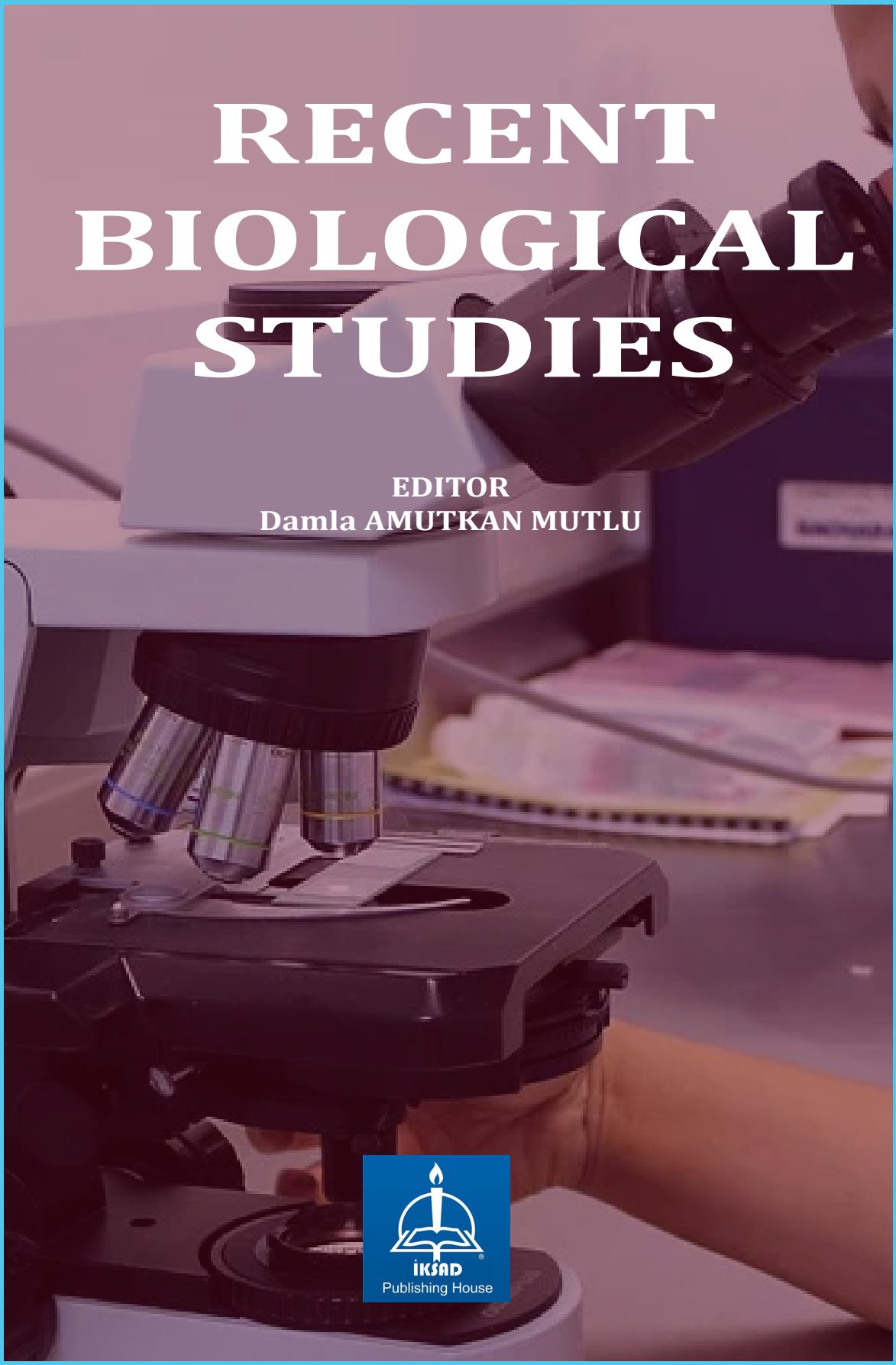
RECENT BIOLOGICAL STUDIES

EDITOR
Damla AMUTKAN MUTLU



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***EPITRIX* FOUDRAS, 1860 (COLEOPTERA: CHRYSOMELIDAE: ALTICINAE) IN TURKEY**

PREFACE

This book entitled “RECENT BIOLOGICAL STUDIES”, prepared by different researchers specializing in various fields of biological science, consists of ten chapters. The topics and applications discussed in this book are tried to be explained together with theoretical information.

I would like to thank the authors who contributed to the emergence of this book with their valuable chapters, and the publishing team who took part in the preparation and publication of this book, and I hope that this book will be useful to you, esteemed researchers, and the students.

With my best regards,

Dr. Damla AMUTKAN MUTLU

CHAPTER 1

AN OVERVIEW OF BIOLOGICAL WARFARE

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INTRODUCTION

Pathogenic microorganisms that cause disease in humans, animals or plants are called biological agents. Biological warfare is a term used for the use of biological weapons. A biological weapon consists of the use of a biological agent to harm any living thing or thing. In the past, the spread of biological agents in biological warfare has been accomplished by aerosol dispersal using spray devices or by combining the agents with a bomb with a ballistic missile warhead (Pal et al., 2017). The gaseous or liquid medium in which the microbiological agent is contained can be formulated to contain additives that increase the agent's survivability and facilitate its diffusion when released into the environment. Fomites can be used to transmit certain biological agents to certain individuals and to spread pathogens. Potential biological warfare agents consist of a long list of all microorganisms that may be pathogenic to humans, plants and animals (Anderson and Bokor, 2012). Although anti-material agents have been investigated for their biological warfare potential, there is no evidence of their use in any national biological warfare program. As an act of war, biological warfare is defined as the use of viruses, bacteria, fungi or their toxins to cause disease, kill or incapacitate humans, animals or plants. Bioterrorism is the covert deliberate release or threat of harmful biological organisms with the aim of causing disease and death or injury among the community, food crops and livestock to intimidate the civilian population or manipulate the government (DaSilva, 1999; Eitzen and Takafuji, 1997; Riedel, 2004).

People had limited knowledge of the cause of illness and what they generally knew was wrong. The biggest problem is that there is no credible evidence that ancient societies either polluted their water supplies or that such pollution could have been effective, or that anyone knew how to deliberately spread the disease. It was difficult to definitively to link between etiologic agent that are spread deliberately and outbreaks was poorly substantiated.

Major epidemics and/or pandemics continue throughout history as disasters that threaten all humanity. An important example plague that caused by *Yersinia pestis* has been responsible for at least 3 widespread pandemics with high mortality rates about 3,000 years ago. In the 6 th century, it was entered from the north-eastern coast of the Black Sea and the Sea of Asov towards central and western Europe, called “Justinian plague”. The second, so-called "Black Death", which took many lives in Europe in the 14th century; and the third one that started in China in the middle of the 19th century and spread all over the world (Thalassinou et al., 2015). Before Pasteur and Robert Koch who demonstrated microorganisms could cause disease in middle of the 19 th century, many people had a limited or no understanding of disease causation, and they ascribed diseases to supernatural causes. Plague and many other diseases were thought to be caused by the negative astrological positions of the stars, polluted air, contaminated wells, poisoning from certain foods, spirit driven out of the earth by earthquakes, witchcraft, and most of all, the wrath of God. It is thought that this is why the Romans used the word veneficium for both the terms poisoning and witchcraft (Carus, 2017).

Thus, it was difficult to differantiate between the symptoms of some diseases caused by poison and diseases caused by microorganisms in past eras. Nonetheless, threat to humans of microbes and their toxins is not new. The origin of the word toxin is Latin, derived from the word "toxos". The Greek word “Toxeuma” meaning “arrow” or ‘archer’ while “toxicos” means “poison” or “toxic substances”. Later, the word toxicos began to be used to mean poison. Toxins are poisonous substances that can also be produced synthetically but are usually produced by various plants and animals. Hellebore and ergot (plant and fungal toxins), dead animals, dead human bodies, contaminated clothing or blankets, and excrement have historically been used as weapons of war (Christopher et al., 1997, Kortepeter et al., 2001). As long ago as 400 B.C., warriors used whatever nature provided. While the Greek general Solon used the laxative hellebore during the siege of Krissa, the Assyrians poisoned the enemy wells with rye ergot (Kortepeter et al., 2001; Mishra and Trikamji, 2014). The Carthaginian general Hannibal (247–182 BC) used plants containing a belladonna-like chemical to incapacitate his enemies. In ancient times, warring parties poisoned wells or used arrows contaminated with toxins obtained from plants during hunting, fishing or fighting. In the Middle Ages, people and animals caught in contagious infectious diseases began to be used as weapons. Trying to weaken your enemies with disease by poisoning their drinking water with dead or rotten animals was one of the earliest warfare techniques. Later, when soldiers started to throw venomous snakes at their enemies, it is one of the forms of biological warfare (Barras and

Greub, 2014; Clark and Pazdernik, 2016; Nepovimova and Kuca, 2019).

Although conventional, chemical, and nuclear weapons have mass destruction, they are expensive and difficult to find. However, short and predictable incubation period, easy access, easy delivery, low production costs, and non-detection by routine security systems make some biological agents to choice for mass destruction and terrorist action (DaSilva, 1999; Riedel, 2004). Biological weapons can be economical as they can be obtained from soil, plants, humans or animals, or they can be highly simple, such as a vial containing a liquid consisting of a biological agent and growth medium, or an envelope with dry powder spores. From the military point of view, *Bacillus anthracis* is so easy to obtain that it could be weaponized for warfare. Anthrax spores can survive for several decades in the environment and it is easy to preserve them (Yuen, 2001). Biological agents are considered attractive weapons for warfare as these are consist of very small particles about of 1-5μ, so they cannot be seen by naked eye. Even when billions of come together, they form a very small package and are easy to transport. Also, very small amount of a pathogenic microorganism can cause morbidity or mortality (DaSilva, 1999; Clark and Pazdernik, 2016). For example, botulinum toxin which is obtained from the spore-forming bacteria *Clostridium botulinum*, is stated to be three million times more potent than sarin gas (Cenciarelli et al., 2013; Pirazzini et al., 2017). While botulinum neurotoxins are the most potent poison known to man, they can be spread as a weapon, as aerosol sprays, in food or water, or by

absorption or injection into the skin, causing widespread casualties (Dhaked et al., 2010; Cenciarelli et al., 2013). The Second World War was the period when the first attempts were made to use this toxin as a weapon in the German and allied armies (Tatu and Feugeas, 2021). Any biological agent which causes little or no immunity, no detection and no prophylaxis or treatment in the community could be considered a potential biological weapon. Biological weapons have features such that the agent may have already been swallowed, inhaled by many at the time of the first casualty, and may cause more casualties despite medical treatments (Kılıç, 2006). In accordance with development of genetic engineering, microbiology, biotechnology and biochemistry, the virulence and potency of biological warfare agents are further improved to make efficient bioweapons (Nepovimova and Kuca, 2019). Attacks with biological weapons using vaccine and antibiotic-resistant bacteria can initiate the emergence and spread of diseases on both an endemic and epidemic scale (Clark and Pazdernik, 2016).

This book chapter provides a summary of wars using biological weapons.

1. BIOLOGICAL WARFARE BEFORE THE GERM THEORY

Bacteria have been used as weapons since ancient times, centuries before the term 'biological warfare' was introduced and the germ theory was developed. Since the causes of many diseases were previously unknown, infectious diseases caused by pathogenic microorganisms are now generally defined as diseases due to chemicals, environmental factors and host immunity (Ryan, 2016). It

was not possible to distinguish between toxin poisoning and infectious diseases until the study of infectious diseases began to be studied as a separate scientific field in the late 19th century. British Army doctor, Thomas Heazel Parke, originally thought that poisonous arrows containing strychnine (from a Strychnos plant species) caused tetanus, as it caused fever and chills. During the wars, various febrile diseases were very common in soldiers. For this reason, many doctors have not been able to distinguish between febrile diseases such as malaria, yellow fever, typhoid fever, and typhus (Carus, 2015). Scientists interested in medical science learned to distinguish these diseases by identifying different pathologies only in the 19th century, with the widespread use of autopsy. In the early 19th century, enough information was available to provide an accurate theory of the transmission of only a few diseases, such as smallpox. For example, in the 19th century, yellow fever caused much controversy. During and after the Spanish-American war, many more soldiers died from yellow fever, malaria, and other diseases than died in the war. In the early 20th century, in Cuba, Walter Reed and colleagues conducted experiments that showed that yellow fever disease can be spread not by poor hygiene but by female *Aedes aegypti* mosquitoes, which carry the virus as a vector. Researchers disproved the germ theory and direct contact as causes of the spread of the disease, by having the soldiers wear contaminated clothing belonging to the patients for a set period of time (Clements and Harbach, 2017).

2. EARLY USE OF BIOLOGICAL AGENTS

Some authors discuss that attempts to use biological warfare date back to prehistoric era. Others suggest that most of the allegent incidents almost certainly never occurred and such claims should be viewed skeptically but it should not denied. It is difficult to distinguish natural epidemics from biological attacks, as there is little information about periods before recorded history. Therefore, it is not easy to determine an exact time when biological weapons are used. Some researchers state that there is evidence of extensive use of poison arrows in prehistoric wars everywhere except Central America and Australia, and that toxins and pathogens may have been used as well. However, the earliest documented evidence of biological warfare appears in Hittite texts between 1500 and 1200 BC, where patients with tularemia were exiled to enemy territory and caused an epidemic (Kortepeter et. al., 2001). It has been reported in some literature that the history of biological warfare began with the Scythian archers. In Classical Greek times, the Scythians, a nomadic tribe, are said to have killed and decomposed young vipers, dipping their arrowheads made of rotting human blood and fluid from vipers to make them more lethal (Carus, 2017). Only a limited number of scientific studies have been conducted on poison arrows to investigate the presence of pathogens. On the other hand, after the emergence of scientific explanations for disease causation, this mixture might have contained the snake's venom as well as *Clostridium perfringens* and *Clostridium tetani*. In the war of Eurymedon in the 3 th century B,C., Hannibal instructed his troops to throw clay pots filled with poisonous snakes

on to the decks of King Eumenes II of Pergamon's ships (Barras and Greub, 2014).

The most plausible instance of biowarfare occurred in 1346. Although not an eyewitness, Tatar force threw bubonic plague-infected bodies into the Blacksea port of Caffa city (now Feodossia, Ukraine/Crimea) as written by Italian chronicler Gabriele de' Mussi. The Mongols, who at that time controlled the area around Caffa, besieged the city in 1345 but were forced to retreat in 1346 as their troops suffered a plague epidemic. Ships from the besieged Crimean city of Caffa returned, bringing the plague to Italy, and for the next four years the Black Death epidemic began, which swept across the Mediterranean Basin, Europe, Asia and China and killed more than 25 million people (Wheelis, 2002). In the 15th century, many different incidents occurred in which diseases and poisons were used during war. For example, in 1495, Spanish armies offered French soldiers wine contaminated with the blood of lepers during the war in Southern Italy. The well-known first case of biological warfare was by the Britain against Native Americans in 1763. That time, British infecting Indians with variola-contaminated clothing to diminish the native Indian population during Pontiac's rebellion. Historians have shown that smallpox and the Seven Years' War were among the most important events of the 18th century. Smallpox was an epidemic that was common during the Middle Ages, posing a serious threat to Native Americans and colonists (Riedel, 2005).

3. USE OF BIOLOGICAL WARFARE AGENTS IN WORLD WAR I (1915-1918)

The establishment of the basic knowledge of microbiology by Louis Pasteur, Robert Koch and later scientists in the late 19th century opened up new possibilities for those interested in the use of biological agents as weapons, which would be selected and designed on a rational basis (Oliveira et al., 2020). Countries began to isolation, production and stocks of specific pathogens and develop weapons that are much more effective. The dangers in this area were recognized in a short time and two international declarations were made (in 1874 Brussels and 1899 The Hague) prohibiting the use of poisonous weapons. During the First World War, the Germans used biological agents, mostly targeting animals, and the French responded on a small scale (Carus, 2017). Germans produced several animal pathogens, particularly *Pseudomonas mallei* (glanders) and *Bacillus anthracis* (anthrax) to infect livestock, horses and mules directly or to contaminate animal feed. World War I armies largely depended on horses and mules to move equipment and supplies, and so the German biowarfare efforts focused primarily on such animals. By this way Germans have attempted to spread disease in United States, Spain, and Argentina. The pathogens that cause anthrax and rumen diseases were usually agents secretly sent from Germany, although small laboratories were also established in Spain and the United States (Frischknecht, 2008). Germany's stocks of biological agents have been identified in Romania, but it is unknown whether they are used. There are also allegations that there were attempts by Germany to spread the

plague in St. Petersburg Russia, and cholera in Italy. However, Germany denied all these allegations. During World War I, the Germans tried attacks the U.S. munitions that manufacturing for the Allies. A subcommittee of the Temporary Mixed Commission of the League of Nations, supporting Germany, declared no concrete evidence that the bacteriological arm of warfare was used in war. However, the document showed evidence that chemical weapons were used in warfare. The horrors of chemical warfare used on the battlefield during World War I became a major political concern at the international level, and efforts were directed at limiting the spread and use of weapons of mass destruction such as biological and chemical weapons. On June 17, 1925, Geneva Protocol that prohibits the use of *Asphyxiating, Poisonous or Other Gases and of Bacteriological Methods of Warfare,* but permission their research and production, was signed. A total of 108 nations, including France, the UK, Italy, Canada, Belgium, Poland, and the Soviet Union had ratified the Geneva Protocol, began research on biological weapons (Carus, 2015). However, USA did not signed the Geneva Protocol until 1975. Several countries began to develop biological weapons soon after its ratification**.** Biological weapons were used during World War II, primarily by the Japanese (Frischknecht, 2008).

4. USE OF BIOLOGICAL WARFARE AGENTS IN WORLD WAR II (1939-1945)

From September 1931 to the end of 1932, after Japan defeated Russia in the Russo-Japanese War in 1905, the Japanese army took full control of Manchuria. While Japanese biological warfare experiments have been conducted at several different locations, the best known is Unit 731 in Manchuria. Their operations were mostly experiments of large-scale biological warfare research program conducted until the end of the World War II (WWII). The program included the development of bombs to be used to spread pathogens, the contamination of reservoirs and wells with deadly pathogens (i.e. *Bacillus anthracis*, *Vibrio cholerae*, *Yersina pestis*, *Neisseria meningitidis*, *Shigella species*, and *Salmonella*), and the sprinkling of the infected fleas and contaminated clothing, food, and drink by plane to the areas they conquered in China. Primarily, Chinese POWs (Prison of War) and civilians were exposed to bombs designed to penetrate the skin of organisms that cause cholera, smallpox, botulism, bubonic plague, anthrax, tularemia, and various venereal diseases, and were left untreated to investigate their various effects. There were no known survivors from these experiments; those who did not die of the infection were killed for autopsy, and all remaining prisoners in the last days of the war were killed to hide the evidence. Many years later, many bones were found near Ishii's laboratory during construction work in Tokyo (Reed, 2006). The Japanese continued to conduct minor biological warfare operations until the end of the war. Although most of the attacks were directed at China, they also tried to cause

epidemics in the Soviet Union and Mongolia. They sent infected individuals across the border in the hope that they would infect others.

After the end of WWII, the Soviets convicted some Japanese biowarfare researchers for war crimes. But the US government gave freedom to researchers in exchange for all knowledge of research on human experimentation, so that murderers and war criminals became citizens and founders of pharmaceutical companies. Although the USA lagged far behind other nations in biological weapons research until the Second World War, with the knowledge of war research, it made very rapid progress after WWII (Christopher et al., 1997).

Contrary to Japanese efforts during the Second World War, it was seen that Germany did not use biological weapons at all, but instead experimental infection studies using prisoners, primarily to study pathogenesis and to develop vaccines and sulfonamide antibiotics. They did some research on the effects of numerous vaccines and medication on inmates infected with hepatitis A virus, *Plasmodium* species and *Rickettsia prowazekii*. The biological weapons programs carried out by the United States and Great Britain during the Second World War unequivocally demonstrated the importance of the airway and, more importantly, the importance of biological aerosols. Between 1942 and 1943, England initiated biological warfare research on Gruinard Island, a small rocky islet off the northwest coast of Scotland. These experiments resulted in heavy contamination of the island with spores of *B. antracis*. Although the island was abandoned after the war, complete decontamination of the island was done from

1986 to 1990 with extensive chemical treatments such as seawater and formaldehyde measures (Riedel, 2004).

In the United States, an aggressive biological warfare program was launched in 1942 under the direction of the War Reserve Service, a non-military agency. The program included a research and development facility in Camp Detrick, Maryland (renamed Fort Detrick in 1956 and known today as the US Army Medical Research Institute of Infectious Diseases [USAMRIID]), test sites in Mississippi and Utah, and a manufacturing facility in Terra Haute, Indiana. Although approximately 5000 bombs filled with *B. anthracis* spores were produced at Camp Detrick, the production facility lacked adequate engineering safety measures (Riedel, 2004).

5. USE OF BIOLOGICAL WARFARE AGENTS AFTER WORLD WAR II

Between the 1950s and 1980s, many allegations of biological warfare attacks were made during the Cold War, in the context of the Korean and Vietnam wars, the invasion of Afghanistan, and the dictatorship of Cambodia. Despite the lack of evidence of the alleged products, the credibility of the United States has been shaken by its disapproval of the 1925 Geneva Protocol, public acceptance of its own aggressive biological warfare program, and suspicions of collaboration with former Unit 731 scientists. Therefore, during the Korean War (1950–1953), communist bloc (Soviet Union, China, and North Korea) claimed that the US employed Biological War in the Korean War. On the other hand, the United States claimed that the Soviet Union and its

allies used biological agents in the late 1970s due to the falling yellow rains in Southeast Asia. (Leitenberg et al., 2012). In later years, the United States denied using such weapons, although it acknowledged that it had the capability to produce such weapons. According to some studies, a defense program was initiated in 1953 to develop countermeasures such as vaccines, antisera, and therapeutic agents to protect American troops from biological attacks. The US military has developed a biological arsenal of numerous biological pathogens, fungi, toxins, and plant pathogens that can then be directed against crops to cause famine (Oludairo et al., 2015).

In the 1950s, various studies were conducted in the USA to test the vulnerability of society to biological factors. These studies focused on improving their ability to resist the release of various microbes and any biological weapon attack on experimental animals, volunteers, and civilians. The US military began open-air testing with two harmless bacteria, releasing bacterial aerosols containing *Aspergillus fumigatus*, *Bacillus globigii*, and *Serratia marcensces* from navy ships, bus stations, and airports off the coast of Virginia and off San Francisco. Contaminating the New York subway system with *Bacillus globigii* (supposedly anthrax-like) to study the spread of a pathogen in a major city was the most notorious test in 1966. In the post-WWII period, the eastern European newspaper stated that Great Britain used biological weapons in Oman in 1957. In 1969, Egypt accused foreign powers, which they called imperialist aggressors, of using biological weapons in the Middle East and causing a cholera epidemic in Iraq (Michalak, 2007).

Concerned about the epidemiological risks and ineffectiveness of the 1925 Geneva Protocol, more than 100 countries, notably the US, UK and Soviet governments, have signed a new Biological and Toxin Weapons Convention (BWC), the first multilateral agreement to categorically ban a class of weapons. This protocol prohibits the development, stockpiling, manufacture or delivery of bacteriological (Biological) and toxic weapons that have no justification for protective or peaceful use. If a state had any agents, toxins, or distribution systems for them, they had nine months from the entry into force of the protocol in March 1975 to destroy existing stockpiles or divert them for peaceful use (Wheelis and Dando, 2002). However, the BWC's presence has not prevented various states from developing biological warfare research programmes, on the contrary, the threat of attack with biological weapons has greatly increased since the BWC was ratified in 1975. Although the Soviet Union signed the protocol, it took an important decision to develop its biological warfare program (Roffey et al., 2002).

In 1973 and 1974, the Soviet Union founded and financed the organization known as Biopreparat, which was designed to carry out the R&D and production of biological weapons, hiding behind legal and civil biotechnology research (Davis, 2002). The Soviet Union has prepared and maintained a large ton of biological warfare agents stockpiles of about 50 agents, including plague, anthrax, glanders, tularemia, brucellosis, Marburg, smallpox, and VEE viruses. From its inception until after the collapse of the Soviet Union in December 1991, the biopreparation program was formally operated (Ministry of

Health and Agriculture, KGB, and Academy of Sciences) (Roffey et al., 2002). Beginning in 1989, the United States and Britain made a joint effort to end the Soviet/Russian BW program (Leitenberg, 2001; Roffey et al., 2002).

CONCLUSION

When we examine the history of attempts to use diseases in biological warfare, it turns out that it is quite difficult to distinguish between a naturally occurring epidemic and a biological warfare attack. However, advances in basic and applied microbiology have led to many advances, including the identification of new pathogens with high virulence factors suitable for aerosol delivery and the development of large-scale fermenters to produce large quantities of pathogenic microorganisms and toxins. In today's scenario, a biological warfare agent is not too deadly to be effective, but rather incompetent and confusing enough to produce the effects it is intended to produce. If the first signs of exposure to biological agents are indistinguishable, the lethality of biological weapons may increase and cause mass destruction without their presence being suspected. It is thought that the increase and production of biological warfare agents will stop when it is possible for the BWC contract to establish control over states with heavy sanctions.

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CHAPTER 2

SYNTHETIC PYRETHROID TOXICITY IN AQUATIC ORGANISMS

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INTRODUCTION

It was not one of the environmental problems until the middle of the 20th century, when chemicals were quite harmful to the environment. Along with Silent Spring by Rachel Carson, it was revealed that organochlorine insecticides have effects on non-target species in the environment, and it aroused great repercussions all over the world. In the process until today, studies on the effects of these chemical substances on organisms continue increasingly. The production, use and distribution of these substances continues or is prohibited by international organizations and conventions, depending on their effects on organisms and environment (Palmquist et al. 2012; Arslan et al. 2021; Arslan and Özeren 2021). After understanding the undesirable and adverse effects of organochlorine insecticides on human and wildlife health, one of the alternative substances, synthetic pyrethroids, was developed using pyrethrum (Palmquist et al. 2012).

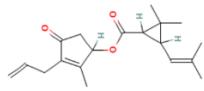
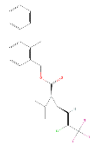
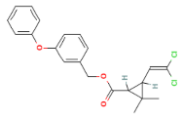
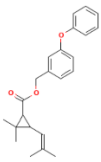
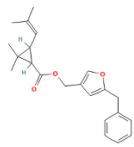
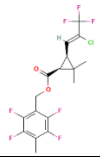
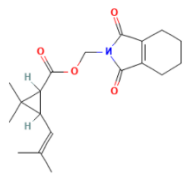
As a native to Dalmatian region, pyrethrum is represented three species: *Chrysanthemum* (*Tanacetum*) *cinerariaefolium*, *C.* (*Tanacetum*) *roseum*, and *C. marshalli* Ascherson. The first originated from Dalmatia has white flowers, the second originated from Persia and Caucasus has red flowers, and the third originated from Persia. Among the three species, *C. cinerariaefolium* has more insecticidal compounds (Hosono, 1950; Katsuda, 2012). Even though the pyrethrum was discovered in Dalmatians, its main insecticidal activities were proved in the 19th century in America and Japan using the dried flowers (Katsuda 2012).

In the mid-20th century, after examining the pyrethrum contents of the species according to their acid and alcohol moiety via analytical methods, pyrethrin I and pyrethrin II, cinerin I and II, and jasmolin I and II were found (Katsuda et al. 1955, 1956; Katsuda 2012). Having chrysanthemic and pyrethroic acids in their structures, pyrethrins are lipophilic molecules but tend to break down in the nature by light (Kaviraj and Gupta 2014).

