N_1 dose (BF applied). The average cob height rates varied between 20 cm and 21 cm (Table 7). Cob length increased one unit in N_1 and N_2 parcels compared to N_0 parcels where BF was applied on the other hand, in those where BF was not applied, while there was an increase in N_1 parcels, it remained the same in N_2 parcels. Cob diameter rates varied between 42 mm and 46 mm (Table 7). The highest cob diameter (46 mm) was achieved with N_2 dose (BF not applied). It is seen that nitrogen application

increases the diameter of the cob. Same results in the samples taken from N_1 and N_2 parcels where BF was applied shows that half dose nitrogen with BF is sufficient. The number of rows in the cob varied between 15 and 16 (Table 7). It can be suggested that BF application has no effect on the number of rows in cob. Llamelo et al. [42] found that different BF created significant differences in plant height, cob height, cob length, cob diameter, thousand grain weight and calculated yield. Marngar and Dawson [43] found that the application of *Azospirillum* inoculation with 150 kg ha^{-1} of nitrogen and 15 kg $ha^{-1}$of $ZnSO_4$ increased plant height, dry weight, amount of grains and grain yield.

Average weight of grain in cob varied between 87,0g $cob^{-1}$and 147,5 g cob^{-1} (Table 7). Grain weights in the cob were determined approximately at the same levels in N_0, N_1 and N_2 where BF was not applied. Nitrogen application had no effect on weight of grain in cob. In BF applied parcels at the N_0 an increase of 24% in weight of grain in cob, 70% in the $N_1$and 20% in the N_2 were observed compared to the parcels where BF was not applied. When parcels where BF applied were evaluated within themselves, an increase of 28% in the N_1 and a decrease of 5% in the N_2 were observed compared to the N_0. Those rates show that BF application positively affects the weight of grain in cob and this effect is clearer at the $N_1$with BF application. It may be suggested that the fact that asymbiotic bacteria in biological fertilizer content are more effective with N_1 nitrogen dose caused this significant increase. In their study investigating the effects of BF, mineral fertilizer and BF+ mineral fertilizer applications on corn agriculture, Namazari et al. [44] determined that BF application positively affected 100 grains weight, cob weight and grain yield, and BF+ mineral fertilizer combination increased the yield components.

Average single cob weight varied between 123,3 g and 182,5 g (Table 7). In N_0, N_1 and N_2 applications where BF was not applied, rates of single cob weight were determined at approximately the same levels by being parallel with the rates of grain weight in the cob. In the BF applied parcels, an increase of 17% in the N_0 dose, 48% in the N_1 dose and 15% in the N_2 dose were observed compared to parcels where BF was not applied. When parcels where BF applied were evaluated within themselves, an increase of 22% in the N_1 and a decrease of 2% in the N_2 were observed compared to N_0 dose. These results show that BF applications positively affects single cob weight as well as grain weigh in the cob. BF and half dose nitrogen application caused the highest increase in single cob weight.

Table 7. The effects of biological fertilizer application on some agronomic properties of corn plant.

Biological fertilizer (g ha^{-1})	**Nitrogen dose (kg N ha^{-1})**	**Plant height (cm)**	**First cob height (cm)**	**Stem thickness (mm)**	**Cob length (cm)**	**Cob diameter (mm)**	**Number of rows in cop (piece)**	**Weight of grain in cop (g cob^{-1})**	**Weight of a single cob (g)**	**Weight of thousand grain (g)**	**Grain yield (kg da^{-1})**
0	**0**	258	107a	16c	20	43bc	15	92,8	127,3	343	761
	125	246	98c	16c	21	44abc	16	87,0	123,3	350	714
	250	253	99c	17ab	20	46a	15	91,6	127,5	343	751

	0	248	101bc	16c	20	42c	16	115,1	149,2	347	944
0,5	**125**	261	105ab	18a	21	45ab	16	147,5	182,5	370	1209
	250	252	98c	17ab	21	45ab	16	109,6	146,2	377	899
Significance		0,114	<0,01	<0,05	0,632	<0,050	0,743	0,182	0,183	0,075	0,182

Thousand grain weight values varied between 343 g and 377 g (Table 7) and determined at the same levels in the N_0. N_1 and N_2 applications where BF was not applied. There was a 2% increase at the N_1 dose alone. On the other hand, in the parcels where BF was applied, there was an increase of 1,2% in the N_0 nitrogen dose, 5,7% in the N_1 dose and 9,9% in the N_2 dose compared to the parcels where BF was not applied. When the parcels BF applied were evaluated within themselves, there was an increase of 6,6% in the N_1 dose and 8,7% in the N_2 dose compared to the N_0 dose. These results show that BF application has a positive effect on thousand grain weight.

Grain yield rates varied between 714 kg da^{-1} and 1209 kg da^{-1}(Table 7). According to the yield rates obtained from the N_0 parcels where BF was not applied, there was a decrease of 6,2% in the N_1 dose and 1,3% in the N_2 dose. In the BF applied parcels, the grain yield increased by 24% in the N_0 nitrogen dose, 69% in the N_1 dose and 20% in the N_2 compared to the parcels where BF was not applied. When BF applied parcels were evaluated within themselves, an increase of 28% in the N_1 dose and 5% in the N_2 dose was observed compared to the N_0 dose. These results show that if nitrogen is sufficient in the soil, the effects of nitrogenous fertilizer on the yield are negative, but the nitrogenous fertilizer application with BF affects the yield positively up to a certain dose. Sarajuoghi et al. [45] reported that the application of nitrogenous and phosphorous fertilizers in different combinations with BF containing *Azotobacter* and *Mycorrhiza* increased the grain yield in corn by 15,78%, and that the applied BF reduced the need for phosphorus and nitrogen fertilizers.

4. CONCLUSIONS and SUGGESTIONS

In this study the efficacy of biological fertilizer at different nitrogen doses was shown by applying 0- 125 – 250 kg ha^{-1} nitrogen doses to the parcels without biological fertilizer (BF_0) and 0,5 g ha^1 dose of biological fertilizer (BF_1). In the evaluation of the studied soil properties, the effect ($P<0,05$) of application subjects on electrical conductivity (EC) and NO_3-N in the first period and the effect on the available Mn in the second period were found to be statistically significant. While the effects on N, P, K, Ca, Mg, Zn, Mn were found to be insignificant in the statistical evaluation of the analysis results on plant samples, it was determined that the effects on Fe and Cu were significant ($P<0,05$). Especially the positive effect of BF application on Fe is remarkable. In all the parcels where BF was applied, significant increases occurred in the Fe content of the plant compared to the parcels without BF. This shows that the applied biological fertilizer increases the availability of the iron element in the soil.

The effects of biological fertilizer application and different nitrogen doses on such agronomic properties as first cob height (P<0,01), stem thickness (P<0,05) and cob diameter (P<0,05) were determined to be significant. Although the effects on other agronomic properties were not statistically significant, the efficiency of the BF application was observed. When the data obtained from the biological fertilizer applied ($BF_{1)}$ and non-applied (BF_0) parcels, where nitrogen was not applied, were compared, (BF_0), increases in the grain weight of the cob were found to be 22,3 g $cob^{-1,}$ single cob weight 21,8 g cob^{-1}, thousand grain weight 4 g and grain yield 183 kg da^{-1} depending on BF application.

In evaluating the results of this study as a whole, it was determined that the best results were obtained from the parcels where half of the biological fertilizer and recommended nitrogen were applied. Therefore, it was concluded that biological fertilizer containing asymbiotic microorganisms may be recommended with the application of 125kg N ha^{-1} nitrogen in corn cultivation, thereby preventing soil degradation and environmental pollution which may occur with excessive use of fertilizers.