Synthetic derivates of pyrethrins have been known as synthetic pyrethroids. Apart from pyrethrins is used in the botanical insecticides, pyrethrins and synthetic pyrethroids are used for various types of pests in agriculture and other areas including mosquito against programs of governments, household and industrial fields. They are applied during pre-harvest and post-harvest times of most crops such as corn, soybean, and tree fruits and most vegetables in the agriculture (EPA 2019).

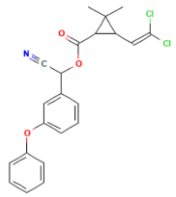
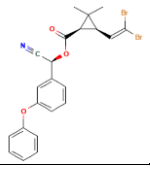
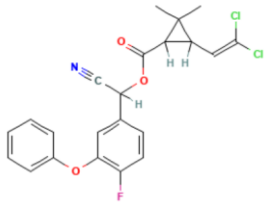
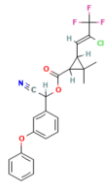
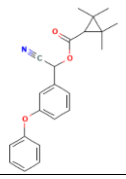
According to their chemical structure, pyrethrins and synthetic pyrethroids are divided into two groups: type 1 and type 2. Type 1 doesn't include alpha-cyano group in its molecule and therefore it causes T-syndrome (tremors). On the other hand, due to containing alpha-cyano group in its molecule, type 2 causes CS-syndrome (choreoathetosis and salivation) (Tordoir et al. 1994; WHO, 2005). However, the effects of the synthetic pyrethroids can be changed between the species (WHO 2005). The examples of Type 1 and Type 2 synthetic pyrethroids are shown in Table 1 and Table 2.

Table 1: The Molecular Formula and Structure of Some Type 1 Synthetic Pyrethroids (modified from Biswas et al. 2019)

Synthetic Pyrethroids	Molecular Formula*	Molecular Structure*
Allethrin	$C_{19}H_{26}O_3$	
Bifenthrin	$C_{23}H_{22}ClF_3O_2$	
Permethrin	$C_{21}Cl_2H_{20}O_3$ or $C_{21}H_{20}Cl_2O_3$	
Phenothrin	$C_{23}H_{26}O_3$	
Resmethrin	$C_{22}H_{26}O_3$	
Tefluthrin	$C_{17}H_{14}ClF_7O_2$	
Tetramethrin	$C_{19}H_{25}NO_4$	

*The molecular formula and structure of chemical are taken from PubChem (https://pubchem.ncbi.nlm.nih.gov/)

Table 2: The Molecular Formula and Structure of Some Type 2 Synthetic Pyrethroids (modified from Biswas et al. 2019)

Synthetic Pyrethroids	Molecular Formula*	Molecular Structure*
Cypermethrin	$C_{22}H_{19}Cl_2NO_3$	
Deltamethrin	$C_{22}H_{19}Br_2NO_3$	
Cyfluthrin	$C_{22}H_{18}Cl_2FNO_3$	
Cyhalothrin	$C_{23}H_{19}ClF_3NO_3$	
Fenpropathrin	$C_{22}H_{23}NO_3$	

*The molecular formula and structure of chemical are taken from PubChem (https://pubchem.ncbi.nlm.nih.gov/)

Synthetic pyrethroids mainly affect the voltage-sodium channels and peripheral nervous system of the organisms. As mentioned above, Type 1 substances make hyperexcitation, prostration, and body tremors (Günal et al. 2021) and Type 2 alter neurosecretory neurons of the organisms (Soderlund and Bloomquist 1989). Even though they are shown less toxicity to mammals, they have adverse effects on aquatic organisms (Lu et al. 2019). Therefore, this chapter aimed to summarize the effects of synthetic pyrethroids on aquatic organisms.

1. AQUATIC ORGANISMS

1.1. Planktons

As microscopic organisms, phytoplankton convert the solar energy into chemical energy and zooplankton pass this chemical energy, also known as food energy, to higher trophic levels in the food chain. The phytoplankton are the base and zooplankton are the second level in the aquatic food chain and/or web. Thus, these organisms are valuable biological indicators of aquatic environment to investigate the possible water quality changes in the environment (Jakhar 2013).

Phytoplanktons play important roles in carbon production, nitrogen, phosphorus, iron, silicon cycles. They use these substances for their metabolic pathways. Then, these substances are released into environment like dissolving in organic matter or sink deep water (Bonachela et al. 2016). Besides, they are primary producers in the aquatic environment, they also the food sources of zooplankton,

aquatic invertebrates and fish (Jakhar 2013). Phytoplankton are affected by residues of pesticides in different aquatic ecosystems (Rumschlag et al. 2021; Yang et al. 2021; Wijewardene et al. 2021). Cypermethrin and lambda-cyhalothrin decreased the photosynthetic percentage of phytoplankton (Ikram and Shoaib 2018). The cypermethrin exposure of three phytoplankton species (Raphidophyceae *Chanottonella marina*, Dinophyceae *Scrippsiella trochoidea*, and diatom *Skeletonema costatum*) resulted in rising the chlorophyll-a contents. While superoxide dismustase (SOD) activities decreased in the early times of exposure to cypermethrin, it was observed that they reached the same level as the control group over time. On the contrary, malondialdehyde (MDA) levels increased at the beginning of the exposure time, but decreased over time to the control group levels (Wang et al. 2011). Tunca et al. (2021) showed that after exposure to cypermethrin and deltamethrin, biomass production and chlorophyll-a content of phytoplankton *Arthrospira platensis*-M2 cells were decreased. Even though cypermethrin caused the increasing SOD and ascorbate peroxidase (APX) activities of the species, glutathione reductase (GR) activity decreased and MDA and hydrogen peroxidase (H_2O_2) levels didn't change. Like cypermethrin, deltamethrin also increased the SOD activity. However, the activities of APX and GR did not change and MDA and H_2O_2 level increased exposure to deltamethrin.

Due to being herbivorous, carnivorous, and omnivorous, zooplankton are placed in one or more trophic levels in the aquatic ecosystems

(Sterner 2009). The fact that it is located between fish and phytoplankton in the aquatic food chain provides the use of zooplankton as an indicator creature. For this reason, in aquatic ecosystem monitoring studies, monitoring of zooplankton is necessary as well as monitoring of phytoplankton and fish (Jeppesen et al. 2011). It was observed that the swimming activity and in heart rate of *Daphnia magna* decreased in lambda cyhalothrin exposure (Bownik et al. 2019). Deltamethrin has been shown to delay the first-generation time and decrease the reproduction rate of *D. magna* (Felten et al. 2020).

1.2. Aquatic Invertebrates

Aquatic invertebrates are organisms that live in many areas, from small ponds to large lakes, from slow-flowing rivers to fast-flowing rivers. The habitats can be vegetative parts at the bottom of the aquatic systems. They live sometimes buried in sand or sediment, sometimes among gravel or debris consisting of wood chips, and sometimes by clinging to large pieces such as rocks in the aquatic environment. They prefer a wide variety of foods such as tree leaves, phytoplankton, small fish and tadpoles. It is one of the creatures that are biologically monitored in aquatic ecosystems, as there are organisms that are very sensitive to environmental pollution in the water system they are in (Bouchard 2004).

In studies investigating the effects of synthetic pyrethroids on aquatic invertebrates, acute toxicity of some synthetic pyrethroids and changes in the biochemical activities of some synthetic pyrethroids in

the movement or tissues of the organisms were observed. The lethal concentration of synthetic pyrethroids and other effects of various aquatic invertebrates are summarized in Table 3 and Table 4, respectively.

Table 3: The Lethal Concentration (LC_{50}) of Some Synthetic Pyrethroids Exposed to Some Invertebrate Species

The groups	The species	Synthetic Pyrethroids	Time	LC_{50}	References
Amphipod	*Gammarus pulex*	Lambda-cyhalothrin	24h	5.69 µg/L	Heckman et al. 2005
		Cypermethrin	24h	0.128 µg/L	Nurum et al. 2011
			48h	0.050 µg/L	
			72h	0.003 µg/L	
			96h	0.029 µg/L	
Mussels	*Unio elongatulus eucirrus*	Cypermethrin	48h	96.50 µg/L	Köprücü et al. 2010
			72h	77.96 µg/L	
			96h	59.20 µg/L	
Crayfish	*Procambarus virginalis*	Cypermethrin	96h	0.0225 µg/L	Lidova et al. 2019
	Procambarus clarkii			0.0425 µg/L	
	Pacifastacus leniusculus			0.045 µg/L	
	Orconectes limosus			0.0475 µg/L	
	Cherax destructor			0.075 µg/L	
Crayfish	*Procambarus virginalis*	Deltamethrin	96h	0.038 µg/L	Lidova et al. 2019
	Procambarus clarkii			0.008 µg/L	
	Pacifastacus leniusculus			0.105 µg/L	
	Orconectes limosus			0.135 µg/L	
	Cherax destructor			0.0015 µg/L	
	Procambarus clarkii	Cypermethrin	24h	0.14 µg/L	Morolli et al. 2006
		Cyfluthrin		0.17 µg/L	
		Deltamethrin		0.22 µg/L	
	Astacus leptodactylus	Permethrin	96h	0.0903 µg/L	Günal et al. 2021

Table 4: The Effects of Synthetic Pyrethroids on Some Aquatic Invertebrates

The groups	The species	Synthetic Pyrethroids	Dose and Time	Effects	References
Nematadote	*Neoplectane carpocapsae*	Fenvalerate	1.0 mg/L 14d	No detected toxicity	Hara and Kaya 1982
Amphipod	*Gammarus pulex*	Cypermethrin	0.003, 0.01, 0.3, 0.1, 1, 3 and 10 µg/L for 30 min	Dose-related slowing of movement	Nurum et al. 2011
Mussels	*Unio elongatulus eucirrus*	Cypermethrin	5, 10,20,40,80,160 µg/L and 1, 24, 48, 72, and 96h	Increased MDA; Decreased glutathione (GSH) and catalase (CAT) in the gill and digestive gland tissues	Köprücü et al. 2010
	Mytilus galloprovincialis	Cypermethrin	50, 100, 200, 400, and 800 µg/L; between beginning of the exposure and 4 h	Reduction of the valve opening activity	Ait Ayad et al. 2011
Crayfish	*Astacus leptodactylus*	Deltamethrin	0.05 µg/L 48h 0.05 µg/L 7d	Decreased total hemocyte counts (THCs) and hemolymph total antioxidant status (TAS); Increased SOD, CAT and glutathione peroxidase (GPx) in gill; Increased GPx in hepatopancreas and SOD in muscle tissues	Yücel Işıldar et al. 2020

Table 4: Continued.

The groups	The species	Synthetic Pyrethroids	Dose and Time	Effects	References
Crayfish	*Astacus leptodactylus*	Permethrin	0,09 µg/L 48h and 96h	Increased THCs; hemolymph potassium and chloride increased; increased MDA values in 96h exposure; epithelial hyperplasia and degenerations of gill lamella; hemocytic infiltration in the vessels; degenerations of tubules in the hepatopancreas	Günal et al. 2021

1.3. Aquatic Vertebrates

The last group of aquatic organisms is aquatic vertebrates, which includes fish, amphibians, reptiles, and mammals. These organisms are located in high trophic layers in the aquatic ecosystem (Kupfer et al. 2006). The acute toxic effect values and effects of synthetic pyrethroids on fish are shown in the Table 5 and Table 6, respectively. The effect of synthetic pyrethroids on some amphibian and mammalian species is also given in the Table 5.

Table 5: The LC_{50} of Some Synthetic Pyrethroids Exposed to Some Fish Species

The species	Synthetic Pyrethroids	Time	LC_{50}	References
Salmo salar	Permethrin	96h	8.8 µg/L	Zitko et al. 1979
			12 µg/L	McLeese et al. 1980
Salmo gairdneri			6.43 µg/L	Kumaraguru and Besmish 1981
			7 µg/L	Holcombe et al. 1982
Pimephales promelas			5.7 µg/L	Mayer and Ellersieck 1986
Labeo rohita	Cypermethrin	96h	0.139 ppm	Kumar Das and Mukherjee 2003
Oreochromis niloticus	Cyfluthrin	48h	25.82 µg/L	Karasu Benli,. 2005
		72h	21.07 µg/L	
	Deltamethrin	48h	4.85 µg/L	Yıldırım et al. 2006
Sciaenops ocellatus	Cypermethrin	96h	8 µg/L	Parent et al. 2011
Fundulus heteroclitus			23 µg/L	
Oreochromis niloticus	Cypermethrin (technical)		10.71 µg/L	Majumder and Kaviraj 2017
	Cypermethrin (commercial)		5.25 µg/L	

Table 6: The Effects of Synthetic Pyrethroids on Some Aquatic Vertebrates

The groups	The species	Synthetic Pyrethroids	Dose and Time	Effects	References
Fish	*Labeo rohita*	Cypermethrin	0.014 and 0.003 ppm for 15, 30, and 45d	No change muscle protein levels, serum protein levels brain alkaline phosphatase activity, AChE decreased, in the kidney tissue, lactate dehydrogenase and succinate dehydrogenase activities were inhibited	Kumar Das and Mukherjee 2003
	Oreochromis niloticus	Deltamethrin	5.0 µg/L 96h	Focal lamellar deformations in gills, hydropic degenerations in liver	Yıldırım et al. 2006
	Cyrinus carpio	Cyfluthrin	10.0 µg/L 48h and 7d	Plasma chloride levels decreased, sodium, cortisol, and phosphorous levels increased, telangiectasis in the gill for 48h and 7d; hydropic degenerations in the liver and hyperemia in the vessels of brain for 48h	Sepici-Dinçel, et al. 2009

Table 6: Continued.

The groups	The species	Synthetic Pyrethroids	Dose and Time	Effects	References
Fish	*Sciaenops ocellatus*	Permethrin	96h	Increased MDA values in exposed groups in liver, no change in acetylcholine esterase in brain	Parent et al. 2011
	Fundulus heteroclitus				
	Oreochromis niloticus	Cypermethrin	1.25 and 2.5 µg/L for 24, 48, 72 and 96h	Hepatic glycogen values and, alkaline phosphatase, AChE, and CAT decreased; plasma glucose level, cid phosphatase, aspartate aminotransferase and alanine aminotransferase increased	Majumder and Kaviraj 2017
Amphibian	*Bufo americanus*	Permethrin	From 0.001 ppm to 2 ppm for 22h or 96h	Slow growth rates of tadpole, delayed metamorphosis, paralysis of tadpole and salamander larvae	Berrill, et al. 1993
	Rana sylvatica				
	Rana pipens				
	Rana clamitans				
	Ambystoma maculatum				
	Rana temporaria		0.1 and 1 µg/L 72h	Increased metamorphosis size	Johansson, et al. 2006
Mammals	*Dolphins*	Tetramethrin, bifenthrin, Lambda-cyhalothrin, deltamethrin, tralomethrin, fluvinalate	-	The mentioned synthetic pyrethroids were detected in the liver of calves, juveniles and adult males as well as breast milk and placenta of females.	Alonso et al. 2012

CONCLUSION

In this study, in which the effects of synthetic pyrethroids on aquatic organisms were investigated by examining the literature, it was found that they are extremely toxic effects on various aquatic organisms. It appears that even at very low doses (µg/L) it can have an impact on populations and thus may affect biodiversity in aquatic life. In addition, it was concluded that it affects cell metabolism, tissue structure, and individual behaviour on organisms in short-term exposure situations. Therefore, the production, distribution and usage of synthetic pyrethroids may be under control by international and national policies.

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CHAPTER 3

EFFECTS OF METAL STRESS ON BIOCHEMICAL RESPONSES IN CROP PLANTS: COMPARISON STUDY IN HYDROPONIC ENVIRONMENT

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INTRODUCTION

Today, it is known that heavy metals (HMs) are a significant threat to public health over the whole world. HMs, also accepted as trace elements (Kabata-Pendia, 2001), have high specific density above 5 g/cm^3 (Duffus, 2002; Li et al 2017). While some of these HMs are necessary nutrients (like zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), magnesium (Mg), etc.) and required for various physiological and biochemical functions for living organisms (WHO/FAO/IAEA, 1996), other metals (including nickel (Ni), mercury (Hg), lead (Pb), cadmium (Cd), arsenic (As), etc.), are known to be as non-essential metals (Chang et al., 1996).

Sources of Heavy Metals

HMs, as one of the major contaminants for the environment, are present in the atmosphere, lithosphere, hydrosphere and biosphere (Krishna and Mohan, 2016). However, they affect ecosystems through various human activities. Therefore, it is not wrong to summarize their sources under two main groups in relation to natural and anthropogenic activities. Although, according to surveyed literature, their sources can be further examined under different categories; (i) Natural sources, such as rocks' weathering, bacterial activity or volcanic bursts (Nagajyoti et al., 2010; Monge et al., 2015), (ii) Agricultural sources, like fertilizers, pesticides and manures (Yanqun et al., 2005; Srivastava et al., 2015), (iii) Industrial sources, for example metal mining and milling processes, coal burning in power plants and paper processing plants (Arruti et al., 2010; Ahmed and

Ahmaruzzaman, 2016), (iv) Domestic sources, for instance treated or untreated municipal waste waters, inorganic and organic wastes (Singh and Kumar, 2017), (v) Atmospheric sources, like leaded gasoline, solid particles or other emissions of factory chimneys (Zhang et al., 2010; Wuana and Okieimen, 2011) and (vi) Other sources, such dust from open dumps, incineration, transportation or traffic emissions (Nagajyoti et al., 2010; Aryal et al., 2017; Gope et al., 2017).

Being Not Degredable

Since, they do not undergo biodegradation, HMs must either be converted to non-toxic compounds or, if this is not possible, physically removed from their environment (Gaur and Adholeya, 2004; Pehlivan et al., 2009). Even some HMs are naturally found in the earth's crust, due to the specific applications mentioned above, the slowly developing metal cycle is accelerating and the wide distribution of HMs in the environment poses a risk to the ecosystem and its inhabitants. For example, HMs are released into the soil, then easily transported to distant areas through air or water during mining activities. Again, during combustion, some of these metals are released into the air, soil and waters. In addition, HMs can be transported to different locations over waters and soils by erosion or acid rain (Sharma et al., 2017).

From Farm - To - Table

Over population and changes in agricultural practices along with developing technology, cause an increase of HM contamination in soil

year by year. For this reason, soils constitute the main areas for HMs released into the environment by the anthropogenic activities mentioned above. Nowadays, with the increasing population growth, the safety of the nutrients from farm - to - table is one of the most important issues to be controlled in terms of public health. However, since most HMs are not degraded by chemical and microbial processes (Kirpichtchikova et al., 2006), their high concentrations remain in the soil for a long time and reach animals and humans via the food chain (Nagajyoti et al., 2010). On the other hand, good management of soil quality is also important to prevent the reduction of land availability for agricultural production, which leads to food insecurity.

Bioaccumulation of Cadmium and Lead

Since 1973, cadmium (Cd) and lead (Pb) are known as the most hazardous HMs according to Global Monitoring Program adopted by the UN. These two HM are also present in USEPA (1997) data, (in descending order of Pb > Cr > As > Zn > Cd > Cu > Hg) as the most common HMs in soils. In fact, Cd and Pb can be distinguished from other pollutants because they do not degrade in nature, but even in minute quantities they can cause various diseases as a result of their tendency to accumulate in living organisms (Pehlivan et al., 2009). Another risk is reduced crop production because of bioaccumulation in the food web (Wuana and Okieimen, 2011).

Sources of Cadmium and Lead Exposure in Environment

The common source for Cd and Pb is the geologic origin (Nagajyoti et al., 2010). Very high concentrations of both of them are found in plants and soils adjacent to smelting works (USEPA, 1997). Fungicides and inorganic or phosphate fertilizers contributed to the varying concentrations of Cd and Pb during agricultural applications (Kelepertzis, 2014). Also, they are added into the soil by sewage sludges (Verkleij, 1993). On the other hand, another common source for these metals are the anti-wear protectants which used in automobiles.

In addition to abovementioned sources, Cd is mostly used in industrial activities, such as production of batteries, alloys, electronic compounds, pigments, paints and plastics, coatings to vessels and other vehicles, stabilizers for polyvinylchloride. Cd is produced as an inevitable byproducts of Zn. It is also present in detergents and refined petroleum products as an impurity. In addition, nuclear power plants, coal-fired power plants and high voltage lines are also effective in the emission of Cd (Zhu et al., 2016; Ahmed and Ahmaruzzaman, 2016) and Cd is associated with vehicle sources in the form of dust from tire wear (Gope et al., 2017; USEPA, 1997).

In the case of Pb, volcanoes are the high-level emitters of this element (Nagajyoti et al., 2010). Also, burning lead containing gasoline releases Pb into the atmosphere and this is the major source of soil contamination (Wuana and Okieimen, 2011). On the other hand, while the industrial sources of Pb include Pb storage batteries, bearings,

cable covers, pipe solders, pigments, ammunitions, caulking and devices to shield X-rays (CDC, 1991), some others can be found in households as toys and an old lead plumbing pipes, some traditional medicines and cosmetics (ATSDR, 1992).

Effect of Heavy Metals on Plants

Due to deposition of HMs from different sources, like mine ores, agricultural and industrial activities (Toth et al., 2016; Sharma et al., 2017), soil become contaminated irreversibly. HMs in soil, with a long residence times, not only affect the soil biology, quality and microbiota, they also adverse effects on plants grown at metal polluted sites. This is a complete 'chicken and egg' situation. Because, while HM toxicity directly effects to flora, the biological reorganization of HMs depends on plant's metabolism (Srivastava et al., 2017).

There are different reasons why the soil can become a heavy metal sink. These can be explained as the acceleration of HM production cycles with increased human activities, their transfer from the locations where they are produced to new areas by wind and other carriers, the transferred materials having a higher HM concentration compared to the new environment, and the environment in the new location may increase the bioavailability of HM based on its chemical form (D'Amore et al., 2005). On the other hand, while some properties of the soil (pH, organic matter, etc.) are important for the bioavailability of metals, toxicity of HMs depends on criteria that originate from the plants (species, size, etc.) or metal itself (such as dose, kind and exposure time) (Tchounwou et al., 2012; Gill, 2014).

Since soils accumulate HM (in one type or in a mixture), they can adversely change the biochemical and physiological processes in plants, such as enzyme activities, membrane permeability and water balance (Sharma and Dubey, 2005). When interacting with the structural components of plants, HMs cause to changes in a reduction in plant growth, leaf chlorosis, decreased rate of seed germination, impeded photosynthetic functions, etc. (Dalcorso et al., 2010). These general effects in plant tissues and organs are, due to existence of HMs, and all related to changes of either structural or biochemical and physiological level (Gamalero et al., 2009). Therefore, researches at structural and ultrastructural level would benefit to uncover the toxicity of HMs and possible plant responses, as much as biochemical and physiological studies.

Together with some others, hydroxyl radicals ($OH^{\bullet}$), singlet oxygen ($^1O_2$), superoxide radicals ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) are known as Reactive Oxygen Species (ROS) and produced when exposure of plants to toxic HMs. These highly unstable intermediates are inseperable part of aerobic life (Wang et al., 2010), as they are chemically derived from molecular oxygen (Tamas et al., 2017). Even ROS are known to be as important signalling molecules and generally in equilibrium with antioxidants in cells (Mittler, 2017), when there is an imbalance between ROS formation and antioxidant molecules, they induces undesired danger called oxidative stress (Venkatachalam et al., 2017).

These reactive molecules can oxidize proteins, carbohydrates, lipids and nucleic acids leading to abnormal processes in protein synthesis, water intake, stomatal movements, etc. (Seregin and Ivanov, 2001). Moreover, by disrupting the redox status in the cells, ROS can damage to the membrane (Anjum et al., 2017) and cause to lipid peroxidation (Venkatachalam et al., 2017). All above situations may cause death of the plant organism at some point (Gonçalves et al., 2007).

To minimize or detoxify the effects of oxidative stress induced by ROS, plants have derived an array of antioxidative defense mechanisms. This mechanism comprise of some antioxidant enzymes (such as glutathione *S*-transferases [GST], glutathione peroxidase [GPX], glutathione reductase [GR], superoxide dismutase [SOD], and others) and non-enzymatic antioxidants (like glutathione [GSH], α-tocopherol [vitamin E], carotenoids, L-ascorbic acid [L-AA] and others) (Gill and Tuteja, 2010) which work individually or in harmony to protect plants cells from toxicity. However, the matureness and species of plant itself, the degree of exposure to heavy metals and their persistence are the main factors that determine the effectiveness of antioxidant defense mechanisms.

GSH

Non-enzymatic antioxidants work with antioxidant enzymes to control oxidative stress by quenching ROS (Noctor and Foyer 1998). GSH (γ-L-glutamyl- L-cysteinyl-glycine), tripeptide, is a low molecular weight thiol compound which has been determined in plant tissues and localized in different cell compartments (Ahmad et al., 2009).

Due to its unique amino acid cysteine having the -SH group, GSH reacts with different electrophiles. Its high water solubility and stability make GSH a suitable substance to defend organisms and to combat various stress conditions, including metal-induced ROS (Viehweger, 2014). In plant cells, being the main source of non-protein reduced sulphur, GSH associated with many different physiological functions, such as cell growth, cell differentiation, cell death, signal transduction, etc. (Rausch and Wachter 2005; Gomez et al., 2004; Szalai et al., 2009).

However, GSHs defensive function is triple; (i) GSH is an important antioxidant (Foyer et al., 2001), (ii) GSH is a co-substrate in detoxification reactions catalyzed by GSTs (Edwards et al., 2000) and (iii) GSH is involved in the synthesis of phytochelatin (PC) and regeneration of L-AA and α-tocopherol (Yadav, 2010). Because of the above mentioned roles, the level of GSH in cells is accepted as an important biomarker of oxidative stress (Noctor et al., 2015).

GSTs

In the cell, ROS production as well as removal should be effectively controlled so that the damage it causes can be kept to a minimum. Therefore, the efficient detoxification of ROS needs the single or synhroniced action of several different antioxidant enzymes in plant cells (Mittler et al., 2004). Beside others, glutathione *S*-transferases (GST) are known to be as one of the scavenging and detoxifying enzymes in the cell. GSTs, which were first shown to exist in plants in the 70's (Frear and Swanson, 1970), are found in all living species.

This multifunctional enzymes participate in antioxidative defence and protects cells from the harmful effects of ROS (Hajime et al., 2005). Also, they catalyse the conjugation of GSH (thiol group) to different lipophilic molecules and forms less active end products where the resulting less toxic and more water soluble *S*-conjugates are transferred into the vacuole in plants (Rea, 1999). Their high expressions under toxic conditions reveals that GSTs are an efficient biomarker (Dasari et al., 2018).

Proteins

Irrespective of the source of stress whether abiotic or biotic, the excess amounts of ROS in cells interact with and cause detrimental results in carbohydrates, lipids, DNA and proteins (Noctor and Foyer 1998). According to the accepted view, proteins are the main targets for HM actions. They may interfere with native folded proteins either by binding to free thiols (or other functional groups) (Da Silva and Williams, 1993) or by displacing the essential metal ions in metalloproteins (Lemire et al., 2013). On the other hand, some recent studies have shown that HMs can inhibit the in vitro refolding of chemically denatured proteins (Jacobson et al., 2012).

Proteins need to be fold into a well defined 3D structure, which is the native conformation, to show their biological functions. The 3D structure is determined by the amino acid sequence of the protein (Anfinsen, 1973). However, as mentioned above, HMs may inhibit protein folding and cause misfolded proteins. These proteins that are cytotoxic can aggregate in the cell or interact with some cellular

components. In fact, chelators cannot recover misfolded proteins in the presence of HMs (Engwa et al., 2019).