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RESEARCH ARTICLE

HISTOLOGICAL STUDY OF STOMACH OF *LYCIASALAMANDRA FAZILAE* AND *LYCIASALAMANDRA FLAVIMEMBRIS*

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ABSTRACT

Histological examinations were conducted in order to describe the tissues on the stomach of the critically endangered *Lyciasalamandra fazilae* (Başoğlu and Atatür, 1974) and *L. flavimembris* (Mutz & Steinfartz, 1995). In this study two adult females, two adult males and one juvenile from Dalyan, Turkey (*L. fazilae*) and one adult male and one juvenile from Marmaris, Turkey (*L. flavimembris*) were examined. Tissue samples were taken from the freshly dead animals found on the road during the night field work. They were taken into alcohol for the histological studies. Tissue samples were fixed in Bouin's solution and routine histological techniques were applied. Hematoxylin and Eosin stains were used to identify cells and structures of the tissues. Stomach is covered with gastric columnar epithelial cells and glandular epithelium which are contained with gastric glands. Tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa were observed as mucosa layers. There was no significant difference between males and females in mucosa layers where gastric glands are situated. The histological structure of stomach of both species between males and females and between adults and juveniles were found to be mostly similar. Results from the literature review has indicated that the stomach histology show a similarity with the other Urodela species.

Keywords: *Lyciasalamandra fazilae*, *Lyciasalamandra flavimembris*, Stomach, Histology

1. INTRODUCTION

The digestive system of amphibians has two major part; a digestive track and digestive glands. The anatomy and histology of the digestive system is flexible and may give different responses to variation in environmental conditions [1]. Digestive track of amphibians begins with the buccal cavity, the pharynx, oesophagus, stomach and small and large intestines. Mostly the general morphology of the digestive track is similar within the amphibians, show some differences in dimensions [2]. Digestion begins in the stomach for the amphibians. Most amphibians are carnivores but in some species their diet varies according to age.

The distribution of *Lyciasalamandra* [3] is restricted to a small, ~350 X 50 km region of southwestern Turkey between Muğla and Antalya, plus three offshore Greek islands. The Turkish Lycian salamanders are currently classified as seven allopatric species *L. antalyana, L. atifi*, *L. billae*, *L. fazilae, L. flavimembris* and, *L. luschani*. The seventh described species, *L. helverseni* inhabits the

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Greek islands of Karpathos, Kasos and Saria opposite the westernmost Anatolian species. Ecologically, Lycian salamanders are all terrestrial, ovoviviparous and aestivating species [4]. They are most active during the cooler winter months from December to March. According to Veith et al. (2001) generalized ecological information on all Turkish *Lyciasalamandra* species can be summarized by as follows; they are restricted to karstic limestone with precipitation exceeding 800 mm annual rainfall, most localities are below 500 m elevation, and typical habitat is pine forest on northerly exposed slopes. However, little is known about the specific ecological microhabitat requirements for each *Lyciasalamandra* species.

Lyciasalamandra flavimembris is an endemic species found around Marmaris and Ula. It spreads at altitudes up to 600 m above sea level. They are found in stony areas (with limestone rocks) within the pine forests or maquis that are not destroyed or modified [5]. The species previously thought to be subspecies of *Mertensiella luschani* were reclassified under the genus Lyciasalamandra. While making this classification, apomorphic base changes that are different from all other Salamandridae in the mitochondrial genome of Lyciasalamandra genus were noticed [3].

L. fazilae classified as Endangered by the IUCN is restricted to the southern Anatolian coast, Turkey Muğla to Fethiye and ranges from sea level up to 1,000 m asl [5]. The species can be distinguished from other Lyciasalamandra species by the fact that the head and body are orange reddish in color. The dorsal bump in the tail base of male individuals can be used for gender identification.