On the other hand, when ROSs reacts with polyunsaturated fatty acids (PUFAs), peroxidation of membrane lipids occurs. The increased PUFA peroxidation decreases the membrane fluidity and secondarily cause damage to proteins in membrane as well, which results in peptide chain fragmentations and cross-linked product aggregations that is considered as one of the oxidative stress' diagnostic marker (Moller et al., 2007).

In general, when cell components come across to HMs' high concentrations, visible injuries could be seen in the plant organisms (Shahid et al., 2014). On the other hand, it is important to carefully monitor HMs in certain critical concentrations, as they can reach dangerous levels in humans via food chains without having any effect on plants. Therefore, in this study, the toxic effects of heavy metals on different biochemical parameters (GSH content, total soluble protein and GST activities) in some barley (*Hordeum vulgare* cv. Kalaycı and cv. Bilgi) and wheat (*Triticum aestivum* cv. Yunus and cv. Bezastaja) varieties were investigated with different concentrations of both single and mixed applications of lead and cadmium chloride.

1. MATERIALS AND METHODS

1.1. Seeds and Seedling Growth

In this study, *Hordeum vulgare* L. (cv. Kalaycı and cv. Bilgi) and *Triticum aestivum* (cv. Yunus and cv. Bezastaja) cultivars were used.

All seeds were obtained from Transitional Zone Agricultural Research Institute (Eskisehir, Turkey) and are registered varieties. Seeds were surface sterilized according to method of Riaz et al., (2017).

For seedling growth, 20 seeds were germinated with dH_2O in a growth chamber for ten days (in dark for 3 days at 22°C± 1°C in the plant growth chamber, followed by 7 days of 16 h photoperiod). Seeds were considered germinated when emerging radicle elongated over than 2 mm. At the end of germination, ten seedlings were transferred to beakers containing 250 ml of Hoagland solution (including 2mM $Ca(NO_3)_2$, 1mM $NH_4H_3PO_4$, 3mM KNO_3, 0.5mM $CuCl_2$, 50mM KCl, 25mM H_3BO_3, 2mM ZnCl2, 0.5mM $(NH_4)_6MO_7O_{24}$, 1mM $MgSO_4$, 2mM $MnCl_2$ and 20mM Na_2Fe-EDTA) (Hoagland and Arnon 1950). Following growth for 3 days in the growth chamber, which beakers were arranged under randomized block design with three replicates, different single $PbCl_2$ and $CdCl_2$ (0, 1.5 and 3.0 mM) and mixed $PbCl_2$ + $CdCl_2$ (1.5 + 1.5 and 3.0 + 3.0 mM) treatments were applied into the nutrient medium. Only distilled water was added to the control sets. Finally, after 16 h photoperiod for another 7 days, seedlings were harvested on 10 days from the beginning of Pb and Cd treatments (Öztetik, 2016). Roots and shoots were separated and pulverized in liquid N_2 for further analysis or stored in pulverized form at -80°C for further studies.

1.2. GSH Determination

Pulverized crude extracts (roots and shoots) were separately homogenized in a ratio of 1:4 w/v, with 5% (w/v) TCA by using

UltraTurrax at 13.500 rpm at 4°C for 3 x 30 s periods. Then centrifugation carried out at 4°C, 12.000 rpm for 15 min and supernatants' pH was adjusted to 4.0 - 5.0 with 1M NaOH. For the determination of GSH contents in crude extracts, Ellman (1959) procedure was used in which absorbance was read at 412 nm and GSH contents calculated from a standard curve prepared by reduced GSH.

1.3. Preparation of Cytosolic Extracts from Plant Materials

Pulverized roots and shoots were separately extracted, in a ratio of 1:3 w/v, with 100 mM pH 7.0 phosphate buffer (including 0.05 mM DTE, 1 mM EDTA and 3.5 % (w/v) PVPP) at 4 °C. The mixture obtained was homogenized for 4 × 30 s. periods by Ultra-Turrax at 13.500 rpm on ice which is followed by centrifugation at 15.000 rpm for 30 min. at 4 °C. Immediately afterward, the supernatant fraction was used for protein and GST activity determinations.

1.3.1. Total Soluble Protein Determination

Protein contents were measured according to the method of Lowry et al. (1951), with crystalline bovine serum albumin (BSA) as a standard.

1.3.2. GST Activity Determination

GST activities with 1-chloro-2,4 dinitrobenzene (CDNB) as substrate were determined spectrophotometrically at 340 nm. To kept constant reaction environment at 25°C, spectrophotometer equipped with thermoregulated cell holder. While incubation mixtures without the enzyme source were used as blanks, the cytosolic fractions (wheats or barleys) were used to start reactions and followed for 3 min. The

reaction rate was calculated using the ε values of CDNB as 9.6 mM^{-1} cm^{-1} (Habig and Jakoby 1981).

2. RESULTS

In the present study, adverse toxic impacts of Pb and Cd solutions were detected on *Hordeum vulgare* L. (cv. Kalayci and cv. Bilgi) and *Triticum aestivum* L. (cv. Yunus and cv. Bezostaja) varieties either by single or mixed treatments. For both shoots and roots, obtained results were compared with the control samples, by focusing on some biochemical variables, like GSH, total soluble protein contents and GST activities. For easy comparison of the effects of HMs on the tested parameters, the obtained values are expressed as a percentage of the control values and the absolute values of the control samples are given in the text.

2.1. Effects of Heavy Metals on GSH Contents

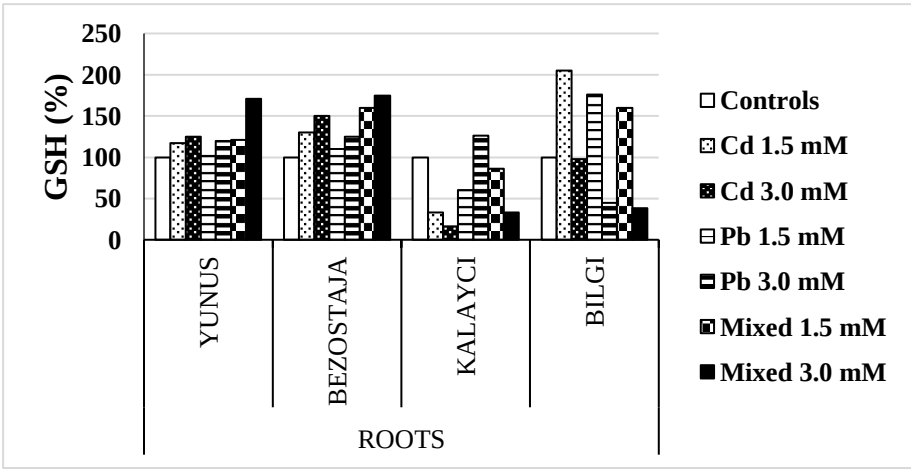

Figure 1A: Effects of Cd and Pb treatments on GSH contents of roots

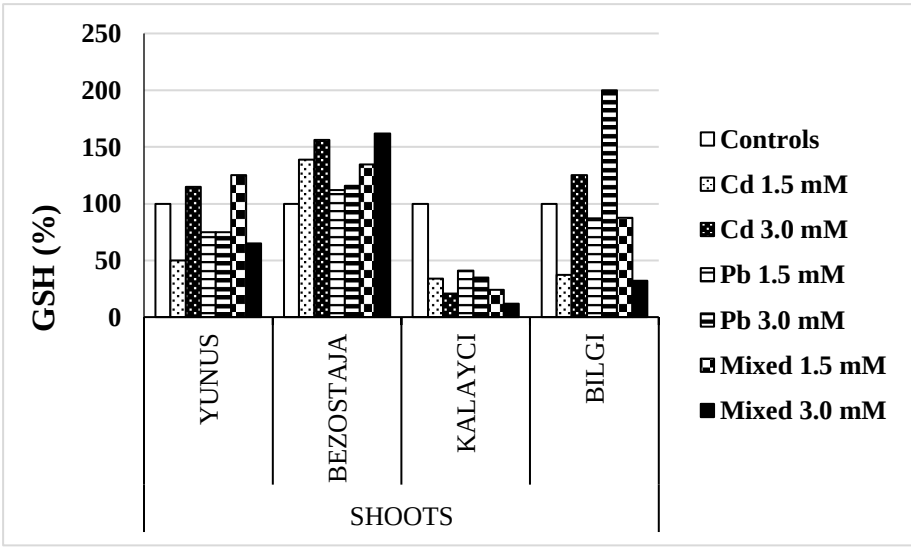

Figure 1B: Effects of Cd and Pb treatments on GSH contents of shoots

Figure 1A and B show the treatments results of the different single and mixed concentrations of Cd and Pb on the GSH content of Yunus, Bezostaja, Kalaycı and Bilgi varieties roots and shoots. The absolute values of GSH contents for control groups of Yunus, Bezostaja, Kalayci and Bilgi samples were calculated as 8.00, 10.12, 10.90 and 14.70 μg mg^{-1} for shoots, 22.55, 19.01, 26.10 and 39.40 μg mg^{-1} for roots, respectively. In general, the roots of plants have higher levels of GSH, although it varies according to the applied HM concentrations and plant varieties (**Figure 1A and B**).

Following treatments, GSH content of the Yunus and Bezostaja roots and Bezostaja shoots were greatly elevated with Pb and Cd at all applied concentration and these are the only plant parts showing dose dependence. By contrast, especially Kalaycı shoots appeared less sensitive to metals exposure (**Figure 1B**), even had lower GSH levels than their own roots. However, the increase in GSH level with single

1.5 Cd in roots and 3.0 mM Pb in shoots were remarkable for Bilgi variety (205 and 200%, respectively), because while 1.5 mM Cd application shown a higher value than combined applications of Pb + Cd for both concentrations and also more effective than Pb itself for the same concentration, 3.0 mM Pb treatment shown again higher value than applications of Pb + Cd for both concentrations. Similarly, all single Cd applications were more effective than single Pb applications for varieties which shown dose dependent pattern.

2.2. Effects of Heavy Metals on Protein Contents

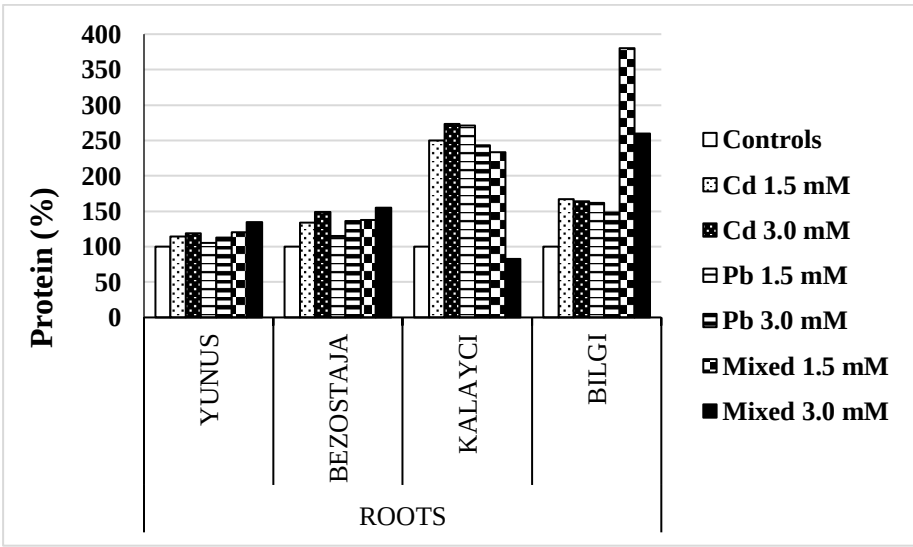

Figure 2A: Effects of Cd and Pb treatments on protein contents of roots

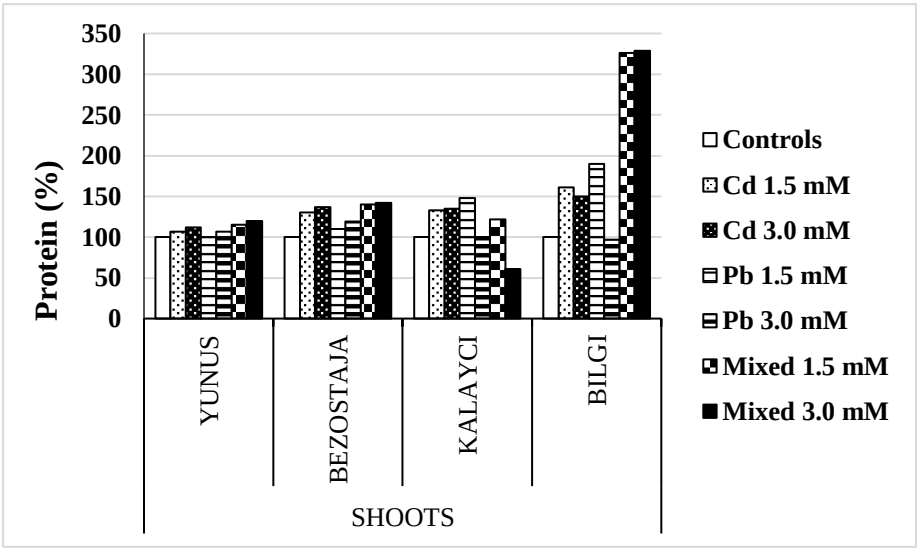

Figure 2B: Effects of Cd and Pb treatments on protein contents of shoots

The treatments results of the different single and mixed concentrations of Cd and Pb on the protein content of Yunus, Bezostaja, Kalaycı and Bilgi varieties roots and shoots were shown in **Figure 2A and B**. The absolute values of protein contents for control groups of Yunus, Bezostaja, Kalayci and Bilgi samples were calculated as 2.00, 0.562, 1.4 and 2.3 mg ml^{-1} for shoots, 1.00, 0.445, 4.4 and 9.7 mg ml^{-1} for roots, respectively. Under HM treatments, the increase in protein content of roots was more significant than that of shoots, compared with the respective controls in general (**Figure 2A and B**).

As mentioned in GSH contents above, HM treatments caused a dose-dependent increase of protein content only in the roots and shoots of Yunus and Bezostaja varieties. Again in these varieties, all single Pb applications were generally less effective than single Cd applications. However, except mixed 3.0 mM application in Kalaycı variety, all

other varieties have shown protein content increases, but without dose-dependent pattern, in respect to their control groups. In another similarity to GSH pattern seen above, Kalaycı shoots were having the less protein amount than their roots. On the other hand, the most significant increase in protein contents were observed in Bilgi variety, with mixed 1.5 and 3.0 mM applications (380% in roots, 326 and 329% in shoots, respectively).

2.3. Effects of Heavy Metals on GST Activities

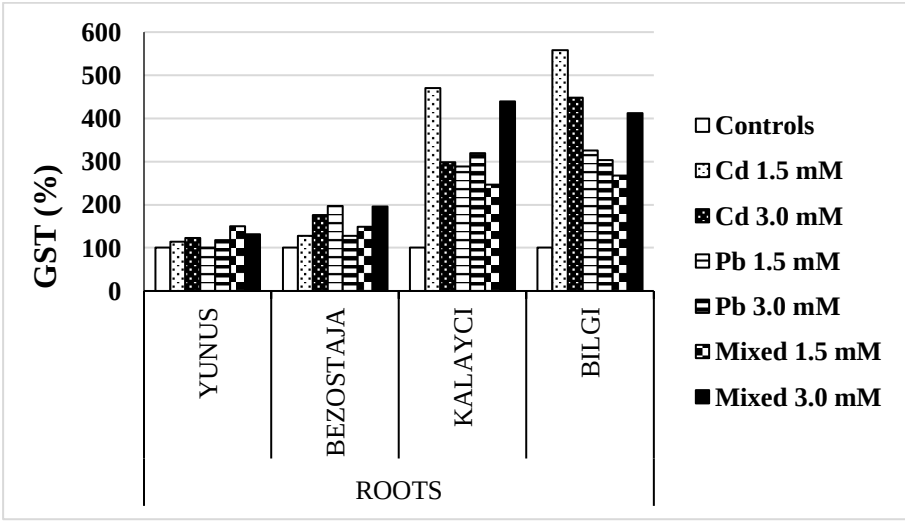

Figure 3A: Effects of Cd and Pb treatments on GST activities of roots

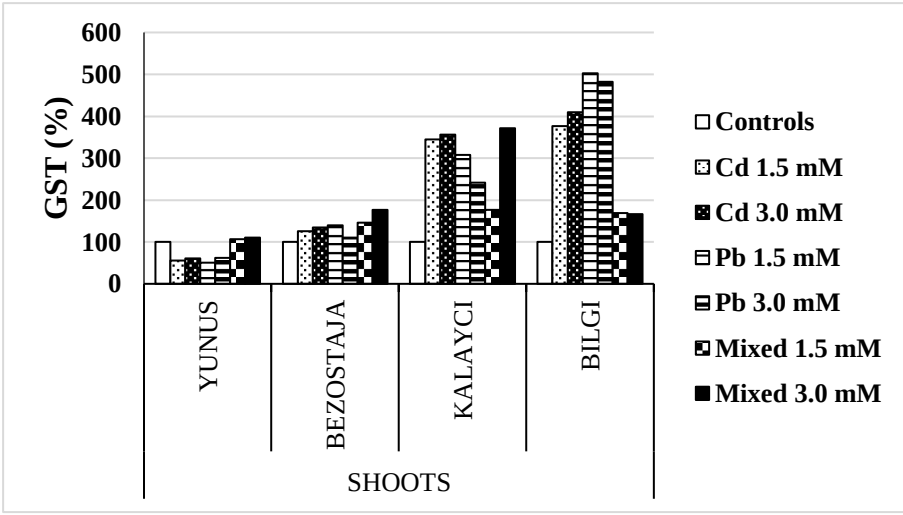

Figure 3B: Effects of Cd and Pb treatments on GST activities of shoots

The data in **Figure 3A and B** show the results of the different single and mixed concentrations of Cd and Pb effects on the GST activities of Yunus, Bezostaja, Kalaycı and Bilgi varieties roots and shoots. The absolute values of GST activities for control groups of Yunus, Bezostaja, Kalayci and Bilgi samples were determined as 600.0, 280.0, 186.8 and 237.4 nmol min^{-1} mg $protein^{-1}$ for shoots, 750.0, 417.0, 362.8 and 582.6 nmol min^{-1} mg $protein^{-1}$ for roots, respectively. As seen from the **Figure 3A and B**, the plant roots had higher GST activity compared to the shoots by differing according to the applied HM concentrations and the plant itself.

In **Figure 3B**, it is clear that all other cultivars showed dose- or non-dose-dependent increases in GST activities relative to controls, with the exception of the shoots of Yunus, which appeared less sensitive to metal exposure than controls and roots, especially for single treatments. After all treatments, the maximum GST activity increase

in roots were observed with 1.5 mM Cd (558%) and in shoots with 1.5 and 3.0 mM Pb (502 and 482% respectively) for Bilgi variety. This is resembling the similar manner in GSH contents mentioned above. However, single Pb applications generally less stimulatory than the Cd applications.

3. DISCUSSION

3.1. GSH Contents

As explained in Introduction section, GSH prevents adverse effects of HM toxicity being as either antioxidant molecule, co-substrate of GSTs or synthesis other antioxidant molecules, such as PCs. Therefore, ample of studies available in the literature regarding the relation between GSH and HMs (Freeman et al., 2004, Shao et al., 2008). However, this relation is not always as an elevation of GSH content by increasing concentrations of HMs (Foyer and Noctor 2005). This is consistent with our current study, a decrease in the GSH content of Kalaycı shoots was observed at all applied HM concentrations compared to the control, which might be attributed to a reduced GSH pool due to increased PC synthesis in cells. On the other hand, some of our results (roots of Yunus, roots and shoots of Bezostaja) have shown a dependence on concentration. These results are in accordance with previous studies reporting the correlation between GSH contents and different concentrations of Pb and Cd metals in *Arabidopsis thaliana*, wheat and barley plants (Guo et al., 2008, Öztetik, 2016). The current study shows that Bezostaja (wheat) and Bilgi (barley) varieties have the highest GSH contents in roots and

shoots. Similar to study of Ozturk et al. (2003) in roots and shoots of two wheat cultivars, our results indicated that GSH contents in roots are more than shoots, as probably metals taken up by roots and cannot be transported into the shoots.

3.2. Protein Contents

Today, it is known that, the increased level of protein oxidation is one of the indexes of the oxidative stress in many plant species (Haluskova et al., 2009). However, plants responds to HM-induced oxidative stress either decreasing (De Dorlodot et al., 2005; Tripathi and Gautam 2007) or increasing (Mittra et al., 2008; Chandra et al., 2009) their total protein contents. In the current study, we observed that with or without dose-dependence, different concentrations of Pb and Cd treatments on wheat and barley varieties increased the shoot and root protein levels compared to the control groups, except 3.0 mM mixed application in Kalaycı variety. This is in aggrement with the abovementioned literature, however, probably Pb and Cd did not work sinergistically at that concentration. Also, in wheat, Bezostaja has a higher protein content than the Yunus variety. On the other hand, the highest protein levels were measured in roots and shoots of Bilgi variety. As reported by Patra et al. (2004), the total protein pool of cells includes not only GSH but also other proteins (PCs, metallothioneins [MTs], etc.) and when induced by HMs, these proteins begin to be synthesized and activated. Therefore, the high protein levels found in Bilgi variety might be attributed to presence of other stress proteins which participate in excess metal storage.

3.3. GST Activities

For antioxidant enzymes, the presence of metal ions is like a double-edged sword. Because, some metal ions are essential for enzymes to show their actions, but can also be inhibitory when excess amounts. For example, it is reported that Cd^{2+} and Pb^{2+} usually produce 50% inhibition in enzyme activities (Kositsin, 1991). These effects of Pb and Cd are supported by others (Verma and Dubey, 2003; Dalcorso et al., 2008) in different antioxidant enzymes and attributed to change in the subunits assembly or decline in enzyme synthesis (Sharma and Dubey, 2005).

On the other hand, there are recent studies reporting the increase in GST activities with various HM treatments in several plant organisms, such as in onion (Hossain et al., 2012), mung bean (Hossain et al., 2010), *Brassica juncea* L. (Szollosi et al., 2009), *Arabidopsis* (Skorzynska-Polit et al., 2010) and some others. In our study, we observed significant GST activity increase in barley roots especially under Cd applications and that is well associated with results of Haluskova et al (2009) whom found similar data by working on *Hordeum vulgare* cv. Jubilant roots. Furthermore, as previously reported for wheat seedlings (Lamhamdi et al., 2011), we measured higher GST activities in roots than shoots with Pb treatments. Also, Bezostaja in wheat and Bilgi in barley has a higher GST activities than Yunus and Kalaycı varieties, respectively. In the current study, we observed high GST activities and GSH contents in general with Cd applications, but especially for the roots of Bilgi variety with 1.5 mM

Cd treatment. Similar results were reported by Zhang and Ge (2008), who found a close correlation between GSH content, Cd level and GST activity in rice seedlings. These observations are well corroborated with our results and as suggest by the authors that these two parameters of the antioxidant defense system may be used as biomarkers of Cd-induced stress.

CONCLUSION

Plants routinely face numerous biotic or abiotic stresses, including HM stress, due to their immobility. Therefore, HMs may cause considerable alterations in physiological and biochemical processes of plants. Plant responses to HM stress depend on numerous factors, such as type and concentration HM, duration or plant itself. With this study it has been shown that, biochemical mechanisms in selected wheat and barley varieties were affected by different single and mixed applications of $PbCl_2$ and $CdCl_2$ applications. According to our results, Bezostaja (wheat) and Bilgi (barley) were more resistant to HM stress than other varieties. It should be seriously considered that these plants, which are used as the main food source, may pose a risk because of their tolerance to HMs and the possibility of transmission of these metals to humans through the food chain. By the presence of high GSH, protein contents, and GST activities which all indicates a general adaptability to stress conditions, we can conclude that some biochemical biomarkers were identified in this study. However, further studies are necessary to understand whether these biomarkers are specific only for HM toxicity. Although, we found a correlation

between GSH content and Cd level and GST activity in Bilgi roots. But, this relationship must be questioned to explain the presence of specific Cd-induced GST isozymes in these or other plants and whether Cd has the ability to selectively increase the activity of this GST isozyme(s).

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CHAPTER 4

ANTIOXIDANT METABOLISM OF PLANTS UNDER UNUSUAL EDAPHIC STRESSORS

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INTRODUCTION

The environment consists of interactions between living and non-living things and is in balance with natural processes (Verma, 2018; Verma, 2020; Xie et al., 2019). Because of their sessile nature, plants cannot escape from biotic and abiotic factors that will stress them, and they are straightly affected to most environmental factors (Doerner, 2020, Imran et al., 2021; Keshan et al., 2021). Plants are frequently subjected of many stressors such as drought, salt, freezing, heat shock, toxic metals/metalloids, flooding/waterlogging, ultraviolet, radiation, air pollutants, nutrient deficiency, pathogen attack, high light and edaphic factors in their habitat (Kamiński et al., 2012; Skoneczny et al., 2019; Hasanuzzaman et al., 2020).

Plants use the cuticle (Han et al., 2019; Yuan et al., 2020; Sanaullah et al., 2021), unsaturated fatty (UFAs) acids (He and Ding, 2020), reactive species scavengers, molecular chaperons and compatible solute as general defense systems against abiotic stressors (He et al., 2018).

1. REACTIVE OXYGEN SPECIES (ROS)

The entity of ROS, which occurs kind of unwelcome by-products, has been known for along time (García-Caparrós et al., 2020). The fact that ROS are important elements of aerobic living conditions first started with the emergence of O_2-evolving photosynthetic organisms and causing oxygen accumulation (Singh et al., 2016). ROS, which emerged on world with the initial atmospheric O_2 three billion years ago, has a constant presence in aerobic life (Mittler, 2017). ROS are

also known to have positive effects on plant growing and improvement by actively participating in the development of germination, apical meristem, flowering, pollen tubes, root hair cells, lateral roots, and, leaves apart from the aspects that damage the basic components of living cells (Mittler, 2017; Noctor et al., 2018). ROS exhibits a dual role at plant biology due to its positive and negative effects (Mittler, 2017).

2. OXIDATIVE STRESS

Oxidative stress in plants occurs in two ways: (i) directly affected by environmental stress or (ii) indirectly caused by its production and accumulation that harm the cell (Xie et al., 2019). The increase of ROS at stressful environment causes DNA damage, protein oxidation, and lipid peroxidation in cells (Abdelaal et al., 2020; García-Caparrós et al., 2020; Kahtani et al., 2020).

Histones and associated proteins normally protect nuclear DNA in plants, but the proximity of histones to ROS production systems makes mitochondrial and chloroplastic DNA sensitive to ROS assault (Tudek et al., 2017).

-ROS compounds such as $^{1}O_{2}$ and $^{*}OH$, which are immensely reactive, create DNA harm by damaging the deoxyribose backbone as well as purine and pyrimidine bases (Agarwal and Khan, 2020; Molinier, 2020; Wang et al., 2021).

-The oxidation process of proteins depend on covalent change of proteins produced by the byproducts of oxidative stress (García-Caparrós et al., 2020).

-Lipid peroxidation is a good marker for determining lipid damage under stress conditions, thus determining the extent of oxidative stres (Gaschler and Stockwell, 2017; Angelova et al., 2021).

Oxidative stress often occurs as a result of biotic or abiotic stress, thus increasing the ROS production containing free radicals, and nonradical molecules (Mehla et al., 2017; Banerjee et al., 2019; Hasanuzzaman et al., 2019). Free radicals are alkoxy radical, hydroxyl radical, hydroperoxyl radical, and superoxide anion, nonradical molecules are also singlet oxygen, and hydrogen peroxide (Singh et al., 2019; Hasanuzzaman et al., 2020).