There are several studies on the histology of Urodela species [6]–[11]. Recent studies were conducted on the histological studies on stomach of *Triturus karelinii*, *Mertensiella luschani* and *Salamandra infraimmaculata* [8], [10]. According to Koca and Karakahya (2014) the stomach tissues of *T. karelinii* and *M. luschani* show similar characteristics. To date from Lyciasalamandra genus only *Lyciasalamandra luschani* (former name: *Mertensiella luschani*) stomach histology was examined [8]. More studies using histochemical, morphological and immunohistochemical have been used to identify the differences of gastrointestinal tube between the anuran species [12]–[16].

In recent years, decreases in amphibian species and populations have been accepted as a problem with the global dimension. The main reasons for this situation are the change, fragmentation or disappearance of habitats. Increasing UV-B radiation in recent years can directly affect amphibians or slow down growth, causing sublethal effects such as making the immune system inoperable. In addition, pollution caused by chemical stressors (pesticides, heavy metals, nitrogen fertilizers, acidification) has also been shown to cause deaths and other sublethal effects (slow growth, developmental or behavioral abnormalities, decreased reproductive success, weakened immune system, hermaphroditism). The objective of this study is to describe the histological characteristics of the stomach of *L. fazilae* and *L. flavimembris* and compare with the previous studies and contribute to the literature in this area.

2. MATERIAL AND METHOD

A total of five specimens of *L. fazilae*, two adult females, two adult males and one juvenile from Dalyan, Turkey and one adult male and one juvenile of *L. flavimembris* from Marmaris, Turkey were collected from the freshly dead animals found on the road during the night field work in 2014-2016. SVL (Snout-vent length) measured from the tip of the snout to the posterior edge of the cloaca, is the standard measure of body size in caudate amphibians [17]. After measurement process, they were

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taken into alcohol for the histological studies. The stomach tissues of seven species were collected and fixed in Bouin's solution for 48 hours. Stomach tissues were dehydrated in ethanol series and embedded in paraffin. Using a microtome (RM 2145; Leica), histological sections of 5 µm were obtained and stained with Hematoxylin and Eosin (HE) (Humason 1962). Stained sections were photographed with a digital camera (Kameram 5) connected to an Olympus light microscope (Olympus CX31).

3. RESULTS

The average SVL and total length of adult *L. fazilae* sampled from Dalyan and *L. flavimembris* sampled from Marmaris were 64.13 and 120.88 respectively (Table 1). The juvenile *L. flavimembris* found from Marmaris was smaller than the *L. fazilae* Dalyan specimen.

Table 1. Snout-vent length (SVL), Total length and sex categories of *L. flavimembris* and *L. fazilae*.

Species	Sex	SVL (cm)	Total length (cm)
L. fazilae	Adult Female	65.69	117.63
L. fazilae	Adult Female	58.5	112.62
L. fazilae	Adult Male	62.35	118.65
L. fazilae	Adult Male	62.79	113.91
L. fazilae	Juvenile	41.09	77.32
L. flavimembris	Adult Female	71.34	141.57
L. flavimembris	Juvenile	35.21	64.72

The stomach of both *L. fazilae* and *L. flavimembris* divided into two distinct parts; the fundus and the pylorus histologically. As for almost most amphibians and reptiles the stomach was histologically divided into two parts; fundic and pylorus regions, we also examined these two parts in both species examined stomach. [18], [19]. Stomach is lined with columnar epithelial cells and glandular epithelium contained with gastric glands. The presence of the simple columnar epithelium in this study also show similarity with the other vertebrates. [20]. Mucosa layers were observed as tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa in both two species (Fig. 1a,b; Fig. 2a,b,c).