Since ROS production is linked to basic metabolic processes, the ROS production sites is very important. Chloroplasts, mitochondria, peroxisomes, apoplasts, cell walls, and plasma membranes are the ROS production areas in plant (Corpas et al., 2015; Choudhary et al., 2020). The main source of ROS production area is chloroplasts in plants (Pospíšil, 2016).

Plants have to balance between ROS and antioxidants seeing protect their cells from the damage of oxidative stress. In normal/unstressed conditions, this balance is in question (Figure 1a). However, under stress conditions that cause damage to plant growth and physiology, ROS production increases and an imbalance occurs with antioxidants

(Figure 1b). ROS production exceeds cellular antioxidant potential under stress conditions.

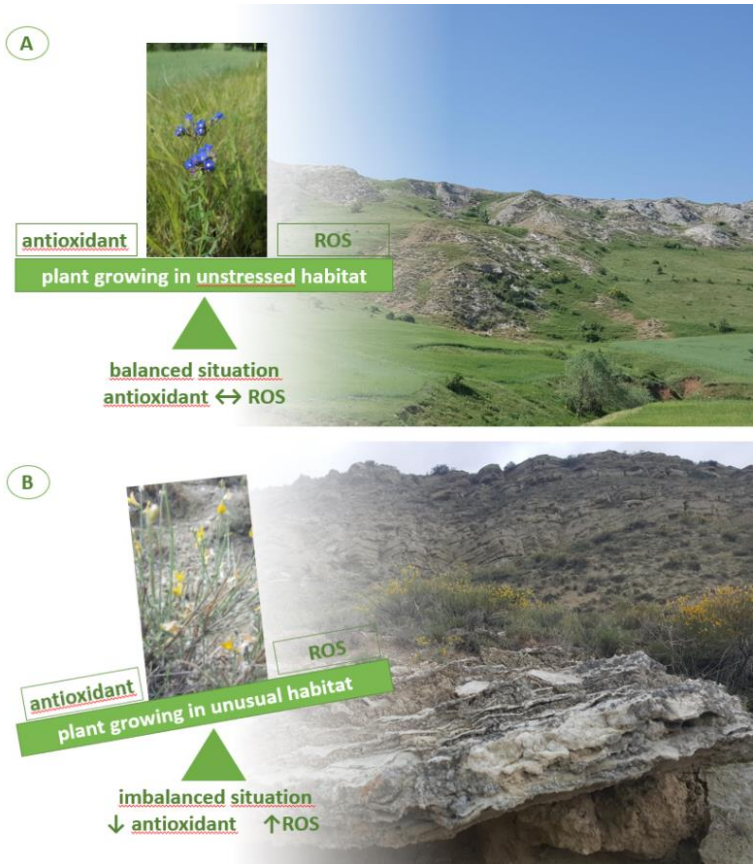

Figure 1: The Relationship between ROS and Antioxidants Depending on the Stress State in Plants. A) Balanced Situation in Unstressed Habitat, B) Imbalanced Situation in Unusual Habitat

3. ROS SCAVENGERS

ROS scavengers that pass the cell from the stressed phase to the unstressed phase under various environmental stress conditions include enzymatic and non-enzymatic systems. Enzymatic scavengers

are ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), and superoxide dismutase (SOD). The non-enzymatic scavengers are α-tocopherols, carotenoids, flavonoids, glutathione, and osmolyte proline (Choudhary et al., 2020; Berwal et al., 2021; Kaur et al., 2021). These enzymatic and non-enzymatic systems reduce harm from oxidative stress (Wituszyńska and Karpiński, 2013; Hancock, 2016; Sewelam et al., 2016).

3.1. Superoxide Dismutase (SOD)

SOD, the first barrier against oxidative damage, is an enzymatic antioxidant found in every cell. The main task of SOD is the conversion or dismutation of nocuous superoxide radicals ($O_2^{\cdot-}$) into hydrogen peroxide (H_2O_2) and oxygen (O_2) (Chung, 2017). SODs, which are also a family of metalloenzymes, are classified into three basic types containing manganese (Mn), iron (Fe), or copper and zinc (Cu/Zn) as prosthetic metals in higher plants (Fridovich, 1975). The number, the locations and type of Cu/Zn-SOD, Mn-SOD and Fe-SOD, which are SOD isozymes, vary in plants (Asada, 1980; Wang et al., 2017). Cu/Zn-SOD localized in chloroplast, cytosol and mitochondria, Mn-SOD in mitochondria and peroxisome and Fe-SOD in chloroplast, peroxisome and mitochondria (Houmani et al., 2016; García-Caparrós et al., 2020; Stephenie et al., 2020).

3.2. Catalase (CAT)

CAT is an enzymatic antioxidant responsible for the dismutation of H_2O_2 into H_2O and O_2 (Liu et al., 2015). CAT localizes in peroxisomes and mitochondria (Palma et al., 2020). Because of the

important role of CAT in H_2O_2 metabolism, peroxisomes are considered equivalent to oxidative organelles (Su et al., 2018; Corpas et al., 2019). Reporting of *CAT1*, *CAT2* and *CAT3*, in the model organism *Arabidopsis thaliana* provides evidence for the presence of CAT isozymes (Frugoli et al., 1996). It is known that the catalase isozymes vary between plants and also according to plant parts. Expression of *CAT1* in pollen and seeds, *CAT2* in photosynthetic parts, and *CAT3* in vascular parts is the proof of this difference (Su et al., 2018; Palma et al., 2020).

3.3. Ascorbate Peroxidase (APX)

APXs, which have five isoforms distributed in different regions such as thylakoid, microsomes, stroma, cytosol and apoplast, reduce H_2O_2 to water by using ascorbate during photosynthesis (Navabpour et al., 2020; Moursi et al., 2021).

3.4. Glutathione Reductase (GR)

GR, which managing for the transformation of oxidized glutathione (GSSG) to reduced glutathione (GSH), is another important enzyme in the adaptation of plants to oxidative stress (Moradbeygi et al., 2020).

CONCLUSIONS

The edaphic factor includes the biological, chemical, and physical properties of the substrate. Changes in the edaphic factor lend the biodiversity seen in the biotic World (Rajakaruna and Boyd, 2008). In unusual edaphic conditions, the rate of endemism is high, and these habitats host rare and endemic plant associations. Therefore,

characteristic features that cause some morphological and physiological modifications are observed in plants grown under extreme conditions (Rajakaruna and Boyd, 2008). Examples of the most conspicuous edaphic habitats are gypsum substrates ($CaSO_4.2H_2O$), limestone forms by precipitation and lithification of $CaCO_3$, saline soils where high concentration of soluble salts, and serpentine soils deriving ultramafic and concerned rocks (Rajakaruna and Boyd, 2008). Gypsum soils and serpentine areas have singular flora consisting of plants that can tolerate the physicochemical challenges imposed by extreme conditions (Mota et al., 2017).

Substrate-based salinity is one of the principal abiotic stresses that causes loss of plant growth, seed germination, vegetative growth, flowering and productivity (Bharti and Barnawal, 2019). Ion toxicity begins as a result of ionic imbalance in plants subjected of excessive salt concentration under extreme conditions, thus water deficit occurs in plants as a result of osmotic stress. As a result of this stress occurring in saline environments, the increase in ROS level triggers toxicity at the cellular level (Luo et al., 2021). The antioxidant system activates against the cellular stress that occurs and protects the plant against the stress conditions. In some plants growing in saline habitats, roots play an prominent role in increasing the uptake of water and nutrients, and preventing salt accumulation. The antioxidant defence metabolism in various plants under extreme conditions are summarized in Table 1.

Table 1: Responce of Antioxidant Defence of Plants under Extreme Edaphic Conditions

Plant species	**Stress type**	**Antioxidant response**	**Reference**
Allium cepa L.	Salinity stress	AsA↑, CAT↑, GSH↑, SOD↑,	Semida et al., 2021
Triticum aestivum L.	Salinity stress (in wheat plants threated with selenium (Se) and silicon (Si))	APX↑, AsA↑, CAT↑, GR↑, GSH↑, SOD↑,	Taha et al., 2021
Triticum aestivum L.	Salinity stress (9.16 dS m^{-1}, in wheat plants treated exogenously with the MLE (3%) and GSH (1 mM) sequences)	CAT↑, POX↑, SOD↑	Rehman et al., 2021
Tetragonia decumbens Mill.	Drought and salinity stress (the treatments included three salt concentrations (50, 100, and 200 mM))	chlorophyll content↓, phenolic content↑	Sogoni et al., 2021
Coriandrum sativum L.	Lead (Pb)-spiked soil stress (the treatments included four levels of Pb (0, 500, 1000, and 1500 mg/kg of soil))	CAT↑, Flavonoid↑, MDA↑, POD↑, SOD↑	Fatemi et al., 2021
Pisum sativum L.	Salinity stress (the treatments included three levels of NaCl (0, 50, and 100 mM))	APX↑, AsA↑, CAR↑, CAT↑, PRO↑, SOD↑	Sofy et al., 2020
Solanum lycopersicum L.	Salinity stress (6.50 dS m^{-1})	carotenoids↑, phenolic compounds↑	Sumulan et al., 2020
Zea mays L.	Cadmium-contaminated calcareous soil stress	APX↑, CAT↑, POX↑, SOD↑, photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids) ↑	Shahkolaie et al., 2020
Glycine max L.	Spherical 38 nm Zinc oxide nanoparticles (ZnONPs) as a novel nanofertilizer (the treatments included one level of Zn (400 mg Zn/kg))	CAT↑, MDA↑, POX↑, SOD↑,	Yusefi-Tanha et al., 2020

Table 1: Continued.

Plant species	Stress type	Antioxidant response	Reference
Capsicum frutescence (L.)	Salt-affected soil (6.74 dS m^{-1}) and deficit irrigation	GSH↑, Osmoprotectants, ascorbate, glutathione, capsaicin, and phenolic contents ↑	Al-Elwany et al., 2020
Sunflower plants	Gypsum treated soil stress (sulfur-based fertilizers, 20 mg kg-1 soil)	CAT↑, GPx↑, SOD↑,	Shafiq et al., 2021
Limonium delicatulum (Girard) Kuntze	Salinity stress in natural conditions due to seasonal changes	MDA↑, Photosynthetic pigments↑ (during the reason season)	Souid et al., 2018
Crithmum maritimum L.	Salinity stress	Phenolic compounds↑	Pereira et al., 2017
Anthyllis cytisoides L., *Cistus clusii* Dunnal, *Gypsophila struthium* subsp. *hispanica* (Willk.) G. López, *Helianthemum syriacum* (Jacq.) Dum.Cours., *Ononis tridentata* L. subsp. *angustifolia*, *Plantago albicans* L., *Rosmarinus officinalis* L., *Teucrium capitatum* L., *Thymus vulgaris* L.	Salt toxicity at gypsum habitats	Total phenolics and flavonoids↑ (the highest in summer)	Boscaiu et al., 2010

AsA: Ascorbic acid, CAR: Carotenoid, CAT: Catalase, GPx: Guaiacol peroxidase, GSH: Glutathione, MDA: Malondialdehyde, MLE: Moringa leaf extract, POX: Peroxidase, PRO: Prolin, SOD: Superoxide dismutase, ↑: increase.

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CHAPTER 5

MODULATION OF MIRNAS IN CANCER THERAPY BY PLANT SECONDARY METABOLITES

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INTRODUCTION

Cancer is one of the most serious public health problems and it is one of the leading causes of morbidity and mortality worldwide. According to Global Cancer Observatory (GLOBOCAN) 2020 report, 19.3 million new cancer cases and almost 10 million deaths from cancer were reported in 2020. The worldwide cancer load is anticipated to be 28.4 million patients in 2040, a 47% rise from 2020 (Sung et al., 2021).

Various traditional attitudes to treat cancer contain chemotherapy or radiotherapy. Nevertheless, these are generally associated with different noxious effects and several drawbacks in clinical practice. Moreover there are growing fears on drug resistance. In the constant seek for reliable and more powerful treatments, phytochemicals (secondary metabolites) are of draw interest (Masika et al., 2016).

Since time immemorial, people have used medicinal plants for the prevention and/or treatment of various diseases and ailments (Michel et al., 2020). Although the popularity of herbal medicines had been dramatically decreased in the western world with the development of synthetic medications in the last 200 years, the recent huge demand for plant-based therapeutic agents as primary health care in well-developed countries, and the sharp upward trend for using traditional remedies and natural-based products indicate the importance of research on the medicinal plants (Rastogi et al., 2016). The massively increased consumption of phytotherapeutic agents has resulted in an

estimate by the WHO, that more than 80% of the population relies solely or largely on medicinal plants for health care (Abdala et al., 2012). Because of the perverse effects of numerous synthetic drugs, plant-derived natural compounds have remarked for academic research such that they may be used as verified helpful anticancer agents (Birudu & Naik, 2014; Choudhari et al., 2020).

Over the years, protein-coding genes were the major focus of cancer study, nevertheless, in last two decades there has been a primary paradigm shift with the rising status of miRNAs and other epigenetic mechanisms as leading actors (Sato et al., 2011). MicroRNAs (miRNAs) are a class of non-coding RNAs, they are short single strands of nearly 20-24 nucleotides in length that are processed from endogenous transcripts, they act a major function in gene expression regulation, by binding to a sequence in the 3'UTR region of their specific target mRNA, ending up gene silencing, which can occur via translational repression and/or mRNA degradation. The discovery of miRNA changes the way in which we understand gene regulation. Bioinformatics analysis results suggest that miRNAs control up to one-third of all human genes (Tétreault & Guire, 2013).

The plethora of published reports in recent years demonstrated that miRNAs act key parts in plenty biological processes, like cell propagation, apoptosis, tumorigenesis, invasion and migration. They play either as oncogenes or tumor suppressors, and modification in their expression designs has been tied to initiation, development and chemoresistance of different cancers. Thus, exploitation of miRNAs

as targets for cancer protection and remedy may be a encouraging perspective (Zhang et al., 2020). Rising data suggest that diverse plant secondary metabolites could possibly regulate the expression of different miRNAs, which are included in oncogenic roles (Li et al., 2010; Phuah & Nagoor, 2014).

1. BIOSYNTHESIS OF MIRNA AND ITS REGULATION ON GENE EXPRESSION

Since the breakthroughs in RNA biology after the unravelling of so-called “junk RNA”, small non-coding RNAs (sncRNAs) have gained particular attention in understanding molecular biology of cell and in advancing clinical applications for novel therapeutic strategies. Three main classes of sncRNAs are known as microRNAs (miRNAs), small interfering RNAs (siRNAs), and piwi-interacting RNAs (piRNAs). sncRNAs are typically ~18-200 nucleotide long, associate with Argonaute (AGO) protein family for their regulatory function and have a role in transcriptional and post-transcriptional gene expression silencing (Ha & Kim, 2014).

MiRNAs are extremely preserved little (~ 22 nt) non-coding RNA particles that modulate gene expression at the post-transcriptional degrees (Mendes et al., 2009). MiRNA binds to the 3′ untranslated region (3′-UTR) of target mRNA, which ends up the inhibition of translation or the degradation of target mRNA (Fig. 1). The miRNAs are primarily transcribed from the nucleus genome as pri-miRNA (Booton & Lindsay, 2014). It is remarkable that every pri-miRNA can

be a pioneer to diverse mature miRNAs. After that, initial operations of pri-miRNA begins with RNase III (Drosha) and its cofactor (DGCR) to form pre-miRNA (circa 60 nt a hairpin) In the following stage, nuclear export of pre-miRNA is provided by exportin 5 and RanGTP. Dicer and TRBP accomplished the other operation in the cytoplasm to make a dsRNA (circa 22 nt) (Bartel, 2004). Finally, later inserting the RISC complex, passenger strand is destroyed, and guide strand (mature miRNA) can be bound to the target mRNA. MiRNAs act their role by demolishing mRNA or restricting the translation (Vazquez, 2006). Former investigations have displayed that a miRNA solo can modulate the expression of many diverse genes by its role. By the way, different miRNAs can synchronically check the expression of one mRNA. On the other hand, most human genes (~ 60%) can be modulated by miRNAs (Kalhori et al., 2020).

Plenty of investigations have given full attention on the evaluation of miRNA expression and have displayed significant alterations in its expression patterns in cancer (Hata & Kashima, 2016; Cansaran-Duman et al., 2021). Due to the main function of miRNAs in the modulation of gene expression, their expression stands to be firmly checked by epigenetic methods (Morales et al., 2017). Connected with different elements, miRNA can have either a tumor suppressive function if the target molecule is an oncogene, or an oncogenic function if the target mRNA is a tumor suppressor gene. Plant-synthesized secondary metabolites can effect expression grade of miRNAs and attend in gene expression modulation alongside effecting

miRNA regulated metastasis, drug resistance, and relapse of cancer (Fig. 2) (Masika et al., 2016).

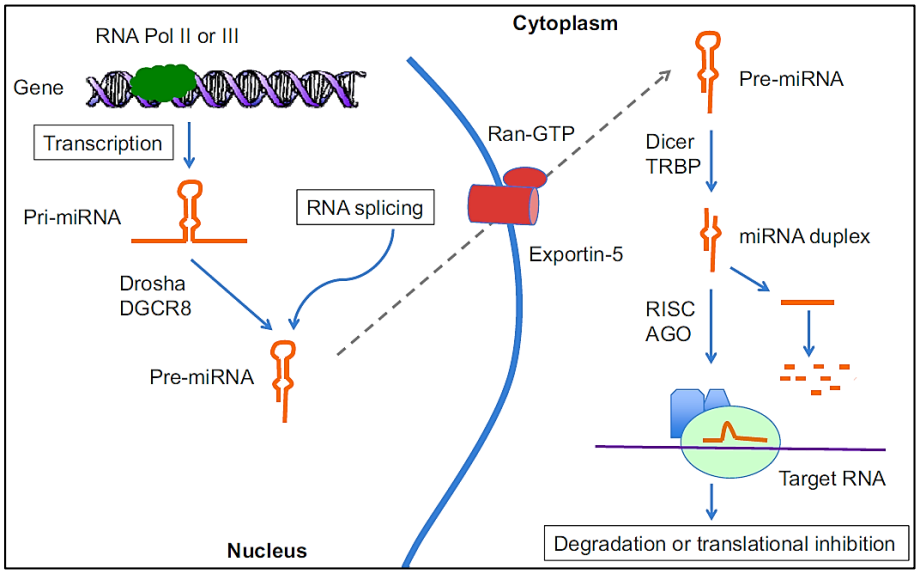

Figure 1: Biosynthesis of miRNA and its modulation of gene expression (Kim et al., 2018).

2. MIRNA AS A TARGET FOR CANCER THERAPY

Taking into consideration that miRNAs act substantial functions in regulation of great deal of gene expressions, cancer progression is extremely connected with miRNAs dysregulations (Wu, 2011). MiRNA and its role in cancer are supported by numerous literatures, it suggests the substitution of tumor suppressive miRNAs or repression of oncogenic miRNAs, which could be utilized to improve new curation approaches (Shanmugapriya & Sasidharan, 2020). MiRNAs are generally classified as tumor suppressors miRNAs or oncomiRs.

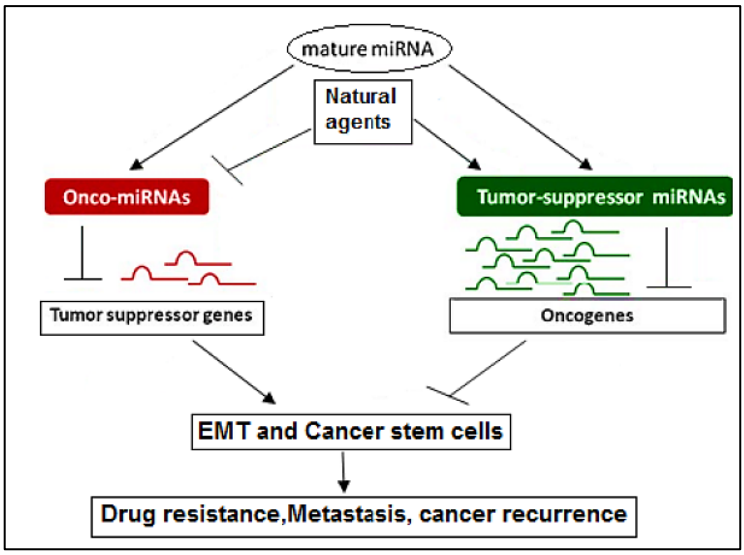

Figure 2: The effect of natural agents on miRNA regulated drug resistance, metastasis and recurrence of cancer (Masika et al., 2016)

2.1. MiRNAs as Oncogenes

OncomiRs, which have been reported to be overexpressed in several cancer studies. Duplication, gene deletion, mutation or epigenetic modulations are some of the causes of this overexpression. Examples of oncomiRs; miR 21, which was overexpressed in majority kinds of cancer, containing breast, ovarian, leukemia, lymphoma and lung cancers. Also, miR-181a and miR-498 which they have been reported in breast cancer (Kim, et. al., 2018). The miR-17-92 group is other miRNA representing oncogenic effect in different cancers (e.g., miR-20a, miR-19b-1, miR-92-1) (Diosdado et al., 2009). Recent evidence has demonstrated that miRNA can regulate metastasis through the regulation of metastasis-associated genes. For instance, miR-200 and miR-205 family have been informed to prohibit EMT (Epithelial-to

Mesenchymal Transition) process in lung and breast cancers (Kim, et. al., 2018).

2.2. MiRNAs as Tumor Suppressors

Tumor suppressors miRNAs are responsible for inhibiting oncogenes, and they are mostly down-regulated in cancers. An example of tumor suppressor miRNA is let-7, which targets many oncogenes like RAS, and myc. MiR-34 family is other example of tumor suppressor miRNA, which targets many genes regulated cell cycle and apoptosis, such as *BCL2*, *CDK4*, and *CCND1* (Kim, et. al., 2018). Moreover, miR-15a and miR-16-1 are tumor suppressor miRNAs have been reported in chronic lymphocytic leukemia, they induced apoptosis in leukemia cells (Mollaei et. al., 2018).

3. REGULATION OF MIRNAS BY PLANT SECONDARY METABOLITES IN HUMAN CANCER CELLS

Latest evidence indicates that miRNAs are extremely included in the different stages of cancer. Thus, targeting miRNAs, which are deregulated in cancer, could be a encouraging perspective for cancer treatment (Li et al., 2010). As from epigenetic changes act significant functions in abnormal expression of different miRNAs in cancer cells, plant-derived secondary metabolites are now being studied for their capability to reverse these alterations that will eventually induce to repression of cancer progression and metastasis (Masika et al., 2016) (Fig. 3).

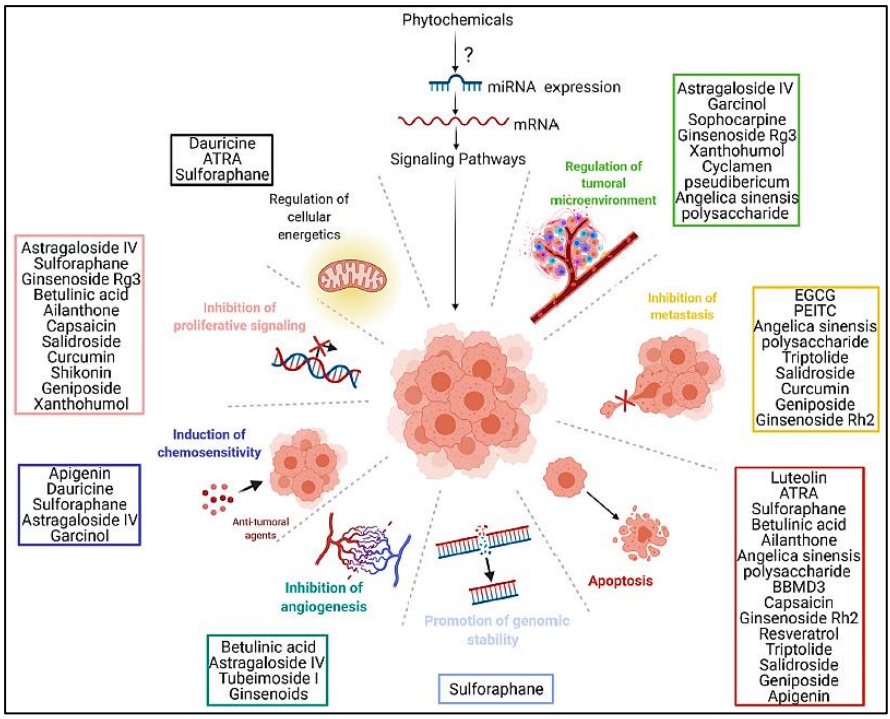

Figure 3: The major types of PSMs and their pathways of action (Sabo et al., 2021).

Most of the anti-cancer medicines recently utilized in cancer treatments have been improved from natural products of plant origin (Karagur et al., 2018). Plant secondary metabolites (PSMs), also known as phytochemicals are originated from primary metabolites with several biological properties (Özay et al., 2016). PSMs are as crucial as primary metabolites in plants because they are related to survival, adaptation, and growth etc. (Kliebenstein, 2013). PSMs are also responsible for medicinal properties of plants to which they belong The number of PSMs in all plants exceeds one hundred thousand (Verma & Shukla, 2015). PSMs are classed into 3 main clusters, according to their biosynthetic pathway: 1) Phenolic

compounds, 2) Terpenes, 3) N comprising molecules (Fang et al., 2011).

Investigations found out that plants exhibit their biological potentials, particularly anti-cancer activities by stimulating apoptosis in cancer cells via modulation of miRNA. Plant-derived chemotherapy has lately reached enormous interest as the PSMs perform lower toxic adverse effects in comparison to that of chemically synthesised anti-cancer medicines (Shanmugapriya & Sasidharan, 2020). According to the comprehensive published letters, different therapeutic plants have been clearly informed to regulate many different miRNAs up to the present. For instance, *Inula viscosa* extract was detected to have important effect in modulating malignant melanoma (MM)-related miRNAs, upregulating miR-579 and miR-524, and downregulating of oncogenic miRNAs, miR-191 and miR-193 that caused EMT and poor prognosis in MM (Colak et al., 2021). *Cyclamen pseudibericum* tuber extract was revealed to hinder cell proliferation in A549 lung cancer cells via up-regulation of miR 200c (Karagur et al., 2018). In a study on colorectal cancer, Alemdar (2016) found that *Nigella sativa* (black cumin) seed extract (NSE), *Olea europeae* (olive) leaf extract (OLE) and *Rubus fruticosus* (blackberry) root extract (RRE) had capability to inhibit colorectal cancer cell proliferation and migration by the changing of the miRNA expressions in two colorectal cell lines (HT-29 and LoVo).

Numerous PSMs display pharmacological features by upregulating certain miRNAs in people. Such as, the bioactive secondary

metabolite purified from the root of *Astragalus membranaceus* was informed to show anti-cancer effect on human osteosarcoma MG63 cells by stimulating apoptosis via up-regulation of miR-133a (Chu et al., 2018). Pterostilbene, the secondary metabolite from the blueberries, was notified to increase anti-cancer effect in breast cancer cells by up-regulating the expression of miR-448, which ulitimately inhibits the expression of NF-κB (Mak et al., 2013). Different such PSM, namely sulforaphane which is abundat in cruciferous plants (e.g., broccoli, kale) was indicated to have anti-cancer feature (Cheng et al., 2020). Sulforaphane demonstrates anticancer activity in MGC803 and BGC823 cell lines (gastric carcinoma) through the up-regulation of miR-124, which straightly targets and inhibits the expression of IL-6 receptor/signal transducer and activator of transcription 3 signalling (Wang et al., 2016).

PSMs could be used as single chemotherapeutic agent or in combination with standard anticancer medicines. The use of PSMs is promising because they could not only decrease toxicity, but also increase the therapeutic efficacy. It is useful since malignant transformation and cancer progression are usually caused by serious genetic alterations and disorganized intracellular signals. This is one of the most plausible explanations why monotherapy often fails in cancer treatment, because specific inhibitors typically target only one protein in a signaling pathway. The effects of PSMs on cancer treatment could be more effective, because they can be used alone or as an adjuvant in combination therapies to overcome drug resistance

and/or reduce drug-induced toxicity, thereby increasing therapeutic effectiveness. However PSMs have been shown to alter the expression profile of certain miRNAs by unknown mechanisms during its antitumor action (Zhang et al., 2020).

Plant phenolics are secondary metabolites that have gained importance as potential anti-cancer compounds. The importance of the diet is confirmed by studies of dietary polyphenols with anti-oxidant activity found in many fruits, vegetables, and plants. The phenolic compounds can also affect the miRNA expression resulting in the up or down regulation of expression of genes involved in many physiological and pathological processes including cancer (Milenkovic, 2013). Polyphenols can modulate the expression of more than 100 miRNAs important for different cellular processes such as in inflammation or apoptosis (Gulyaeva & Kushlinskiy, 2016). Phenolic acids are a subclass of plant phenolics, furtherly divided into benzoic and cinnamic acids, that are associated with potent anticancer abilities in various in vitro and in vivo studies (Abotaleb et al., 2020). A study on the effects of gallic acid on chondrosarcoma cells (CC) showed that this phenolic acid reduces viability, inhibits migration and induces apoptosis. In addition, miR-518b was up-regulated in CC treated with gallic acid, suggesting its implication in apoptosis and inhibition of migration, but no molecular pathway was directly described (Liang et al., 2014).

Terpenes, which also known as isoprenoids/terpenoids, constitute the largest group of PSMs in plants (Verma & Shukla, 2015). α-pinene,

which is a monoterpene found in the resin of coniferous plants, was reported to induce G2/M phase cell cycle arrest and inhibit miR-221 expression with downstream upregulation of CDKN1B/P27 and down-regulation of CDKN1C/P57 in HCC cells (Yang et al., 2016). Alkaloids are a class of PSMs with structures containing at least one nitrogen atom and immensely structurally diverse. Solanine is a toxic alkaloid found in some very common members of the Solanaceae family. Wu et al. (2018) informed that solanine up-regulated miR-138 with anti-proliferative and chemosensitizing effects in lung and esophageal cancer respectively.

Latest researches have exhibited that PSMs, including curcumin, genistein, resveratrol, ginsenosides and others could change the expression of certain miRNAs, which may lead to increased sensitivity of cancer cells to conventional agents, and thereby inhibition of tumor growth (Biersack, 2016). Curcumin, which is a principal phenolic compound isolated from the rhizome of *Curcuma longa* has been notified to display anti-cancer activity in pancreatic cancer cell line via regulation of miRNAs. The miRNA microarray showed a significant up-regulation of miR-22 and down-regulation of miR-199a* in curcumin treated BxPC-3 human pancreatic carcinoma cell line as compared to the untreated cell line (Sun et al., 2008).

CONCLUSION

MiRNAs are abnormally expressed in most cancers and this has been correlated along with cancer initiation and progression; thus, miRNAs show very appealing and novel targets for cancer therapy. Plant secondary metabolites (PSMs) show a unique capability to change the level of miRNAs involved in regulation of cancer pathobiology by modulating the expression of miRNAs. As a result, PSMs can be exploited for designing therapeutic approaches in combination with conventional therapies to develop cancer treatment and suppression strategies. Elucidating the molecular mechanisms underlying the therapeutic effect of PSMs by identifying the essential regulatory miRNAs and their targets will facilitate the efforts to maximize the role of PSMs in cancer therapy.

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CHAPTER 6

LUCERNE LEAF BEETLE (*Gonioctena fornicata* chevrolat, 1836)

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INTRODUCTION

Gonioctena Chevrolat, 1836 (Coleoptera: Chrysomelidae, Chrysomelinae), which has about 110 species, is one of the most special genera of the Chrysomelinae subfamily. It has a wide distribution in the Palaearctic and Oriental regions, with 4 local species in the Nearctic region (Cho, 2019). Both adults and larvae of the breed feed on plants belonging to various birch (Betulaceae), Self-Tubers (Cannabaceae), Legumes (Fabaceae), Rosegils (Rosaceae) and Willows (Salicaceae), but each species usually has a narrow range of host plants (Jolivet and Hawkeswood, 1995).

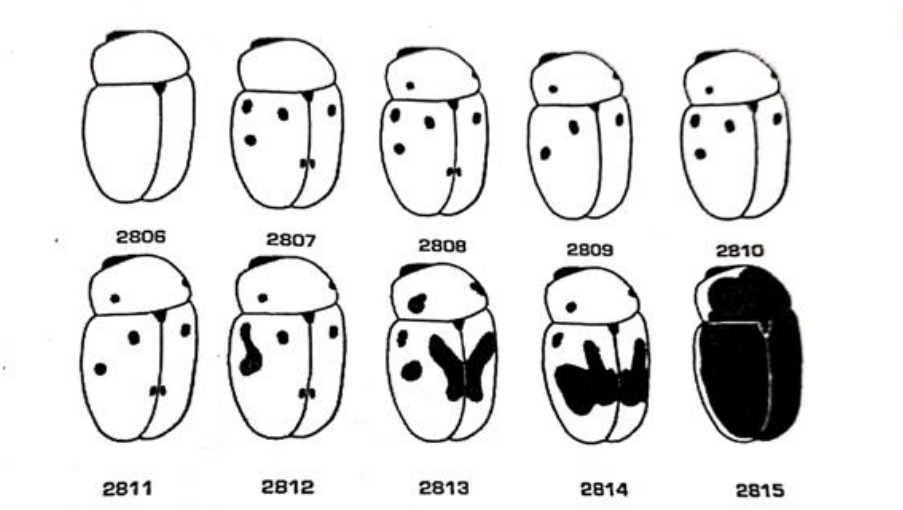

Figure.1. *Gonioctena* (*Spartomena*) *fornicata*, variations (after WARCHALOWSKI 1994): 2806 – ab. *septempunctata* BECHYNÉ, 1947; 2807 – ab. *nigriceps* CSIKI, 1953; 2808 – ab. *tetraspilota* BECHYNÉ, 1945; 2809 – ab. *triangularis* BECHYNÉ, 1945; 2810 – ab. *sexpunctata* PANZER, 1796; 2811 – typical form; 2812 – ab. *vittipennis* CSIKI, 1953; 2813 – ab. *conjuncta* ENDRÖDI, 1957; 2814 – ab. *bohumilae* BECHYNÉ, 1945; 2815 – ab. *picea* WEISE, 1884. (Warchalowski, 2010)

A large number of *Gonioctena* species, especially Palearctic members, are highly variable in color and similar in external morphology. *Gonioctena* elytra has 10 regular large black dots but can appear in

individuals with different patterns (figure.1). Thus, several existing key and regional reviews are largely dependent on the male genitalia, for example, Europe (Warchałowski 2003), Russia (Medvedev 1992), Korea with China (Gressit & Kimoto 1963) and Japan (Takizawa 2007). Palearctic species have been extensively studied and twelve new species have recently been described from Italy, Nepal, China, Korea and Japan (Cho and Borowiec 2016).

Figure 2: General appearance and genital structure of *Gonioctena fornicata* (Anonymous, 2021).

In the genus *Gonioctena*, the body is large or medium in size, squat-oval or elongated oval, bulging. The head is wide. Clypeus is separated from the forehead by a thin, arc-shaped line. Mandibles large and weakly bent. Antennae are short. From the 6th segment onwards, it gradually thickens towards the apex and is generally flattened. Pronotum highly transverse, basally equally wide with elytra, and convex. The disc of the elytra bears regular rows of punctuation, sometimes even in pairs. Legs are short and thick. Tibias

well developed, apically enlarged. At least four spurs and claws bear teeth on the apex of the middle and hind legs (Figure 2) (Biçer 2013).

Gonioctena fornicata (Synonym: Phytodecta fornicatus Brüggemann, 1873; *Gonioctena sexpunctata* Joakomov, 1904, *Phytodecta fornicata ab. sexpunctata* Zivojnovic, 1950), has no feathers except for the mouthparts. Pronotum densely punctate, small, prominently punctate. There are two black spots on the lateral side of the pronotum and it is hairless. Basal of pronotum slightly narrower than basal of elytra. Elytra punctuation is regular, large, ten rows. Between the rows are fine and irregular dots. The elytra are brown with four round black spots on each elytron. The elytra completely covers the abdomen from above. The legs are completely brown, with very short, sparse, yellowish-white hairs. Nails are simple. The underside of the body is hairless and brown (Biçer 2013).

Lucerne leaf beetle [(*Gonioctena fornicata* (Brüggem) (Coleoptera, Chrysomelidae)] is harmful to plants belonging to Fabaceaea family, especially alfalfa. Both adults and larvae of this species are harmful and cause significant crop losses in alfalfa.

The first record of the presence of the pest in our country belongs to Alkan (1946). Later, Bodenheimer (1958) described the pest as a Lucerne leaf beetle, stating that its damage is especially important in clover, that larvae and adults feed on the leaves and stems of clover, that there is a risk of outbreaks, in which case the damage will be much greater. Then, as a result of the researches carried out in the Western, Inland and Southeastern Anatolian provinces of our country

with different studies discussed in our country, it was determined that clover is an important pest.

1. STUDIES ON *GONIOCTENA FORNICATA* IN *ALFAALFA* PLANT

Bodenheimer (1958) stated that *Gonioctena fornicata* was a significant pest in clover in Central Anatolia. With its study of the bioecology of the pest, it has determined that the pest, which spends the winter in adult hood, started to operate at 10-12 °C in March. She stated that the adults started to lay eggs shortly after mating, they laid their eggs on the leaves of the host plants in groups of 6 and 12, and the hatched larvae fed on the leaves. She determined that the larval period lasts for 2-3 weeks, then pupae in the soil. He stated that the larvae and adults are harmful by gnawing on the leaves and stems of the clover, the damage is important and precautions must be taken against the pest (Figure 3).

Figure 3: The damage caused by *Gonioctena fornicata* on alfalfa plant.

In a study conducted on the decaying and biology of the lucerne leaf beetle in Ankara province between 1974 and 1976, it was found that the insect was found in the Central, Ayaş, Beypazarı, Nallıhan,

Kızılcahamam, Kırıkkale, Çubuk and Polatlı districts of Ankara province. The biology of the insect was investigated in laboratory conditions of 22.5 ± 1oC and 60-70% humidity and it was determined that the larval period was 9.64 days (Kovancı 1982). Tamer et al. (1997) detected *Gonioctena fornicata* in the survey studies conducted in Ankara and Konya provinces in 1990 and 1991 in order to determine the distribution areas and densities of harmful and beneficial species in alfalfa and sainfoin fields.

According to Yıldırım et al. (1996) studied the population density of *G. fornicata* in alfalfa. In their studies in Erzurum province, it is stated that the lucerne leaf beetle migrates to clover after leaving its wintering places and it causes product losses as a result of feeding on alfalfa. As a result of this faunistic and systematic study carried out on Chrysomelinae (Coleoptera, Chrysomelidae) species collected from the provinces of Artvin, Erzincan and Erzurum in 1992-1996 and previous years, 34 species belonging to 12 genera were identified. Among these, it is stated that *Gonioctena fornicata* (Brügg.) causes damage to alfalfa (Arslan and Özbek 1999).

Atay and Çam (2006), in their study to determine the species belonging to the Chrysomelidae family in Tokat province, stated that G. fornicata is found on clover in Tokat and its surroundings, adults and larvae feed heavily on the plant and they pierce the leaves in places.

They stated that the average number of eggs per female of *Gonioctena fornicata*, in laboratory conditions, was 243±213 (47-665) and the development period lasted an average of 37.4±4.4 days (Coşkuncu and Gençer 2006).Efe and Özgökçe (2014) created a life schedule of *Gonioctena fornicata* under 25±1ºC constant temperature, 60±5% relative humidity and 16:8 light-dark period in a climate cabinet. Life table parameters of the Euler-Lotka iteration calculated according to the equality and Intrinsic rate of increase (rm), 0.015; Net reproductive rate (R_o), 180.25 females/female; Mean generation time (T_o) 353.03 days; Gross reproductive rate (GRR), 287.93 females/female; Doubling time (T_2) 47.11 day; Finite rate of increase (λ), 1.02 females/female was calculated. Female and male development periods according to the companions are 5.07 eggs, respectively; 2.85 days: $larva_1$, 2.83; 2.72 days: $larva_2$, 2.10; 2.36 days: $larva_3$, 2.15; 2.29 days: $larva_4$, 4.46; 4.29 days: pupa, 7.92; They state that it is 7.69 days. In addition, preoviposition, oviposition, postoviposition and generation time were recorded as 305.53, 32.73, 30.07 and 331.60 days, respectively. Images of different biological periods of *G.fornicata* are given in Figure 4.

Barış et al. (2020), In their study of the prevalence and intensity of *G. fornicata* in Bartin, Bolu and Zonguldak provinces, they found that all areas where pests are prevalent in all three provinces were 16.22-34.86%. While the population follow-up in Zonguldak province stated that the pest spent the winter under an average of 5-10 cm of soil in the alfalfa areas as an adult, Kovancı (1982) found that *G. fornicata*

adults wintered in the soil at a depth of 1-20 cm (5.2 cm on average) in his study of alfalfa fields in Ankara province (Figure 5).

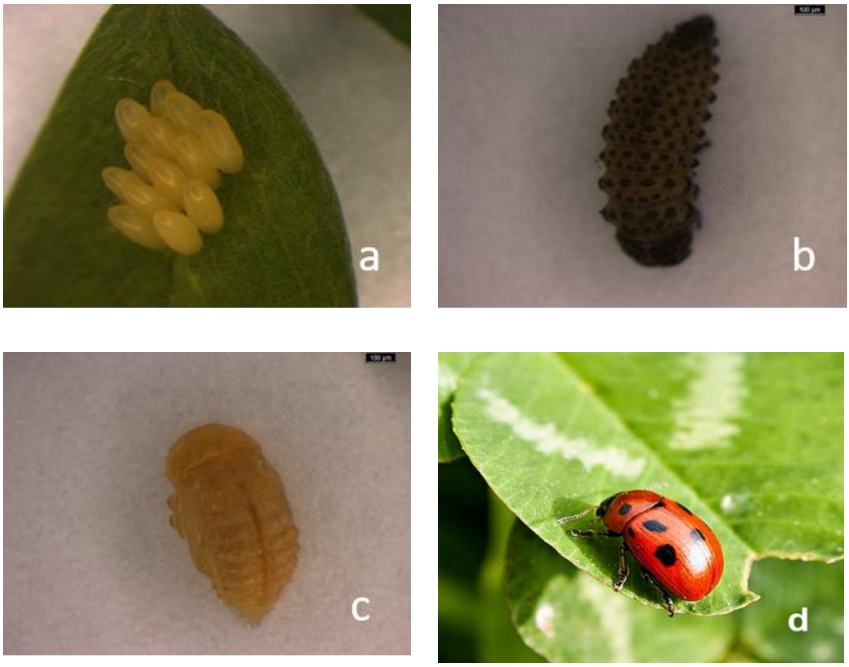

Figure 4: Biological periods of *Gonioctena fornicata* a. Egg, b. Larva, c. Pupa and d. Adult

Figure 5: *G. fornicata* adult overwintered in the soil

Studies have been carried out in our country to determine the natural enemies and effectiveness of the insect. He stated that *P. fornicata* was parasitized by *Metarrhizium sp*. during the prepupa and pupae periods, and by its adults *Aspergillus sp*. and *Metarrhizium sp*. (Hive 1982).

Atay (2018) identified *G. fornicata*'s Tachinid (Diptera: Tachinidae) parasitots and parasitization rates in Tokat province. In his study, he found that the species *Meigenia mutabilis* (Fallén, 1810) and *Macquartia tenebricosa* (Meigen, 1824) (Diptera: Tachinidae) parasitize *G. fornicata*. Parasitization rates for *Meigenia mutabilis* and *M. tenebricosa* were 3.61-3.69% and 1.07-0.50%, respectively. According to the results obtained, *M. mutabilis* indicates that G. fornicata is a more effective parasitoid (Figure 6).

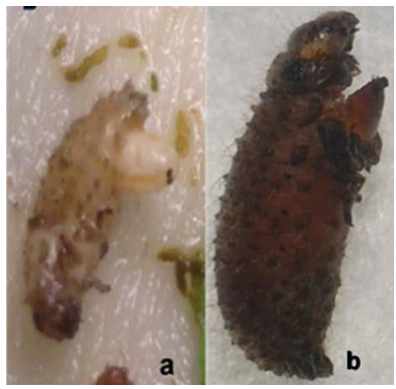

Figure 6: a) *Meigeenia mutabilis* larvae emerging from *G.fornicata* larvae, and b) the puparium of *Macquartia tenebricosa* inside the host larval skin (Atay 2018)

Baysal et al. (2018) They state that they have isolated *Baeuveria bassiana* entomopatogen from *G. fornicata* adults who have wintered in alfalfa fields of Tokat province. *B.bassiana* entomopatogen was

similarly isolated from *G. fornicata* adults who wintered in alfalfa fields of Bolu province (Yucel 2021).

Caglayan et al. (2020) found some of the EPNs they detected in their studies for the detection of entomopathogen nematodes (EPNs) in alfalfa fields sensitive to insect insots in their studies against *G.fornicata* and determined a high mortality effect (Caglayan et al. 2021).

Many studies have been carried out on the lucerne leaf beetle in the world besides our country.

Lustun and Panu (1968), *Phytodecta fornicata* spent the winter in the soil at a depth of 10-15 cm, Brovdii (1976) *Phytodecta fornicata* (*Gonioctena fornicata*) is found in central and southern Europe and is found in the European part of the Soviet Union, in the Transcarpathian, Chernovtsy and Odessa regions of Ukraine, and in Moldavia, where it causes serious damage to clover.

The insect winters in the soil as adults at a depth of 5-20 cm or more. Adults become active at a depth of 10 cm in spring, when the air temperature is above 20°C and the soil temperature is at least 11°C, and the days are sunny and warm. The exit from the soil occurs in a period of 15 days or more, and insects feed on the young leaves of clover. In Ukraine and Moldova, both adults and larvae feed on *M. sativa*, *M. falcata*, *M. romanica* and *M. lupulina*, and sometimes *trifolium repens* and *T. pratense*. The female lays eggs in clusters of up to 23 (sometimes up to 27) on the lower surface of the nutrient plant or on undergrowth weeds. During the entire spawning period of

30-50 days, the female produces up to 1000 eggs (2344 eggs/female were recorded in Yugoslavia). Embryonic development lasts 5-7 days, and the larval period lasts 19-27 days. In Ukraine, they give fertilization once a year.

Bournoville et al. (1984), *Hypera variabilis* (*Phytonomus heiabilis* Herbst.) in the fields of the pest species found in alfalfa in 18 European countries, especially towards the southern parts of Europe. (Coleoptera: Curculionidae) *Subcoccinella vigintiquattuorpunctata*, *Colaspidema atrum* (Olivier,1790) (Coleoptera: Chrysomelidae) and *Phytodecta fornicata* (Bruggem.) [*Gonioctena fornicata*] (Coleoptera: Chrysomelidae) is harmful.

Mardulyn et al. (1997) In phylogenetic analysis of the *Gonioctena* genus from allozyme data, they stated that the *G.fornicata* species is located in the sub-genus Spartomena (Figure 7)

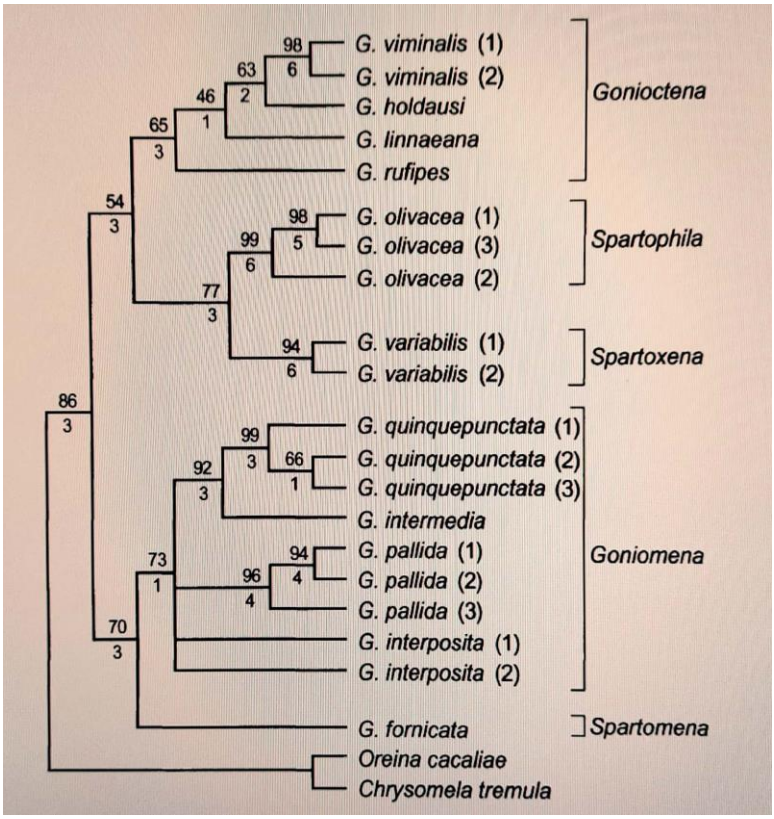

Figure 7: The three most-parsimonious trees (length= 351) by Gonioctena allozyme (17 gene loci) data (Mardulyn et al. 1997).

Bronskikh (1987) reports that the adults and larvae of *G. fornicata* feed on the leaves, flowers and leaf buds of alfalfa, young sprouts and the ends of their stems.

György et al. (2007) found that *G.fornicata* is harmful in alfalfa and tricolour plants. In their study, they found that the insect wintered as an adult at a depth of 10-26 cm and when the soil temperature exceeded 7-8 °C, they reached the surface of the soil and reached the highest number of individuals as of mid-April. They refer to young

leaves and shoots, flower buds and even flowers. They found that clover and other lice (common yarrow, white candlestick, dandelion) lay their eggs on the back of the lower leaves in 8-10 pakaets, and that the resulting larvae feed on shoots, buds and young leaves.

They state that the larvae of *G. fornicata* are pupae in the soil at a depth of 5-6 cm and rise to the surface of the soil as adults in about 8-14 days. After feeding for 15-22 days, the new adults went to the ground at the beginning of August and found that they spent the winter there.

Atanasova and Semerdjieva (2009) stated that the damage done by the species *Phytonomus heirabilis* Hrbst., 1795 and *Phytodecta fornicata* Brugg., 1873, was significant as a result of their work in Bulgaria. Fiera et al. (2013) conducted surveys in rapessace and alfalfa in Romania and Moldova. They found that *Gonioctena fornicata* was the cmmon harmful species, especially in alfalfa, and the beneficial species was *Coccinella septempunctata.* In addition, they stated that sampling was carried out in species belonging to Heteroptera, Homoptera, Diptera, Hymenoptera, Thysanoptera, Orthoptera and Araneae.

Gonioctena fornicata was listed as an alarm by the North American Plant Protection Organization in 2001. It has been reported that the pest in question is a significant pest of clover in parts of Europe, it is able to consume the product completely. It is noted that both adult and larvae are harmful, adults feed especially on the leaves of host plants,

while larvae are harmful in the whole plant, including the leaf stem and plant trunk (Anonymous 2015).

2. RESULTS

The Lucerne leaf beetle is an important pest of the alfalfa plant. Biology, population tracking, natural enemies and damage status have been determined in nature and in the laboratory regarding the pest that prefers alfalfa as the main host in the life cycle. Studies carried out in Ankara and Konya provinces are an important indicator in terms of global warming and its impact on insects. In 1974-1976, Hive mentioned G.fornicata as a significant pest, while Tamer and his friends stated that in 1990-1991 the pest was not dense. In their study in 2016-2017, they did not detect the pest in Ankara province. In a period of about 35 years, the pest has not been detected in this area.

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CHAPTER 7

GENERAL INFORMATION ABOUT SUBCOCCINELLA VIGINTIQUATUORPUNCTATA LINNAEUS, 1758 (COLEOPTERA: COCCINELLIDAE)

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INTRODUCTION

24-spot ladybird *Subcoccinella vigintiquatuorpunctata* (linnaeus 1758) (Coleoptera: Coccinellidae) is among the pests of alfalfa in our country. Both adults and larvae of this species are harmful and cause significant crop losses in alfalfa. This pest is confused with other beneficial Coccinellidae species and therefore it becomes difficult to control.

S. vigintiquatuorpunctata are in the subfamily Epilachninae. The current systematic situation is as follows.

Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Coleoptera
Family	Coccinelliade
Subfamily	Epilachninae
Genus	Subkoksinella
Species	*Subcoccinella vigintiquatuorpunctata* (Linnaeus, 1758)

Within this subfamily are commonly found beneficials. It is reported to be a common species in Europe, North Africa, Western Asia, Russia, Siberia and Asia as a pest (Horion, 1961). It has been

determined that the pest has more than 70 hosts, especially in alfalfa (Anonymous, 1974, Richardas et al. 1976). Its presence in our country was first detected by Bodenheimer (1958), who called the pest the "Clover beetle". In later survey studies, Elmalı (1996) investigated this species on maize plant, Isikber and Karci (2006), On wheat in Konya and Kahramanmaraş, Çevik (1996), on walnut, Tamer (1997), on alfalfa, Kaya (2009) on the apple, Ozbek and Cetin (1991) on alfalfa, sainfoin, lentils and some foreign herbs have determined.

The adult and larvae of the 24-Point weasel feed on the leaves of the host plants. They eat the lower epidermis layer and parenchyma of the leaves, in turn, the upper epidermis remains in the form of a membrane. As a result of feeding, the leaves look like lace and can cover the whole plant when the damage is intense (Figure 1).

A B

Figure 1: 24 point ladybug (*Subcoccinella vigintiquatuorpunctata*) (a) laceration damage (b) The form of damage to the plant

Studies have reported that the pest causes a decrease in leaf yield in alfalfa by 40% to 60% and significant losses in seed yield occur (Keresi and Sekulic, 2005). Adults overwinter in the soil near host

plants (Marriner, 1927; Tanasijevic, 1958; Richards et al., 1976; Wheeler and Henry, 1981; Baldwin, 1988). It is stated that especially the pest feeds on alfalfa and its damage is important.

Alfalfa (Medicago sativa L.) (Fabales: Fabaceae) is a perennial forage plant from the legume family. Alfalfa is the most commonly cultivated forage plant in our country, with 15.714.381 tons in an area of 6.501.107 decares (Anonymous, 2016). While the ratio of alfalfa in the total arable land varies between 5-30% in countries such as the USA, France, Italy and Argentina, where livestock and forage crops agriculture is developed, this rate is approximately 2.50% in our country. One of the most important problems of animal husbandry in our country is the difficulties in providing sufficient and quality feed. Alfalfa, which has the most intensive production among forage crops, has an important place in our country. Although its production changes according to years, it is produced around 15 million tons. This production is affected by many diseases and harmful organisms. Clover, which has an important place in forage crops, is affected by diseases and harmful organisms, which are among these harmful organisms. Especially in recent years, it is gaining more and more importance due to the sudden increase and fluctuations in the populations of insects due to climate change. It has been observed that the studies on the alfalfa leaf beetle, which causes widespread damage to alfalfa and cause uncontrolled spraying, are limited in our country. For this reason, it is aimed to collect data obtained from studies on pests both in our country and abroad.