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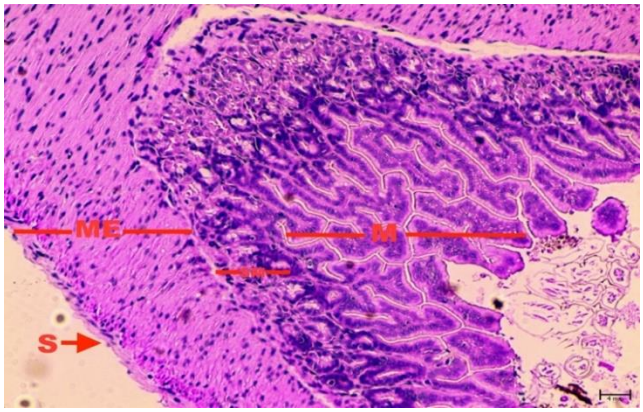

Figure 1a. Histological sections of the mucosa *L flavimembris* (Juvenile). Muscularis externa (ME), submucosa (SM), mucosa (M), serosa (S).

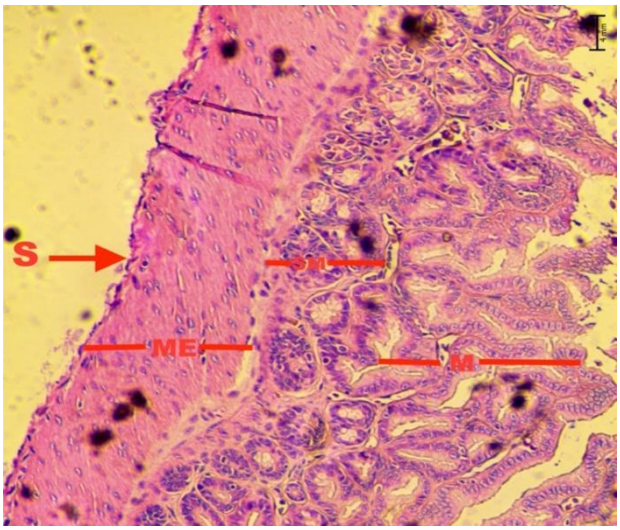

Figure 1b. Histological sections of the mucosa *L. flavimembris* (Female). Muscularis externa (ME), submucosa (SM), mucosa (M), serosa (S).

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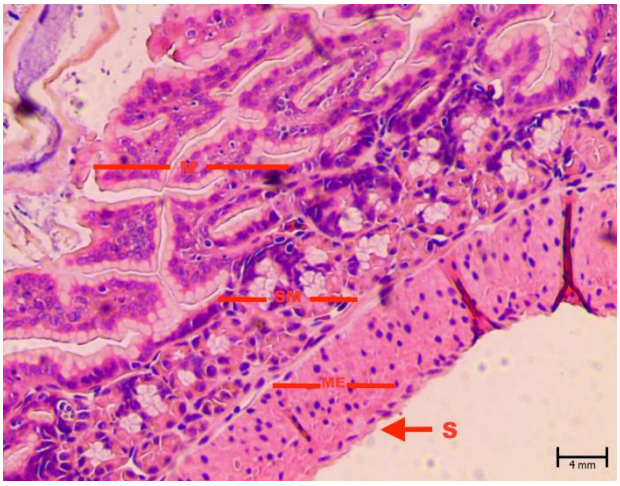

Figure 2a. Histological sections of the mucosa *L. fazilae* (Juvenile). Muscularis externa (ME), submucosa (SM), mucosa (M), serosa (S).

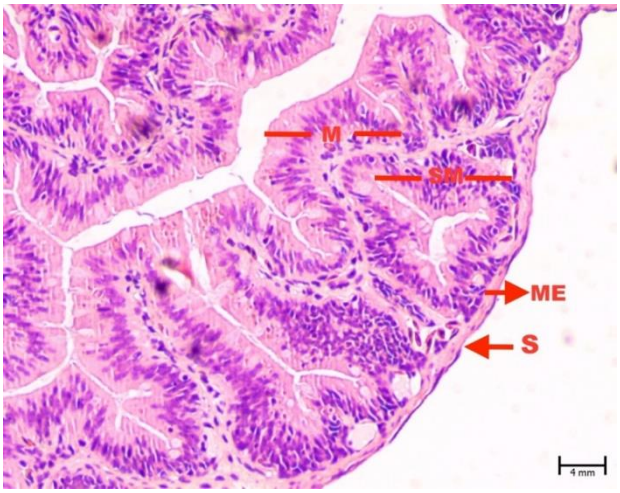

Figure 2b. Histological sections of the mucosa *L. fazilae* (Male). Muscularis externa (ME), submucosa (SM), mucosa (M), serosa (S).