1. DEFINITION, BIOLOGY, FORMS OF DAMAGE

Studies on the biology of the pest have been carried out both in our country and abroad. The first study on pest in our country was conducted by Bodenheimer. Accordingly, Bodenheimer (1958) stated that the Clover bug, Subcoccinella vigintiquattuorpunctata, is widely found in other parts of our country, especially in Central Anatolia, and its biology is unknown. It has been determined that the pest, which spends the winter in its adult period, started its activity in April and is commonly causing damage to legumes. He noted that the adults begin to ovulate from May, lay their eggs on the leaves of host plants in groups of 50, and the larvae that occur feed on the leaves.

He determined that the larvae pupae on the host after an estimated 4 weeks, and can produce one or more offspring per year. He stated that a method of struggle against the pest was not known. Later on, the description of the genre was made by Uygun (1981). The adults of this species are hemispherical, 3-4 mm long, and their body is covered with fine light colored hairs. The number and pattern of stains on Eyltra vary considerably. It is in colors ranging from yellowish-red to brown-red, and there are normally 24 black spots. However, it is reported that there are individuals who are completely unblemished, light-colored, with combined spots or even completely black.

Kovanci, B., 1982. He worked on the survey and biology of alfalfa leaf beetle between 1974-1976 in Ankara. According to this, he stated that the adults emerged in April-May, and that they lay their eggs in clusters after feeding for a short time. In addition, in laboratory

conditions (22.5±1oC; 60-70% RH) larva development time was 17.8 days, pupal period was 5.2 days, 30±1oC; 50-55% RH stated that the larval development time is 11.44 days and the pupal period is 3.75 days. In addition, it was determined that females lay eggs in clusters of 1-25, and an average female could lay 545 eggs.

Later, studies were carried out by Barış et al. Accordingly, they carried out studies on the biology of the pest in the provinces of Bartın, Zonguldak, Ankara and Konya. They stated that the pest is especially common in the clover fields of Zonguldak and Bartın provinces in the Western Black Sea Region, which is a humid region. In their biology studies in Zonguldak province, they stated that the pest was seen in clover fields from March to April, they mate after a feeding in the winter, and then they can lay their eggs in clusters on both the lower and upper surface of the leaves. It has been determined that there are lace-like symptoms as a result of the pest feeding on the leaves. In addition, these symptoms were found to cover the entire leaf area and cause damage to the plant (Figure 1).

They determined that the first larvae were seen in April and the first pupae were seen in May. They stated that the new generation adults that emerged from the eggs were fed but did not lay eggs and after a short time they moved to the soil to spend the winter. They determined that the pest gives only one generation per year. In addition, they made biology and morphological measurements of the pest in the laboratory (Figure 2).

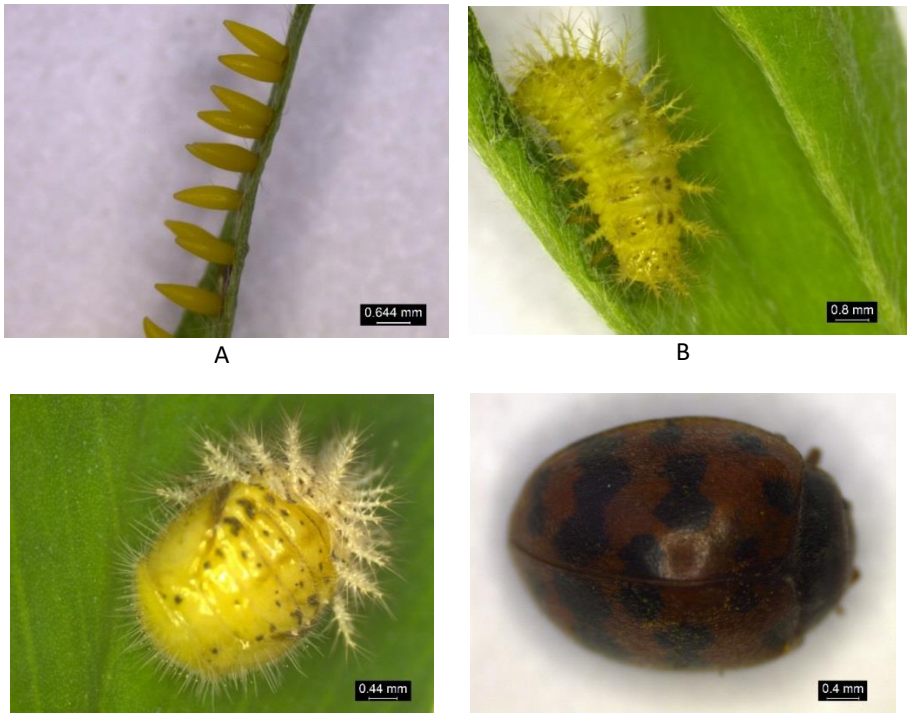

Figure 2: 24-spot ladybird *Subcoccinella vigintiquatuorpunctata* (a) Egg; (b) Mature larva (c) Pupa, (d) Adult.

Studies on the biology of the pest abroad have not been examined. It has been reported that Subcoccinella vigintiquattuorpunctata was first detected in the state of Pennsylvania in the USA, and then it was widely encountered in the states of New Jersey, Ohio, Maryland and West Virginia. Although this pest was detected on clover in Europe, it was detected on Coronilla varia (Fabales: Fabaceae), Saponaria officinalis (Caryophyllales: Caryophyllaceae), Lychnis alba (Caryophyllales: Caryophyllaceae) and Arrhenatherum elatius (Poales: Poaceae) in the USA. They reported that overwintering adults are active in April and early May, mating individuals lay eggs towards

the end of May, and each female lays 50 eggs. They found that the eggs hatched in a few days, the larval period lasted for 30 days, the pupal period lasted around 6-7 days, and there were pupae on the plant. Anonymous (1974), Anonymous, (1974), Subcoccinella vigintiquattuorpunctata has been reported to be harmful to alfalfa in Hungary, Yugoslavia, the Soviet Union, the Netherlands, Germany and Turkey. Information about the life cycle in Yugoslavia is given. According to this, they stated that they give 2 offspring per year, the adults spend the winter on clover and surrounding plants, both adults and larvae feed on the leaves, and the adults usually lay their eggs on the lower surfaces of the leaves. Richards et al. (1976) reported that Subcoccinella vigintiquattuorpunctata is a phytophagous pest, causing damage to various cultivated plants and wild plants in Britain. They determined that the adults were seen towards the end of June, and the time to adulthood in the laboratory was around 6 weeks.

Ali and Szelenyi (1979) stated that there are over 70 hosts of Subcoccinella vigintiquattuorpunctata, among which they are particularly harmful to alfalfa. They determined that both adults and larvae feed on clover leaves and there is loss in leaf yield as a result of feeding, and besides the direct damage, it also causes a decrease in the seed yield of the product. They stated that the pest was found in Europe, certain parts of Turkey and Russia.

Wheeler and Henry (1981) studied the biology of Subcoccinella vigintiquattuorpunctata in alfalfa between 1974-1979 in the US state of Pennsylvania. They stated that the pest, which spends the winter in

the adult period, begins to appear from the end of March to the beginning of April, and the larvae from April to the end of June. Towards the middle of June, the adults, which were formed from the larvae, were widely detected. As its natural enemy, the larval parasitoid Tetrastichus sp. (Hymenoptera: Eulophidae) have determined. Miczulsk et al. (1993) conducted studies in both field and laboratory conditions against Subcoccinella vigintiquattuorpunctata, a pest of alfalfa, in the Lubnin Region of Poland between 1986-1991. They determined that the pest gives offspring once a year, the ratio of male and female individuals is 0.61, 1 in the field, and this rate is 0.91, 1 in the laboratory. Although its natural enemy is low in density, a larval parasitoid Tetrastichus sp. (Hymenoptera: Eulophidae) have determined.

Keresi and Seculis (2005) stated that one of the most important pests of alfalfa in Serbia is Subcoccinella vigintiquattuorpunctata, it gives 2-3 generations a year and winters in the adult period. They stated that both adults and larvae are harmful and there is a loss of 40-60% in product yield, and there are also significant losses in seed yield.

Later, Wang et al (2014) examined the morphological characters of the pest in detail (Figure 3).

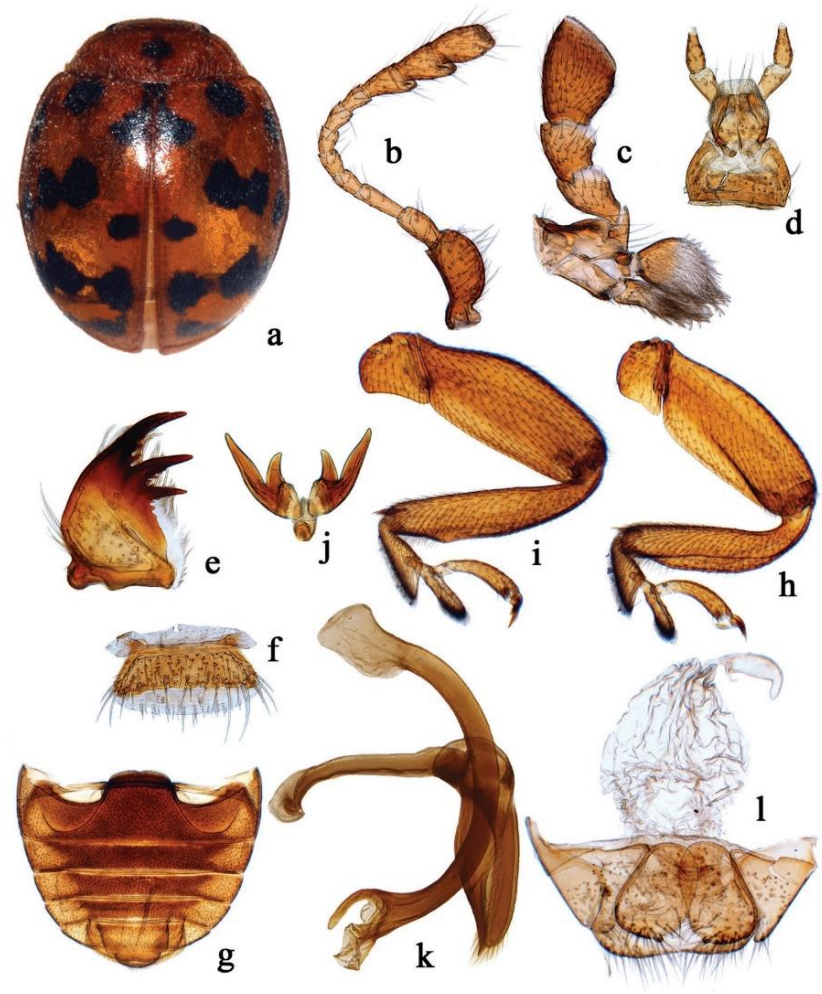

Figure 3: Morphological characters of the genus *Subcoccinella*. a-i *Subcoccinella vigintiquatuorpunctata* Linnaeus, 1758 From China. **a** dorsal habitus; **b** antenna **c** maxilla **d** labium **e** mandible **f** labrum **g** abdomen **h** front leg **i** hind leg **j** tarsal claw **k** male genitalia: penis and tegmen **l** female genitalia: coxites and spermatheca.

2. RESULTS

When the studies on pests in our country are evaluated, limited data have been reached. In particular, limited data were found on the biology and control of the pest. As a result of the studies, it has been determined that the adults, whose winter is under the ground, can emerge from the end of March to the beginning of May, although it varies according to the regions. It was determined that the population of the pest increased in April and May and reached its highest rate in June. Although the eggs of the pest are usually found on the lower surface of the leaves, it has been determined that they are laid in groups of 1-25 on the upper surface, usually with spaces between them. Larval period of the pest was determined from the end of April to June. Adult period was determined during March and July, the population increased from April until June and then it was determined that the population decreased in July.

Similar findings were obtained in studies conducted abroad, similar to the results performed in our country. Wheeler and Henry (1981) studied the biology of *Subcoccinella vigintiquattuorpunctata* in alfalfa between 1974-1979 in the US state of Pennsylvania. They stated that the pest, which spends the winter in the adult period, begins to appear from the end of March to the beginning of April, and the larvae from April to the end of June. They found that adults consisting of larvae towards the middle of June were common.

Anonymous, (1974) reported that *Subcoccinella vigintiquattuorpunctata* was first detected in the state of Pennsylvania in the USA, and then it was widely encountered in the states of New Jersey, Ohio, Maryland and West Virginia. They reported that overwintering adults are active in April and early May, mating individuals lay eggs towards the end of May, and each female lays 50 eggs. They found that the eggs hatched in a few days, the larval period lasted for 30 days, the pupal period lasted around 6-7 days, and there were pupae on the plant. They determined that adults and larvae feed on the leaves, and switch to wintering again in autumn towards the end of summer. As a result of the studies, it was determined that the adults migrated to 5-10 cm soil to spend the winter. Studies abroad have also found that adults spend the winter in the soil near the host plants (Marriner, 1927; Tanasijevic, 1958; Richards et al., 1976; Wheeler and Henry, 1981; Baldwin, 1988).

In natural enemy studies, no natural enemies were detected. However, in the observations made in the field, it has been seen that some spider and ant species feed on pests as a result of coincidence. In studies conducted in our country and abroad, no natural enemies have been detected against the adult stage of the pest. Wheeler and Henry (1981) conducted studies on the biology of *Subcoccinella vigintiquattuorpunctata* in alfalfa between 1974-1979 in the US state of Pennsylvania and as its natural enemy, the larval parasitoid Tetrastichus sp. (Hymenoptera: Eulophidae) stated that they were detected. Tetrastichus spp. is present in our country and has been defined as a hyperparasitoid (Kılınçer 1982, Avcı and Özbek, 1990).

In this study, the findings obtained from studies on pests both in our country and abroad are given. It is seen that there is a need for studies on the biology of the pest and the information about its control is limited.

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CHAPTER 8

THE MALE REPRODUCTIVE SYSTEM OF ORTHOPTERA

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INTRODUCTION

Male insects have a very high mating capacity because they have a high sperm count and transfer millions of spermatozoa to the female's reproductive tract during mating. In addition, male insects can increase their reproductive chances with the frequency of mating. Various mechanisms can increase the chance of reproduction in insects. There are some behaviors in male insects such as courtship and offering nuptial gifts to the female, as in grasshoppers such as *Schistocerca* (Orthoptera, Acrididae). These behaviors increase the chance and capacity of reproduction in insects by the fact that females can mate several times before or after oviposition, thus increasing mating competition between males. In order to gain an advantageous position in competition with spermatozoa, male insects also transfer some nuptial gifts called spermatophylax to females. These substances are antiaphrodisiacs that prevent females from mating with other males later (Von Helversen and Von Helversen, 1991; Reinhold and Heller, 1993; Reinhold and Von Helversen, 1997; Sottile et al., 2010; Dushimirimana et al., 2012).

1. THE MALE REPRODUCTIVE SYSTEM IN ORTHOPTERA

The spermiolytic activity is conducted in the reproductive tracts of both female and male insects. This process includes portions of mesoderm origin in male insects. Although the male reproductive system structures in the Orthoptera order have some differences between insect species, it generally consists of a pair of testis, a sperm duct emerging from the testis (vas deferens), a pair of seminal vesicles

where the spermatozoa are stored, an ejaculatory duct where the vas deferens open, and a pair of accessory glands connected to the ejaculator duct (Viscuso et al. al., 1996, 1999, 2014; Liu et al., 2005; Jones et al., 2013). The male reproductive system of *Poecilimon cervus* Karabag, 1950 (Tettigoniidae), *Gryllus sigillatus* (Walker, 1869) (Gryllidae), *Gryllus campestris* Linnaeus, 1758 (Gryllidae) and *Melanoplus sanguinipes* (Fabricius, 1798) (Acrididae) that are Orthoptera species has a pair of testis, a pair of vas deferens, accessory glands and an ejaculator duct. However, no seminal vesicle structure was observed in the male reproductive system of *P. cervus*, while others have (Nandchahal, 1972; Jones et al., 2013; Polat, 2016; Gökçe Bayram and Candan, 2021).

1.1. Testis

The structure, shape and number of testis can vary in male insects. Although similar structures are generally seen in species belonging to the Orthoptera order, there are some differences. In *P. cervus* and *G. sigillatus*, there is a pair of testis located dorso-laterally in the abdomen at the level of the midgut. In *P. cervus*, each testis is flattened, oval, and yellow in color, while they are large, white, and inverted pear-shaped in *G. sigillatus*. *Xenacanthippus hainanensis* Tinkham, 1940, *Leptacris vittata* (Fabricius, 1787), *Paregonista infumata* Willemse, *Euthystira yuzhongensis* Zheng, 1984, *Chrysochraon dispar dispar* (Germar, 1834), *Acrida cinerea* (Thunberg, 1815), *Phlaeoba antennata* Brunner von Wattenwyl, 1893, and *Acrida lineata* (Thunberg, 1815) from the Acrididae family have

long shuttle-shaped follicles in their testis (Nandchahal, 1972; Liu et al., 2005; Jones et al., 2013; Polat, 2016).

Each testis consists of units called follicles that contain the stages of sperm formation. The number and shape of follicles differ between insect taxa and can therefore be used as a taxonomic character. In Orthoptera, each testis generally contains more follicles than in some other orders. Approximately 300-400 follicles in the form of a thin long tube in the testis are held together by a thin connective tissue sheath. However, the testis of some species contains few follicles. For instance, *Orphulella punctata* (De Geer, 1773) (Acrididae) males have only 4 follicles in their testis (Liu et al., 2005; Jones et al., 2013; Silva et al., 2018). In *P. cervus*, there are many oval-shaped follicles in each testis. Similarly, in *M. sanguinipes*, each testis contains numerous follicles. In *G. sigillatus*, there are approximately 154 follicles in each testis, but these follicles are seen as shuttle-shaped, while *G. campestris* males have about 140 spindle-shaped follicles in their testis. Similarly, it has been reported in some studies in the literature that the number of follicles is too high in the testis of *Pseudochorthippus paralellus paralellus* (Zetterstedt, 1821) (Acrididae) and *Poecilimon ataturki* Ünal, 1999 (Tettigoniidae) (Nandchahal, 1972; Liu et al., 2005; Jones et al., 2013; Polat, 2016; Polat et al., 2019; Amutkan Mutlu et al., 2021; Gökçe Bayram and Candan, 2021).

In histological studies with male insects, it is seen that there are many cysts in each follicle in testicular sections and there are gametes in these cysts at various maturation stages. The distal region of the testicular follicle is the part of the germ cells. They are called as spermatogonia. The first cells to form in this region are spermatocytes after spermatogonia. The spermatogonia undergo mitosis and formed spermatocytes increase in number and move towards the middle parts of the follicle. Later, spermatocytes undergo meiosis and haploid spermatids appear. As a result of the differentiation of the spermatids, mature spermatozoa are formed, and this stage is called spermiogenesis. At this stage, the tail begins to elongate from round spermatids. In the early stage of spermiogenesis, the mitochondrial nebenkern begins to form in some species such as *Locusta migratoria* L. 1758 (Acrididae) (Szölössi, 1975; Tikku and Saxena, 1990; Chapman, 2013; Klowden, 2013; Polat, 2016; Polat et al., 2019; Amutkan Mutlu et al., 2021).

At the last stage of this maturation stage, in some species such as *Tylopsis liliifolia* (Fabricius, 1793) (Tettigoniidae), spermatozoa are aggregated from the head to form bundles called spermatodesm before they are transmitted to the reproductive tract of the female insect. The morphological and structural organization of spermatodesm has a number of changes in different species. For example, while there are 2 types of ultrastructural organization especially in Acrididae and Tettigoniidae families, spermatodesm does not occur in Grylloidea family. The spermatozoa can be seen freely in the sperm ducts or seminal vesicles of male individuals belonging to this family.

However, the spermatodesm structure obtained from the female spermatheca and those in the male reproductive canal from even the same species show great differences. In males, before being transferred to the female, a mucous capsule covers the sperm's head (acrosome and nucleus) and the tails remain exposed. As in the Tettigoniidae family, after the formation of spermatodesm in many Orthoptera families, it gains an elastic sheath while being transferred from the male reproductive tract to the female reproductive tract. This sheath is produced as a result of the activities of the male reproductive accessory glands and is called spermatophore. The spermatophore has two parts, the small ampulla containing sperm and the larger jelly-like spermatophyllax. During transfer from male to female, the spermatodesm is rearranged inside the spermatophore. As a result of rearrangement, the tassel-shaped spermatodesm are transferred to the female's spermatheca with mating (Viscuso et al., 1998, 2001, 2002, 2012, 2016; Lay et al., 1999; Marchini et al., 2009; Sottile et al., 2010; Viscuso and Vitale, 2015).

Spermiogenesis in *L. migratoria* has 10 differentiation stages. In the first 3 stages, the mitochondrial nebenkern structure is formed. Mitochondrial nebenkern structure is also seen in spermatid in *Poecilocerus pictus* (Fabr.) (Acrididae), *P. cervus*, *P. ataturki* and *P. paralellus paralellus.* With the completion of spermatogenesis, spermiogenesis, which is the differentiation stage, begins and the cells take the shape of a spear and form spermatozoa (Szölössi, 1975; Tikku and Saxena, 1990; Polat, 2016; Polat et al., 2019; Amutkan Mutlu et al., 2021).

1.2. Sperm Transport Channels

In insects, sperm ducts are structures that allow spermatozoa to be transported from the testis. The external morphology and length of these channels may vary between species. In Acrididae the sperm canal is almost straight, in Tettigoniidae it is mostly spiral. The initial area of the sperm ducts is the region where they connect with the testis follicles. Some of the sperm ducts coming out of here remain inside the testis. This part of the sperm duct is called the vas efferens or intratesticular duct. Each follicle is connected to the vas deferens by a very thin vas efference. The vas deferens is the part of the sperm duct outside of the testis (Liu et al., 2005; Jones et al., 2013; Viscuso et al., 2014).

In the male reproductive system of Orthoptera species such as *G. sigillatus, G. campestris,* and *P. cervus,* a long, white vas deferens that emerges from the middle of each testis connects the testis to the ejaculatory duct. In *P. cervus,* the vas deferens appears to be composed of 2-3 layers of cubic epithelial cells. In *P. parallelus parallelus*, on the other hand, single-layered epithelial tissue is prominent. Epithelial cells have round nuclei with large euchromatin. In the lumen of the sperm duct, some spermatozoa are seen in bundles in the secretory substance. In *P. cervus,* connective tissue surrounds the epithelial tissue in the vas deferens, and muscle tissue is the outermost one. It has also been reported that there are trachea and tracheole sections in the connective tissue (Nandchahal, 1972; Polat, 2016; Polat et al., 2019; Gökçe Bayram and Candan, 2021).

In some insect species, the part of the sperm duct to the point where it opens into the ejaculatory duct expands. This structure, called the seminal vesicle, is of mesodermal origin. The sperm duct and seminal vesicles are responsible for collecting and storing sperm before mating, and producing the secretion that will keep the spermatozoa alive until they are transferred to the female. In Tettigoniidae, the seminal vesicles are tubular. Some insect species, such as *P. cervus*, do not have a seminal sac (Viscuso et al., 1999, 2014; Polat, 2016).

1.3. Accessory Glands

In male insects, accessory reproductive glands are the source of various secreted proteins that play a role in improving the male reproductive potential. In the genital canals of male insects, spermatozoa are found in this protein-containing fluid secreted from the mesoderm or ectoderm-derived accessory glands attached to the genital canal, and from the epithelial cells in the canal wall. Together with this secreted fluid, it ensures that the sperm mass is transported from the reproductive system of male insects to the spermatheca of the female. In many insects and Orthoptera, transport of spermatozoa occurs by the production of spermatophores. Male reproductive accessory glands have many functions such as preventing females from accepting another male after mating, stimulating oviposition, protecting the sperm in the spermatheca after mating, and producing the spermatophore. In addition, the secretion of these glands contains substances that affect the behavior and physiology of females. In some insects, such as *L. migratoria*, mating has an accelerating effect on

female oviposition. This acceleration takes place by transferring the secretions in the spermatophore of the male reproductive accessory glands to the female. Similarly, in the reproductive accessory glands of *M. sanguinipes* males, the protein content increases and the oviposition-stimulating factor is produced in the first 14 days (Friedel and Gillott, 1976; Gillott and Friedel, 1976; Lange and Loughton, 1985; Sturm and Pohlhammer, 2000; Viscuso et al., 2001; Braswell et al., 2006; Marchini et al., 2009; Sturm, 2012; Viscuso and Vitale, 2015; Polat et al., 2020).

One of the important tasks of the accessory glands of insects is to produce a nuptial gift for the female. In most insects, during mating, sperm is transferred to the female along with other substances as a spermatophore or nuptial gift to increase the number and quality of offspring. The insect may produce its nuptial gift to the female in the male reproductive accessory glands, it may obtain it by hunting, or it may be a body part of the male insect. While these nuptial gifts may stimulate egg laying in some females, they may also be used as nutrients in some females. In addition, these substances may have aphrodisiac properties or may be anti-aphrodisiac substances that suppress re-mating of the female beetle (Reinhold and Von Helversen, 1997; Heller et al., 1998).

The structure of the male reproductive accessory glands, the number and shape of the parts of the glands may vary between insect groups. The male reproductive accessory glands of *Locusta migratoria migratorioides* R. F. (Acrididae), an Orthoptera species, lie on both

sides of the ejaculator duct. Each accessory gland has a total of 15 tubules consisting of 3 different tubule types as hyaline, white and opaque. In *G. sigillatus*, accessory glands consist of 6 groups of tubular structures with different colors and sizes. Male reproductive accessory glands in *Platycleis intermedia* (Serville, 1839) (Tettigoniidae), *T. liliifolia, Steropleurus elegans* (Fischer 1853) (Tettigoniidae), *P. ataturki,* and *Rhacocleis annulata* Fieber, 1853 (Tettigoniidae) are divided into 2 groups of different sizes. It was observed that the secretion of the 2nd group of tubules modificates spermatodesm cap structure. There are a total of 10 accesory gland tubules in *P. parallelus parallelus,* which are divided into two different types according to their sizes. The length and the way they open into the ejaculator canal divide the male the accessory glands of *Bolivarius siculus* (Fischer) (Tettigoniidae) into 2 main groups with many fringe-shaped tubes. The first group tubules are located in the more anterior region. the male reproductive accessory glands of the desert locust *Schistoerca gregaria* (Forskål, 1775) (Acridida) are divided into 9 different types according to the characteristic of the glandular epithelium and the secretion. In males of *P. cervus*, the accessory glands consist of fringe-shaped tubules of various sizes and are divided into 2 groups according to the size of these tubules. The first set of tubules is larger and longer, while the second set of tubules is shorter and smaller. The second set of tubules can also be examined under two groups according to their color and size under the stereomicroscope. The arrangement and structure of the male accessory glands may vary within the same family, subfamily, and

even within the same genus. This change in the biochemical features of the secretion is associated with different functions in the secretions of the glands, such as feeding the sperm cell, suppressing the female's acceptance of other insects or stimulating egg laying (Odhiambo, 1970; Nandchahal, 1972; Glitho and Huignard, 1990; Gallois and Cassier, 1991; Viscuso et al., 2001; Marchini et al., 2009; Vitale et al., 2015; Polat et al., 2020; Amutkan Mutlu et al., 2021).

Although the cytological and histological structures of male reproductive accessory glands varies among various insect groups, rough endoplasmic reticulum is generally seen as well developed in the epithelial cells as in *P. parallelus parallelus* and *Acheta domesticus* Linnaeus, 1758 (Gryllidae). The well-developed Golgi complex and the rough endoplasmic reticulum in the epithelial cells of the accessory glands are associated with high protein synthesis and secretory capacity. In the first groups of tubules of *P. cervus*, there is a single layer of squamous epithelial tissue around the lumen. In the second groups of tubules, a single-layered squamous epithelium is seen in the proximal region, while towards the distal region, the cells first take a cubic and then a cylindrical shape. There is abundant rough endoplasmic reticulum in the cytoplasm of the cells *P. cervus*. The epithelium of the long hyaline glands *M. sanguinipes* in newly formed males has 1-3 layers. But the cells are rearranged to form a single layer within about 24 hours. In addition, rough endoplasmic reticulum proliferation accelerates and Golgi complexes begin to develop. Approximately 3 days before the insect reaches sexual maturity, accessory gland secretion production from epithelial cells begins. In *S.*

gregaria, the rough endoplasmic reticulum, Golgi complex, polyribosome, and mitochondria develop in the epithelial cells during the maturation of the accessory glands which is regulated by the corpus allatum hormones. In *B. siculus*, each secretory tubule is histologically quite similar. The walls of the tubules consist of a single layer of squamous or cylindrical epithelial cells with highly basophilic cytoplasm. Epithelial cells, which can be, have a and a large nucleus. The epithelial tissue is surrounded by muscle tissue from the outside of the accessory glands in species of Orthoptera such as *P. cervus and B. siculus* (Odhiambo, 1971; Kaulenas et al., 1979; Couche and Gillott, 1987; Marchini et al., 2009; Jones et al., 2013; Polat, 2016; Polat et al., 2020).

Different types of accessory glands are responsible for producing secretions with different chemical and physical properties. For example, in *P. cervus*, there is a dense secretory material in the lumens of both the first and second order tubules in the male reproductive accessory glands. In the lumen of the first group of tubules, there are spherical or ovoid secretory granules with inward depressions while there are spherical or polygonal shaped granules in the lumen of the second group of tubules. In *S. gregaria*, 2 of the 3 tubules produce acidic lipoprotein complex, mucopolysaccharide or mucoprotein secretion, while the third type tubules function as seminal vesicles. In *L. migratoria migratorioides*, the secretion of each tubule is also different. The lumen of the white tubular has granular material produced by the Golgi. The lumen of the opaque glands contains homogeneous material unpacked by the Golgi and

paracrystalline material, which is crystallizes in the lumen. Hyaline tubules also have only homogeneous material in their endoplasmic reticulum. In the adult of *A. domesticus*, male reproductive accessory glands produce secretions containing a mixture of proteins (Odhiambo, 1969; Kaulenas et al., 1979; Gallois and Cassier, 1991; Polat, 2016).

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CHAPTER 9

CHECKLIST OF CLYTRINAE FROM TURKEY (CHRYSOMELIDAE)

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INTRODUCTION

Turkey is one of the countries with the richest biodiversity. The family Chrysomelidae is one of the most important and species-rich families of the order Coleoptera. The Chrysomelidae family is represented worldwide by more than 2,500 breeds and more than 38,000 species belonging to 19 sub-families (Seeno & Wilcox, 1982). Estimates suggest the number of species is thought to be more than 60,000 (Jolivet, 1988; Reid, 1995; Suzuki, 1996). Members of the Chrysomelidae family, which spreads worldwide outside the Arctic region, are represented by approximately 560 genera and 3500 species in the Palearctic region, and 91 genera and 930 species in Turkey (Löbl & Smetana, 2010; Ekiz et al., 2013; Özdikmen et al., 2014).

Clytrinae, one of the relatively large subfamily of leaf beetles (Chrysomelidae) in terms of species, is represented in our country by 8 genera and a total of 73 species. Knowing the areas of spread of the species provides important basic information for many areas of science (Ekiz, 2015).

Since 2014, after many faunistic and systematic studies, this number has increased to 78 species with 2 new records and 3 new species. Increasingly, we see that such studies have increased over the years in our country. Ali Gök, Didem Coral Sahin, Ebru Gül Aslan, Ebru Unal, Ergin Turantepe, Esat Pehlivan, Halil Bolu, Hüseyin Özdikmen, Jan Bezdek, Kadir Bostan, Lev Nikandrovich Medvedev, Neslihan Bal, Özgür Durmuş Kaya, Renato Regalin, Serdar Tezcan, Suat Mikhail

and Yusuf Karsavuran, Turkey Clytrinae fauna contributed to the researchers.

Özdikmen and Bal, with their study in 2016, added *Cheilotoma cankiriensis* Özdikmen & Bal, 2016 species Clytrinae to the fauna of Turkey. İn the same year, Özdikmen, Bal ve Kıyak added L. *atkaracalarica* Özdikmen, Bal & Kıyak, 2016 and *L. medvedevi* Warchalowski, 1985 Clytrinae to the fauna of Turkey. In 2021, Özdikmen, Bolu and bal made their studies and recorded the *labidostomis medvedevi* from Kayseri province. Thus, this is the 2nd time this species has been registered from Turkey. Özdikmen, Bal and Şahin added the *S. concolor concolor* (Fabricius, 1792) subspecies to the faunistic list as a new record for our country, with their work in 2020. According to Bezdek and Regalin's work in 2017, *L. leonardii* Bezdek & Regalin, 2017 have added a new species to the fauna of Turkey Clytrinae. The newly created list, including the new registrations added for the provinces as a result of these studies and the work of the researchers, is listed below.

1.SUBFAMILY CLYTRINAE Kirby, 1837

1.1.Genus *Cheilotoma* Chevrolat, 1836

1.1.1.Subgenus *Cheilotoma* Chevrolat, 1836

***C. beldei* Kasap, 1984;** Provinces in Turkey: **TR-A:** Ankara, Bolu, **Çankırı**, Eskişehir, Isparta, Nevşehir, Samsun, Sivas (Kasap 1984, 1987b; Gök 2003; Warchałowski 2003; Medvedev 2004; Özdikmen 2011; Özdikmen & Mercan, 2014; Özdikmen & Bal, 2016)

***C. cankiriensis* Özdikmen & Bal, 2016;** Provinces in Turkey: TR-A: **Çankırı** (Özdikmen & Bal, 2016)

***C. erythrostoma* Faldermann, 1837**

***C. e. erythrostoma* Faldermann, 1837;** Provinces in Turkey: **TR-A:** Ankara, Bolu, Erzurum, Kastamonu, Konya, Samsun (Regalin 2002a; Medvedev 2004; Özdikmen 2011; Özdikmen & Mercan, 2014)

***C. musciformis* (Goeze, 1777);**

***C. m. musciformis* (Goeze, 1777);** Provinces in Turkey: **TR-A:** Ankara, Konya (Gruev & Tomov 1979, 1984; Özdikmen 2011; Özdikmen & Mercan, 2014)

***C. voriseki* Medvedev & Kantner, 2003;** Provinces in Turkey: **TR-A:** Adıyaman (Medvedev & Kantner 2003; Medvedev 2004; Özdikmen & Mercan, 2014)

1.2. Genus *Clytra* Laicharting, 1781

1.2.1.Subgenus *Clytra* Laicharting, 1781

***C. aliena* Weise, 1897;** Provinces in Turkey: **TR-A:** Ankara, Kastamonu, Sivas (Weise 1897; Kasap 1987b; Warchałowski 2003; Özdikmen et al. 2010; Ekiz et al., 2013; pers. comm., 2013; Özdikmen & Mercan, 2014)

***C. laeviuscula* Ratzeburg, 1837;** Provinces in Turkey: **TR-A: ADA**, Afyon, Ankara, Ardahan, Çankırı, Denizli, Erzincan, Erzurum, Isparta, İzmir, Kahramanmaraş, Kayseri, Konya, Karabük, Kars,

Sakarya, Sivas (Tomov and Gruev 1975; Gruev & Tomov 1984; Kasap 1987b; Aydın & Kısmalı 1990; Aslan 1997; Aslan & Özbek 1998a; Campobasso et al. 1999; Gök 2003; Özdikmen 2011; Ekiz et al., 2013; pers. comm., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bal & Şahin, 2020)

***C. quadripunctata* (Linnaeus 1758)**

***C. q. quadripunctata* (Linnaeus 1758);** Provinces in Turkey: **TR-A:** Ankara, Bolu, Erzurum, Kütahya (Tomov & Gruev 1975; Gruev & Tomov 1984; Aslan & Özbek 1998a; Özdikmen 2011; Ekiz et al., 2013; pers. comm., 2013; Özdikmen & Mercan, 2014)

1.2.2.Subgenus *Clytraria* Semenov, 1903

***C. atraphaxidis* (Pallas, 1773)**

***C. a. atraphaxidis* (Pallas, 1773);** Provinces in Turkey: **TR-A:** Ankara, Amasya, Artvin, Aydın, **Çankırı**, Denizli, Erzincan, Eskişehir, Erzurum, Isparta, İzmir, Kahramanmaraş, Kastamonu, **Kayseri**, Konya, Kars, Manisa, Mersin, Nevşehir, Nığde, Tokat, **Şanlıurfa** – **TR-E:** Edirne (Gül-Zümreoglu 1972; Tomov & Gruev 1975; Gruev & Tomov 1984; Kasap 1987b; Aydın & Kısmalı 1990; Aslan & Özbek 1998a; Gök 2003; Gruev 2005a; Maican 2007; Sen & Gök 2009; Özdikmen et al. 2010; Özdikmen 2011; Ekiz et al., 2013; pers. comm., 2013; Özdikmen & Mercan, 2014; Bal, Özdikmen & Kıyak, 2018; Özdikmen & Şahin, 2021, Özdikmen, Bolu & Bal, 2021)

***C. novempunctata* Olivier, 1808;** Provinces in Turkey: **TR-A:** Adana, **Afyon**, Amasya, Ankara, Antalya, Artvin, Aydın, Bartın, Bolu, Burdur, **Çankırı**, Çorum, Denizli, Elazığ, Erzurum, Gaziantep, Gıresun, Isparta, İzmir, Hatay, Kahramanmaraş, Karaman, Kastamonu, Kayseri, Konya, Karabük, Manisa, Mardin, Mersin, Muğla, Nığde, Osmaniye, **Sakarya**, Siirt, Sinop, Sivas, Tokat, Tunceli, **Şanlıurfa**, Yozgat, Usak – **TR-E** (Gül-Zümreoglu 1972; Tomov & Gruev 1975; Gruev & Tomov 1984; Kasap 1987b; Aydın & Kısmalı 1990; Aslan & Özbek 1998a, 2009; Gök 2003; Gök & Çilbiroğlu 2003, 2005; Warchałowski 2003; Gruev 2004, 2005a; Sen & Gök 2009; Özdikmen et al. 2010; Özdikmen 2011; Ekiz et al., 2013; pers. comm., 2013; Özdikmen & Mercan, 2014; Bal, Özdikmen & Kıyak, 2018; Özdikmen, Bolu & Bal, 2021)

***C. valeriana* (Ménétriés, 1832)**

***C. v. valeriana* (Ménétriés, 1832);** Provinces in Turkey: **TR-A:** Aksaray, Ankara, Antalya, Aydın, **Çankırı**, Eskişehir, Erzincan, Erzurum, Gümüşhane, İzmir, Kayseri, Kırıkkale, Konya, Kars, Manisa, Mersin, Nevşehir, Nığde, Osmaniye, Sivas, Usak, Yozgat – **TR-E** (Tomov & Gruev 1975; Kasap 1987b; Aydın & Kısmalı 1990; Aslan & Özbek 1998a; Warchałowski 2003; Özdikmen et al. 2010; Özdikmen 2011; Ekiz et al., 2013; pers. comm., 2013; Özdikmen & Mercan, 2014; Bal, Özdikmen & Kıyak, 2018)

***C. v. taurica* Medvedev, 1961;** Provinces Spread in Turkey: **TR-A** (Özdikmen & Mercan, 2014).

1.2.3.Subgenus *Ovoclytra* Medvedev, 1961

***C. binominata* Monros, 1953;** Provinces in Turkey: **TR-A:** Adana, Denizli, Isparta, İzmir, Manisa, Mersin (Tomov & Gruev 1975; Aydın & Kısmalı 1990; Regalin 2002b; Warchałowski 2003; Sen & Gök 2009; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

***C. bodemeyeri* Weise, 1900**

***C. b. bodemeyeri* Weise, 1900**; Provinces in Turkey: **TR-A:** Ankara, Antalya, Bilecik, **Çankırı**, Erzurum, Gaziantep, Hatay, Isparta, **Kayseri**, Konya, Kırşehir, Mersin, Muğla, Nığde, Osmaniye (Weise 1900a; Tomov & Gruev 1975; Kasap 1987b; Aydın & Kısmalı 1990; Aslan & Özbek 1998a, 2009; Warchałowski 2003; Gök & Çilbiroğlu 2005; Sen & Gök 2009; Özdikmen et al. 2010; Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Mercan, 2014; **Bal, Özdikmen & Kıyak, 2018, Özdikmen & Şahin, 2021**).

***C. nigrocincta* (Lacordaire, 1848)**

***C. n. nigrocincta* (Lacordaire, 1848);** Provinces in Turkey: **TR-A:** İstanbul, İzmir, Mersin (Sahlberg 1913; Warchałowski 2003; Özdikmen et al. 2010; Regalin & Medvedev 2010; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

***C. ovata* (Lacordaire, 1848)**

***C. o. ovata* (Lacordaire, 1848);** Provinces in Turkey: **TR-A**. The provincial distribution of subspecies is unknown. However, it is likely that only S should be distributed in Turkey. (Medvedev & Kantner

2002; Warchałowski 2003; Regalin & Medvedev 2010; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

***C. o. borealis* Medvedev & Kantner, 2002;** Provinces in Turkey: TR-A Remarks: Provincial distribution of the subspecies is unknown. However, it must be distributed very likely only in S Turkey (Medvedev & Kantner 2002; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

***C. rotundata* Medvedev, 1961;** Provinces in Turkey: **TR-A** (Özdikmen et al., 2010) Remarks: Provincial distribution of the species is unknown. However, it must be distributed very likely only in S Turkey (Özdikmen & Mercan, 2014).

***C. weisei* Monros, 1953;** Provinces in Turkey: **TR-A:** Eskişehir, **Tunceli** (Warchałowski 2003; Regalin & Medvedev 2010; Ekiz et al., 2013; pers. comm., 2013; Özdikmen & Mercan, 2014; Aslan, Kaya & Ünal, 2020).

1.3.Genus *Coptocephala* Chevrolat, 1836

***C. destinoi* Fairmaire, 1884**; Provinces in Turkey: TR-A: Adana, Ankara, Antalya, Ardahan, Aydın, Balıkesir, Burdur, Canakkale, **Çankırı**, Erzincan, Eskişehir, Erzurum, Gümüşhane, Hatay, Isparta, İzmir, Kahramanmaraş, Kastamonu, Kayseri, Konya, Kars, Mersin, Muğla, Osmaniye, Sivas, **Şanlıurfa**, TR-E: Edirne (Fairmaire 1884; Tomov & Gruev 1975; Gruev & Tomov 1979; Warchałowski 2003; Maican 2007; Özdikmen 2011; Ekiz et al., 2013; pers. comm., 2013;

Özdikmen & Mercan, 2014; Bal, Özdikmen & Kıyak, 2018, Özdikmen, Bal & Şahin, 2020)

***C. fallaciosa* Fairmaire, 1884;** Provinces in Turkey: TR-A: Hatay (Fairmaire 1884; Regalin & Medvedev 2010; Ekiz et al., 2013; Özdikmen & Mercan, 2014)

***C. gebleri* (Gebler, 1841);** Provinces in Turkey: TR-A: Adana, **Afyon**, Ankara, **Aydın**, **Bursa**, Çanakkale, **Çankırı**, **Diyarbakır**, **Eskişehir**, Erzurum, **İstanbul**, **Kastamonu**, **Kocaeli**, Kars, Mersin, Rize, Tokat, **Şanlıurfa** (Tomov & Gruev 1975; Gruev & Tomov 1984; Kasap 1987a; Aslan & Özbek 1998a; Warchałowski 2003; Maican 2007; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Bal, Özdikmen & Kıyak, 2018; Özdikmen, Bal & Şahin, 2020)

***C. simillima* Lodewyckx, 1995** Provinces in Turkey: TR-A: Erzincan (Lodewyckx 1995; Ekiz et al., 2013; Özdikmen & Mercan, 2014)

***C. unifasciata* (Scopoli, 1763)**

***C. u. unifasciata* (Scopoli, 1763);** Provinces in Turkey: TR-A: Adana, Afyon, Amasya, Ankara, Antalya, Aydın, Balıkesir, Bartın, Çankırı, **Elazığ**, Erzincan, Erzurum, Hatay, Isparta, İzmir, Kahramanmaraş, Kastamonu, Kayseri, Konya, Karabük, Mersin, Muğla, Nevşehir, Niğde, Osmaniye, Sivas – TR-E: Çanakkale (Kasap 1987a; Aydın & Kısmalı 1990; Aslan & Özbek 1998a, 2009; Gök 2003; Gök & Çilbiroğlu 2003; Sen & Gök 2009; Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Aslan, Kaya & Ünal, 2020).

1.4.Genus *Labidostomis* Germar, 1822

1.4.1.Subgenus *Labidostomis* Germar, 1822

***L. atkaracalarica* Özdikmen, Bal & Kıyak, 2016;** Provinces in Turkey: **TR-A: Çankırı** (Özdikmen, Bal & Kıyak, 2016)

***L. asiatica* Faldermann, 1837;** Provinces Spread in Turkey: **TR-A:** Adana, Afyon, Amasya, Ankara, Aydın, Balıkesir, Bilecik, Bolu, **Çankırı**, Elazığ, Eskişehir, Erzurum, Düzce, Isparta, İzmir, Kastamonu, Kayseri, Konya, Karabük, Manisa, Mersin, Muğla, Nığde, Osmaniye, Sinop, Trabzon, Zonguldak (Sahlberg 1913; Tomov & Gruev 1975; Warchałowski 1985b, 2003; Kasap 1987a; Aydın & Kısmalı 1990; Aslan & Özbek 1998a; Gök 2003; Gök & Çilbiroğlu 2005; Sen & Gök 2009; Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bal & Kıyak, 2016)

***L. axillaris* Lacordaire, 1848;** Provinces in Turkey: **TR-A:** Erzurum – TR-E: Edirne, İstanbul (Aslan and Özbek 1998a; Regalin 2002b; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

***L. basanica* Sahlberg, 1913;** Provinces in Turkey: **TR-A: Çankırı**, Diyarbakır, Erzurum, **Gaziantep** (Regalin 2002a; Aslan and Warchałowski 2005a; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bal & Kıyak, 2016)

***L. beckeri* Weise, 1881;** Provinces in Turkey: **TR-A: Bartın**, **Düzce**, Erzurum, **Zonguldak** (Aslan & Özbek 1998a; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bal & Kıyak, 2016).

***L. brevipennis* Faldermann, 1837;** Provinces in Turkey: **TR-A:** Bingöl, **Çankırı**, **Hakkari, Elazığ**, **Erzincan**, **Kon**ya, Malatya, Şırnak (Warchałowski 1985b, 2003; Regalin 2002a; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bal & Kıyak, 2016; Aslan, Kaya & Ünal, 2020)

***L. cyanicornis* (Germar, 1822);** Provinces in Turkey: **TR-A:** Adana, **Afyon**, Düzce, **Elazığ**, **Kayseri**, Konya (Warchałowski 1985b; Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Aslan, Kaya & Ünal, 2020, Gök & Bostan, 2020, Özdikmen, Bolu & Bal, 2021).

***L. decipiens* Faldermann, 1837;** Provinces in Turkey: **TR-A:** Adana, Amasya, Ankara, Antalya, **Çankırı**, Gaziantep, Hatay, İzmir, Kahramamaraş, **Kayseri**, Konya, Malatya, Mersin, Nığde, Osmaniye, Şanlıurfa (Tomov & Gruev 1975; Gruev &Tomov 1979; Kasap 1987a; Aydın & Kısmalı 1990; Warchałowski 2003; Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

***L. diversifrons* Lefèvre, 1872;** Provinces in Turkey: **TR-A:** Karaman, Kayseri, Kilis, Konya, Nevşehir, Nığde (Aslan & Özbek 1998a; Warchałowski 2003; Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bal & Kıyak, 2016).

***L. elegans* Lefèvre, 1876;** Provinces in Turkey: **TR-A** Remarks: The provincial distribution of the species is unknown. However, at least E and SE should probably be distributed in Turkey. (Regalin & Medvedev 2010; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

***L. hebraea* Lacordaire, 1848;** Provinces in Turkey: **TR-A:** Hatay (Regalin 2002a; Warchałowski 2003; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

***L. humeralis* (Schneider, 1792);** Provinces in Turkey: **TR-A:** Çorum, İzmir, Bolu (Tomov & Gruev 1975; Gruev & Tomov 1984; Aydın & Kısmalı 1990; Warchałowski 2003; Gruev 2004; Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

***L. karamanica* Weise, 1900;** Provinces in Turkey: **TR-A:** Adana, **Ankara**, Antalya, Bilecik, **Çankırı**, **Kayseri**, Konya, Mersin, Isparta (Weise 1900a; Tomov and Gruev 1975; Warchałowski 1985b, 2003; Kasap 1987a; Gök 2003; Gök and Çilbiroğlu 2005; Sen and Gök 2009; Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bal & Kıyak, 2016).

***L. kaszabi* (Medvedev, 1962);** Provinces in Turkey: **TR-A: Afyon**, **Isparta**, Konya (Medvedev 1962; Gruev & Tomov 1979; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bal & Kıyak, 2016; Özdikmen, Bal & Şahin, 2020).

***L. korbi* Weise, 1902;** Provinces in Turkey: **TR-A:** Konya (Weise 1902; Warchałowski 1985b; Kasap 1987a; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

***L.leonardii* Bezdek & Regalin, 2017** Provinces in Turkey: **TR-A: Isparta** (Bezdek & Regalin, 2017)

***L. longimana* (Linnaeus, 1760);** Provinces in Turkey: **TR-A:** Ağrı, Ankara, Ardahan, Balıkesir, Bayburt, Bilecik, Bolu, **Çankırı**, Düzce,

Eskişehir, Erzurum, Gümüşhane, Isparta, Kahramanmaraş, Kastamonu, Kayseri, Kırıkkale, Konya, Kars, Nevşehir, Nığde, Osmaniye, Samsun, Siirt, Sivas, Yozgat, Zonguldak - **TR-E:** Edirne, Kırklareli, Tekirdağ (Tomov and Gruev 1975; Gruev & Tomov 1979, 1984; Kasap 1987a; Aydın & Kısmalı 1990; Aslan & Özbek 1998a; Gök 2003; Özgen & Tok 2009; Özdikmen 2011; Ekiz et al., 2013; pers. comm., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bal & Kıyak, 2016).

***L. lucida* (Germar, 1824);** Provinces in Turkey: **TR-A:** Antalya, **Elazığ**, Erzurum (Aslan and Özbek 1998a, 2009; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Aslan, Kaya & Ünal, 2020).

***L. maculipennis* Lefèvre, 1870;** Provinces in Turkey: **TR-A:** Amasya, Ankara, Antalya, **Çankırı**, **Elazığ**, Erzurum, İzmir, Kayseri, Kırıkkale, Konya, Nevşehir, Nığde, Sivas, Van, Yozgat (Lefevre 1870; Tomov & Gruev 1975; Warchałowski 1985b, 2003; Kasap 1987a; Aydın & Kısmalı 1990; Aslan & Özbek 1998a, 2009; Özdikmen 2011; Ekiz et al., 2013; pers. comm., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bal & Kıyak, 2016; Aslan, Kaya & Ünal, 2020).

***L. medvedevi* Warchalowski, 1985;** Provinces in Turkey: **Isparta**, **Kayseri** (Özdikmen, Bal & Kıyak, 2016, Özdikmen, Bolu & Bal, 2021)

***L. mesopotamica* Heyden, 1886;** Provinces in Turkey: **TR-A:** Aksaray, Ankara, Antalya, Bilecik, Bursa, **Çankırı**, Denizli, Erzincan, Eskişehir, Erzurum, Hatay, Isparta, İzmir, Kahramanmaraş,

Kastamonu, Kayseri, Kocaeli, Konya, Kırşehir, Malatya, Mersin, Muğla, Muş, Nevşhir, Niğde, Sivas, **Tunceli**, **Şanlıurfa**, Yozgat (Weise 1897, 1900a; Gruev & Tomov 1979; Warchałowski 1985b, 2003; Kasap 1987a; Aydın & Kısmalı 1990; Aslan & Özbek 1998a; Gök 2003; Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bal & Kıyak, 2016; Aslan, Kaya & Ünal, 2020).

***L. metallica* Lefèvre, 1872**

***L. m. metallica* Lefèvre, 1872;** Provinces in Turkey: **TR-A: Çankırı**, Iğdır, **Kayseri** (Warchałowski 1985b, 2003; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bal & Şahin, 2020, Özdikmen, Bolu & Bal, 2021).

***L. oertzeni* Weise, 1889;** Provinces in Turkey: **TR-A: Afyon,** Amasya, Ankara, Antalya, Çankırı, Eskişehir, Erzurum, Isparta, İstanbul, İzmir, Kahramanmaraş, **Kayseri**, Manisa, Mardin, Muğla, Nığde – **TR-E:** İstanbul, Kırklareli (Weise 1900b; Tomov & Gruev 1975; Warchałowski 1985b, 2003; Kasap 1987a; Aydın & Kısmalı 1990; Gök 2003; Gök & Çilbiroğlu 2003; Gruev 2005a; Aslan et al. 2009; Özdikmen 2011; Ekiz et al., 2013;Özdikmen & Mercan, 2014; Özdikmen, Bal & Kıyak, 2016; Gök & Bostan, 2020).

***L. pallidipennis* (Gebler, 1830);** Provinces in Turkey: **TR-A:** Ankara, Artvin, **Çankırı**, Denizli, Erzurum, İstanbul – **TR-E:** İstanbul (Kasap 1987a; Aslan 1997; Aslan & Özbek 1998a; Gruev 2005a; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bal & Kıyak, 2016).

***L. peregrina* Weise, 1900;** Provinces in Turkey: **TR-A:** Aksaray, **Burdur**, Erzincan, Erzurum, Isparta, **Kayseri**, Kırşehir, Mersin, Nevşehir (Warchałowski 1985b, 2003; Aslan & Özbek 1998a; Gök 2003; Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bal & Kıyak, 2016; Özdikmen, Bolu & Bal, 2021).