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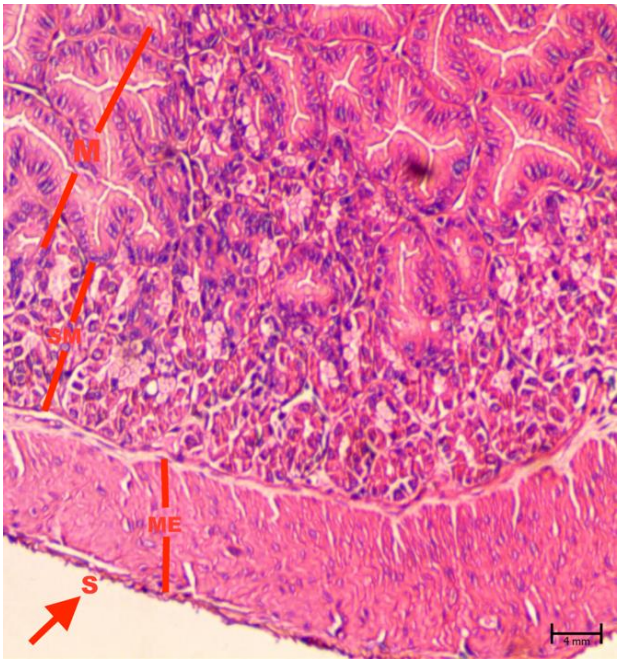

Figure 2c. Histological sections of the mucosa *L. fazilae* (Female). Muscularis externa (ME), submucosa (SM), mucosa (M), serosa (S).

Mucous secreting columnar epithelium covers the mucosa membrane. Fundic glands are mostly simple tubular type, besides pyloric glands are usually branched tubular type (Fig.3). Internal and external muscle layers form the muscular membrane of the stomach.

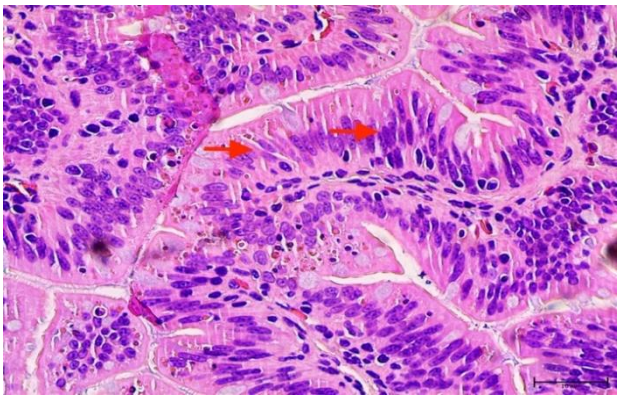

Figure 3. Gastric mucosa of *L. fazilae*. Parietal cells (arrow).

4. DISCUSSION AND CONCLUSION

There was no significant difference between males and females in mucosa layers where gastric glands are situated. The histological structure of stomach of *L. fazilae* between males and females and between adults and juveniles were found to be mostly similar.
It was reported that there is no fundus in amphibians [21] but in further studies based on the morphological and histological features, the region around the entrance of the oesophagus called as cardiac area and the anterior slight dilation is called as fundus, the major part called as corpus and the posterior part called as pylorus.
To date this is the first study describing the histological characteristics of the stomach of *L. fazilae* and *L. flavimembris.* The present description of the stomach revealed similar histological characteristics between *L. fazilae*, *L. flavimembris* and *Mertesnsiella luschani* [8]. And also, the histological characteristics of stomach for *L. fazilae and L. flavimembris* showed a similarity compared to other amphibians [8], [10], [15], [18], [20].

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