***L. propinqua* Faldermann, 1837;** Provinces in Turkey: **TR-A:** Adana, Aksaray, Amasya, Ankara, Antalya, Bolu, Çankırı, Erzincan, Erzurum, Gümüşhane, İstanbul, İzmir, Kahramanmaraş, Kastamonu, Kayseri, Kocaeli, Konya, Karabük, Mersin, Nevşehir, Nığde, Sakarya, Samsun, Sivas, Trabzon – **TR-E** (Medvedev 1970; Gül-Zümreoglu 1972; Tomov & Gruev 1975; Gruev & Tomov 1984; Warchałowski 1985b, 2003; Kasap 1987a; Aydın and Kısmalı 1990; Aslan & Özbek 1998a, 2009; Ulusoy et al. 1999; Gruev 2004; Gruev 2005a; Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

***L. rufa* (Waltl, 1838);** Provinces in Turkey: **TR-A:** Adana, Afyon, Amasya, Ankara, Antalya, Bilecik, Bolu, Bursa, **Çankırı**, Çorum, Denizli, Erzincan, Eskişehir, Erzurum, Gaziantep, Isparta, İstanbul, İzmir, **Kayseri**, Konya, Kütahya, Manisa, Nevşehir, Niğde, Osmaniye – **Tr-E:** İstanbul, Kırklareli (Sahlberg 1913; Medvedev 1970; Tomov and Gruev 1975; Gruev and Tomov 1979, 1984; Warchałowski 1985b, 2003; Kasap 1987a; Aslan and Özbek 1998a; Gök 2003; Gök and Çilbiroglu˘ 2003; Gruev 2005a; ¸Sen and Gök 2009; Özdikmen 2011; Ekiz et al., 2013; pers. comm., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bal & Kıyak, 2016; Özdikmen, Bolu & Bal, 2021).

***L. subfasciata* Weise, 1885;** Provinces in Turkey: **TR-A:** Hakkari, Van (Weise 1898; Warchałowski 1985b, 2003; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

***L. sulcicollis* Lacordaire, 1848;** Provinces in Turkey: **TR-A:** Ankara, **Çankırı**, İstanbul, **Kayseri**, Konya, Nevşehir, Yozgat – **TR-E:** İstanbul (Warchałowski 1985b, 2003; Kasap 1987a; Gruev 2005a; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bal & Kıyak, 2016).

***L. testaceipes* Pic, 1904;** Provinces in Turkey: **TR-A:** Diyarbakır, Gaziantep, Hatay (Warchałowski 1985b, 2003; Özdikmen & Mercan, 2014)

1.5. Genus *Lachnaia* Chevrolat, 1836

1.5.1.Subgenus *Lachnaia* Chevrolat, 1836

***L. sexpunctata* (Scopoli, 1763)**; Provinces in Turkey: **TR-A:** Adana, Amasya, Ankara, Antalya, Bilecik, Bursa, Burdur, Çorum, Isparta, **İzmir**, Kayseri, Konya, Kütahya, Mersin, Osmaniye, Sakarya, Yozgat – **TR-E** (Weise 1884a; Medvedev 1970; Tomov & Gruev 1975; Gruev & Tomov 1984; Kasap 1987a; Gök 2003; Gök & Çilbiroğlu 2003, 2005; Warchałowski 2003; Gök & Gürbüz 2004; Gruev 2004, 2005a; ¸Sen & Gök 2009; Özdikmen 2011;Ekiz et al., 2013; pers. comm., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bal & Şahin, 2020).

1.6.Genus *Macrolenes* Chevrolat, 1836

***M. dentipes* (Olivier, 1808);** Provinces in Turkey: **TR-A:** Aydın, Balıkesir, Bursa, Canakkale, Isparta, İzmir, Manisa, Muğla (Gruev &Tomov 1979; Aydın & Kısmalı 1990; Gruev 2005b; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

1.7. Genus *Smaragdina* Chevrolat, 1836

***S. affinis* (Illiger, 1794)**

***S. a. affinis* (Illiger, 1794);** Provinces in Turkey: **TR-A: Kayseri**, Samsun (Tomov & Gruev 1975; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bolu & Bal, 2021).

***S. amasina* (Pic, 1897);** Records in Turkey: **TR-A:** Amasya (Özdikmen & Mercan, 2014).

***S. aurita* (Linnaeus, 1767)**

***S. a. aurita* (Linnaeus, 1767);** Provinces in Turkey: **TR-A:** Bolu, İstanbul, Sinop – **TR-E** (Tomov & Gruev 1975; Gruev & Tomov 1984; Gruev 2005a; Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

***S. biornata* (Lefèvre, 1872)**

***S. b. biornata* (Lefèvre, 1872);** Records in Turkey: **TR-A:** Aksaray, Amasya, Ankara, Bolu, Çankırı, Çorum, Erzincan, Erzurum, Gümüşhane, Isparta, İzmir, Kahramanmaraş, Kastamonu, Kayseri, Konya, Kırşehir, Nevşehir, Osmaniye, Samsun, Sivas, Yozgat (Weise 1884a; Tomov & Gruev 1975; Kasap 1987b; Aydın & Kısmalı 1990;

Aslan & Özbek 1998a, 2009; Lopatin 2002; Gök 2003; Warchałowski 2003; Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

***S. b. angorensis* (Lopatin, 2002);** Provinces in Turkey: **TR-A:** Ankara, **Çankırı, Kayseri, Konya, Nİğde** (Lopatin 2002; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bal & Şahin, 2020; Özdikmen, Bolu & Bal, 2021).

***S. chloris* (Lacordaire, 1848)**

***S. c. chloris* (Lacordaire, 1848);** Provinces in Turkey: **TR-A:** Ankara, **Diyarbakır** (Gruev and Tomov 1979, 1984; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bolu & Bal, 2021).

***S. concolor* (Fabricius, 1792)**

***S. concolor concolor* (Fabricius, 1792);** Provinces in Turkey: **TR-A: Osmaniye** (Özdikmen, Bal & Şahin, 2020)

***S. djebellina* (Lefèvre, 1872);** Provinces in Turkey: **TR-A:** Hatay, **Hakkkari** (Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Medvedev, 2015).

***S. flavicollis* (Charpentier, 1825);** Provinces in Turkey: **TR-A:** Amasya, **Ankara**, Bıngöl, Çorum, Isparta, Mersin, Sivas, Yozgat (Sahlberg 1913; Tomov & Gruev 1975; Kasap 1987b; Gök 2003; Warchałowski 2003; Gruev 2004; Aslan & Warchałowski 2005a; Gök & Çilbiroğlu 2005; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bal & Şahin, 2020).

***S. graeca* (Kraatz, 1872);** Provinces in Turkey: **TR-A:** Amasya, Antalya, Bolu, Erzurum (Medvedev 1970; Aslan & Özbek 1998a; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

***S. hypocrita* (Lacordaire, 1848);** Provinces in Turkey: **TR-A: Afyon**, Ankara, Bolu, Bursa, Çankırı, Çorum, **Diyarbakır**, Düzce, Eskişehir, Gaziantep, Gümüşhane, Hatay, İstanbul, **Kayseri**, Kastamonu, Kütahya, Osmaniye, Samsun, Sinop, Tokat, Trabzon – **TR-E:** İstanbul (Medvedev 1970; Tomov & Gruev 1975; Gruev & Tomov 1979, 1984; Kasap 1987b; Warchałowski 2003; Gruev 2004, 2005a; Özdikmen & Aslan 2009; Özdikmen 2011; Ekiz et al., 2013; pers. comm., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bal & Şahin, 2020; Özdikmen, Bolu & Bal, 2021, Özdikmen, Bolu & Bal, 2021).

***S. judaica* (Lefèvre, 1872);** Provinces in Turkey: **TR-A:** Adana, Isparta, **Kayseri**, Mersin, Osmaniye (Sahlberg 1913; Medvedev 1970; Warchałowski 2003; Sen & Gök 2009; Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bolu & Bal, 2021).

***S. laeviceps* Abeille de Perrin, 1895;** Provinces in Turkey: **TR-A:** Hatay (Abeille de Perrin 1895; Özdikmen & Mercan, 2014).

***S. limbata* (Steven, 1806);** Provinces in Turkey: **TR-A:** Adana, Afyon, Amasya, Ankara, Antalya, Aydın, Balıkesir, Bilecik, Bursa, Bolu, Burdur, Çanakkale, Çankırı, Çorum, Denizle, Diyarbakır, Düzce, Erzincan, Eskişehir, Erzurum, Gaziantep, Hakkari, Hatay, Isparta, İstanbul, İzmir, Kahramanmaraş, Kastamonu, **Kayseri**, Konya, Karabük, Kütahya, Manisa, Mersin, Muğla, Niğde, Osmaniye, Sakarya, Samsun, Sinop, **Şanlıurfa**, Usak, Yozgat, Zonguldak – TR-

E: İstanbul, Kırklareli, Tekirdağ (Sahlberg 1913; Medvedev 1970; Tomov & Gruev 1975; Gruev & Tomov 1979, 1984; Kasap 1987b; Aydın & Kısmalı 1990; Aslan &Özbek 1998a; Gök 2003; Warchałowski 2003; Gök & Gürbüz 2004; Gruev 2004, 2005a; Gök & Çilbiroğlu 2005; Aslan et al. 2009; Sen & Gök 2009; Özdikmen 2011; Ekiz et al., 2013; pers. comm., 2013; Özdikmen & Mercan, 2014; Özdikmen, Bolu & Bal, 2021; Özdikmen, Bolu & Bal, 2021).

***S. persica* Pic, 1911;** Provinces in Turkey: **TR-A:** Kahramanmaraş (Medvedev 1975; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

***S. salicina* (Scopoli, 1763);** Provinces in Turkey: **TR-A:** Amasya, Ankara, Bolu, İstanbul, Karabük, Samsun, Sinop – **TR-E** (Medvedev 1970; Tomov & Gruev 1975; Gruev &Tomov 1984; Gruev 2005a; Özdikmen & Okutaner 2007; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

***S. scutellaris* (Lefèvre, 1872);** Provinces in Turkey: **TR-A:** Ankara, **Elazığ** (Medvedev 1970; Warchałowski 2003; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Aslan, Kaya & Ünal, 2020; Özdikmen, Bolu & Bal, 2021).

***S. tibialis* (Brullé, 1832);** Provinces in Turkey: TR-A: Amasya, Ankara, Balıkesir, **Bartın**, Bolu, Bursa, Çankırı, Çorum, Düzce, Isparta, İstanbul, İzmir, **Karaman**, Kastamonu, Konya, Karabük, Kütahya, Manisa, **Mersin**, Muğla, Sakarya, **Sinop, Sivas, Zonguldak** – TR-E: Edirne, İstanbul, Kırklareli (Medvedev 1970; Gruev & Tomov 1984; Kasap 1987b; Aydın & Kısmalı 1990; Gök 2003; Gök & Çilbiroğlu 2003, 2005; Warchałowski 2003; Gruev 2004, 2005a;

Sen & Gök 2009; Özdikmen 2011; Ekiz et al., 2013; pers. comm., 2013; Özdikmen & Mercan, 2014; Özdikmen et al., 2021).

***S. unipunctata* (Olivier, 1808);** Provinces in Turkey: TR-A: Şanlıurfa (Lopatin 2002; Warchałowski 2003; Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

***S. vaulogeri* (Pic, 1895);** Provinces in Turkey: TR-A: **Artvin**, **Düzce**, **Elazığ**, Gaziantep, Hatay, Isparta, Mersin, Osmaniye (Warchałowski 1993, 2003; Gök & Ayvaz 2000; Gök 2003; Gök & Çilbiroğlu 2003; Özdikmen 2011; Ekiz et al., 2013; Özdikmen & Mercan, 2014; Gök & Turantepe, 2019; Aslan, Kaya & Ünal, 2020; Özdikmen, Bal & Şahin, 2020).

***S. viridana* (Lacordaire, 1848)**

***S. v. viridana* (Lacordaire, 1848);** Provinces in Turkey: TR-A: Antalya, Bolu, Diyarbakır, Gaziantep, Hatay, Kahramanmaraş, Konya, Siirt (Medvedev 1970; Warchałowski 2003; Özdikmen 2011; Özdikmen & Mercan, 2014).

***S. xanthaspis* (Germar, 1824);** Provinces in Turkey: TR-A: Afyon, Amasya, Ankara, Antalya, Artvin, Balıkesir, Bartın, Bursa, Bilecik, Bolu, Çankırı, Çorum, Düzce, Erzurum, Isparta, Kahramanmaraş, Kastamonu, Kayseri, Konya, Karabük, Nığde, Ordu, Sakarya, Samsun, Sinop, Sivas, Trabzon, Yozgat, Zonguldak - TR-E: Edirne (Medvedev 1970; Tomov & Gruev 1975; Gruev & Tomov 1979, 1984; Kasap 1987b; Aydın & Kısmalı 1990; Aslan & Özbek 1998a; Gök 2003; Warchałowski 2003; Gruev 2004, 2005a; Aslan et al. 2009;

Özdikmen 2011; Ekiz et al., 2013; pers. comm., 2013; Özdikmen & Mercan, 2014).

1.8.Genus *Tituboea* Lacordaire, 1848

***T. arabica* (Olivier, 1808)** Provinces in Turkey: **TR-A:** Hatay, Kahramanmaraş (Özdikmen & Okutaner 2007; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

***T. macropus* (Illiger, 1800)** Provinces in Turkey: **TR-A:** Adana, Aksaray, Ankara, Antalya, Artvin, Aydın, Bolu, Canakkale, Çankırı, Çorum, **Diyarbakır**, **Elazığ**, Erzincan, Erzurum, Hatay, Isparta, İzmir, Kahramanmaraş, Kastamonu, Kayseri, Kilis, Konya, Karabük, Kars, Mersin, Muğla, Nevşehir, Niğde, Osmaniye, Sivas – **TR-E:** Edirne, İstanbul (Gruev and Tomov 1984; Kasap 1987a; Aydın & Kısmalı 1990; Aslan & Özbek 1998; Regalin 2002b; Gök 2003; Warchałowski 2003; Özdikmen & Aslan 2009; Özdikmen 2011; Ekiz et al., 2013; pers. comm., 2013; Özdikmen & Mercan, 2014; Aslan, Kaya & Ünal, 2020; Özdikmen, Bal & Şahin, 2020).

***T. sexmaculata* (Fabricius, 1781)** Provinces in Turkey: **TR-A:** Hatay (Pic 1897; Warchałowski 2003; Ekiz et al., 2013; Özdikmen & Mercan, 2014).

3. RESULTS

Both foreign and Turkish researchers contributed to the fauna of Turkey Clytrinae, and the total number of species belonging to 8 genera was increased to 78. The species that have been newly

registered for the provinces and the province in which they have been newly registered are given in the following table.

Table1: Species and New Record Province

Species	New Record Province
***Cheilotoma beldei* Kasap, 1984**	ÇANKIRI
***Clytra laeviuscula* Ratzeburg, 1837**	ADANA
***Clytra atraphaxidis atraphaxidis* (Pallas, 1773)**	ÇANKIRI, KAYSERİ, ŞANLIURFA
***Clytra novempunctata* Olivier, 1808**	AFYONON, ÇANKIRI, SAKARYA, ŞANLIURFA
***Clytra valeriana valeriana* (Ménétriés, 1832)**	ÇANKIRI
***Clytra bodemeyeri bodemeyeri* Weise, 1900**	ÇANKIRI, KAYSERİ
***Clytra weisei* Monros, 1953**	TUNCELİ
***Coptocephala destinoi* Fairmaire, 1884**	ÇANKIRI, ŞANLIURFA
***Coptocephala gebleri* (Gebler, 1841)**	AFYONON, AYDIN, BURSA, ÇANKIRI, DIYARBAKIR, ESKİŞEHİR, İSTANBUL, KASTAMONU, KOCAELİ, ŞANLIURFA
***Coptocephala unifasciata unifasciata* (Scopoli, 1763)**	ELAZIĞ
***Labidostomis asiatica* Faldermann, 1837**	ÇANKIRI
***Labidostomis basanica* Sahlberg, 1913**	ÇANKIRI, GAZİANTEP
***Labidostomis beckeri* Weise, 1881**	BARTIN, DUZCE, ERZURUM, ZONGULDAK
***Labidostomis brevipennis* Faldermann, 1837**	ÇANKIRI, HAKKARİ, ELAZIĞ, ERZİNCAN, KONYA
***Labidostomis cyanicornis* (Germar, 1822)**	AFYONON, ELAZIĞ, KAYSERİ
***Labidostomis decipiens* Faldermann, 1837**	ÇANKIRI, KAYSERİ
***Labidostomis diversifrons* Lefèvre, 1872**	ÇANKIRI
***Labidostomis karamanica* Weise, 1900**	ANKARA, ÇANKIRI, KAYSERİ
***Labidostomis kaszabi* (Medvedev, 1962)**	AFYONON, ISPARTA
***Labidostomis longimana* (Linnaeus, 1760)**	ÇANKIRI
***Labidostomis lucida* (Germar, 1824)**	ELAZIĞ
***Labidostomis maculipennis* Lefèvre, 1870**	ÇANKIRI ELAZIĞ
***Labidostomis medvedevi* Warchalowski, 1985**	ISPARTA, KAYSERİ
***Labidostomis mesopotamica* Heyden, 1886**	ÇANKIRI, ŞANLIURFA, TUNCELİ
***Labidostomis metallica metallica* Lefèvre, 1872**	ÇANKIRI, KAYSERİ
***Labidostomis oertzeni* Weise, 1889**	AFYONON, KAYSERİ
***Labidostomis pallidipennis* (Gebler, 1830)**	ÇANKIRI

***Labidostomis peregrina* Weise, 1900**	BURDUR, KAYSERİ
***Labidostomis rufa* (Waltl, 1838)**	ÇANKIRI, KAYSERİ
***Labidostomis sulcicollis* Lacordaire, 1848**	ÇANKIRI, KAYSERİ
***Lachnia sexpunctata* (Scopoli, 1763)**	İZMİR
***Smaragdina affinis affinis* (Illiger, 1794)**	KAYSERİ
***Smaragdina biornata angorensis* (Lopatin, 2002)**	ÇANKIRI, KAYSERİ, KONYA, NIGDE
***Smaragdina chloris chloris* (Lacordaire, 1848)**	DIYARBAKIR
***Smaragdina concolor concolor* (Fabricius, 1792)**	OSMANİYE
***Smaragdina djebellina* (Lefèvre, 1872)**	HAKKARİ
***Smaragdina flavicollis* (Charpentier, 1825)**	ANKARA
***Smaragdina hypocrita* (Lacordaire, 1848)**	AFYONON, DIYARBAKIR, KAYSERİ
***Smaragdina judaica* (Lefèvre, 1872)**	KAYSERİ
***Smaragdina limbata* (Steven, 1806)**	KAYSERİ, ŞANLIURFA
***Smaragdina scutellaris* (Lefèvre, 1872)**	ELAZIĞ
***Smaragdina tibialis* (Brullé, 1832)**	BARTIN, KARAMAN, MERSİN, SINOP, SIVAS, ZONGULDAK
***Smaragdina vaulogeri* (Pic, 1895)**	ARTVİN, DUZCE, ELAZIĞ
***Tituboea macropus* (Illiger, 1800)**	DIYARBAKIR, ELAZIĞ

Since 2014, after many faunistic and systematic studies, this number has increased to 78 species with 2 new records and 3 new species. Increasingly, we see that such studies have increased over the years in our country.

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CHAPTER 10

EPITRIX FOUDRAS, 1860 (COLEOPTERA: CHRYSOMELIDAE: ALTICINAE) IN TURKEY

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INTRODUCTION

Flee beetles of the genus *Epitrix* Foudras, 1860(Coleoptera: Chrysomelidae: Alticinae) is distributed in all over the world except Australia and Antarctica with nearly 180 species. Most of the species in the group are native to South and Central America (Döberl 2010). It includes 17 species in Palearctic Region. According to Özdikmen et al. (2014) and Özdikmen (2014), the genus is represented with only 7 species in Turkey. In addition, four species of Epitrix (E. cucumeris, E. subcrinita, E. papa and E. tuberis) are on the eppo A1 and A2 quarantine species lists and and fortunately, they have not been found in Turkey (URL 1) (Coral Sahin et al., 2015).

Epitrix species are similar in appearence which makes their idetification difficult. They are tiny as 1.5-2.0 mm long, generally dark, oval, convex and hairy species. The Known host plants are usually in the Solanacea family, including potatoes, tomatoes (*Solanum lycopersicum*), eggplant (*Solanum melongena*), land repulsion (*Solanum nigrum*), cut leafy thrust (*Solanum triflorum*) and prickly apple (*Datura stramonium*). (URL 2). Below the soil surface, larva develops, feding on the root system and adults of the group live above the surface feeding on leaves like most of the flea beetles (Seeno and Andrews, 1972).

Due to their economic value, the genus of *Epitrix* is an important group. Epitrix species in the Alticini tribe are generally known as potato flea beetles in the world and have been recorded to cause harm to plants belonging to the Solanaceae family in particular.

The aim of this paper is to summarize the knowledge about genus *Epitrix* species in Turkey which includes seven species, provide a key for identification and habitus figures and mention about the importance of the group.

1. Genus *Epitrix* Foudras, 1860

1.1. *E. abeillei* (Bauduer, 1874)

Records in Turkey: Adana

Chorotype: Centralasiatic-Mediterranean Remarks: The species has so far only been recorded in Turkey from the Mediterranean Region. (Özdikmen, 2014).

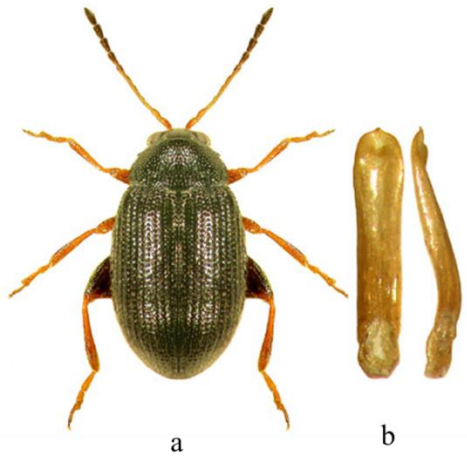

Figure 1: *E. abeillei* (Bauduer, 1874) a. Habitus b. Aedegus (URL 3).

1.2. *E. atropae* Foudras, 1860

Records in Turkey: Erzurum

Chorotype: Europeo-Mediterranean

Remarks: The species has so far only been recorded in Turkey from the Eastern Anatolia Region (Özdikmen, 2014).

Figure 2: *E. atropae* Foudras, 1860 a. Habitus b. Aedegus c. Spermatheca (URL 3;Warchalowski, 2010)

1.3. *E. caucasica* Heikertinger, 1950

Records in Turkey: Erzincan, Erzurum.

Chorotype: Turano-Mediterranean (Turano-Anatolian).

Remarks: Until now, the species has only been recorded in Turkey from the Eastern Anatolia Region. (Özdikmen, 2014).

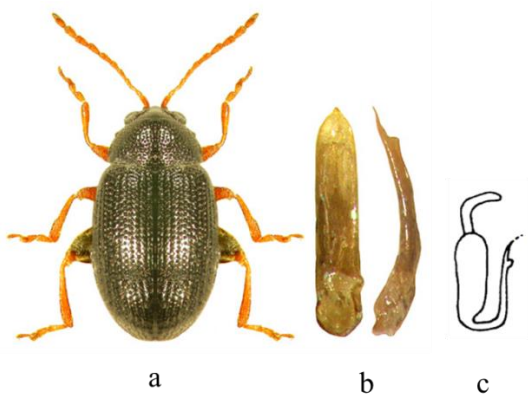

Figure 3: *E. caucasica* Heikertinger, 1950a. Habitus b. Aedegus c. Spermatheca (URL 3; Warchalowski, 2010).

1.4. *E. dieckmanni* Mohr, 1968

Records in Turkey: Isparta

Chorotype: SW-Asiatic

Remarks: The species has so far only been recorded in Turkey from the Mediterranean Region. (Özdikmen, 2014).

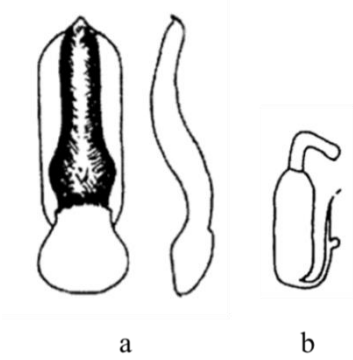

Figure 4: *E. dieckmanni* Mohr, 1968 a. Aedegus b. Spermatheca (Warchalowski, 2010)

1.5. *E. hirtipennis* (Melsheimer, 1847)

Records in Turkey: Ankara, İzmir, Mardin, Zonguldak (Ozdikmen et al. 2017)

Chorotype: Turano-Mediterranean (Turano-Apenninian) + Nearctic + Neotropical

Remarks: *E. hirtipennis* is known as a tobacco pest and It is also known as a pest of tobacco, potato, tomato and eggplant around World. This species feeds on pea, cabbage, pepper and radish aswell (Capinera, 2001). Originally it is a American species. It has been distributed from Canada to Mexico and in 1984, it entered to Europe from coasts of Italy. Turanlı & Kısmalı, 1996 (a,b) reported that tomato, cucumber, melon, Russian turnip and some weeds and ornamental plants are othet host plants. An epidemy has been occured in 1993 and it caused lost of serious products in tobacco production area (Turanlı & Kısmalı, 1996a,b; Coral Sahin et al., 2015).

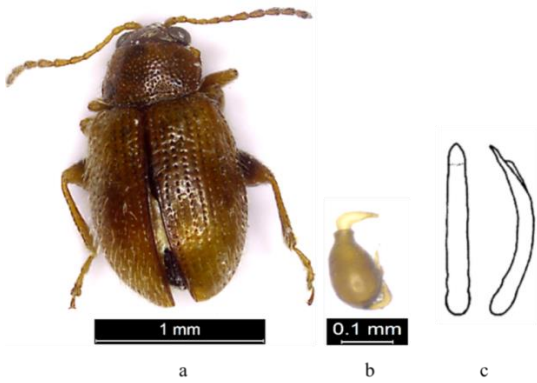

Figure 5: *E. hirtipennis* (Melsheimer, 1847) a. Aedegus b. Spermatheca (Warchalowski, 2010)

1.6. *E. intermedia* Foudras, 1860

Records in Turkey: Erzurum, Erzincan, Giresun, Samsun

Chorotype: S and E-European

Remarks: The species has been recorded in only 2 Turkish regions, the Black Sea Region and the Eastern Anatolia Region (Özdikmen, 2014).

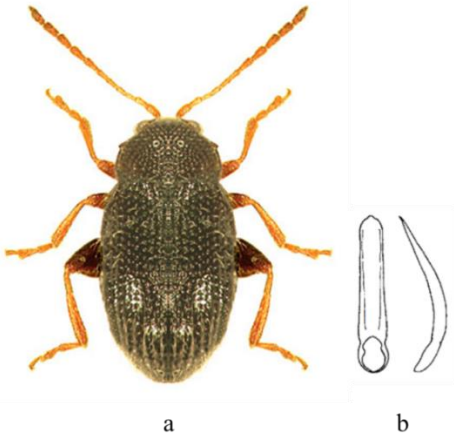

Figure 6: *E. intermedia* Foudras, 1860 a. Habitus b. Aedegus (URL 3; Warchalowski, 2010)

1.7. *E. pubescens* (Koch, 1803)

Records in Turkey: Balıkesir, Bilecik, Denizli, Düzce, Eskişehir, Erzurum, İstanbul, Kırıkkale Chorotype: Sibero-European.

Remarks: The species was recorded only from 4 Regions of Turkey. However, it has not been recorded from the Eastern Anatolia Region, the Mediterranean Region and the SoutheastErn Anatolia Region to date. It is known that this species is a pest of the Solanaceae family (Furth 1979; Aslan, 1997).

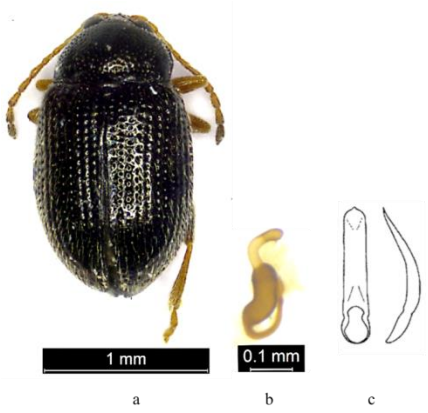

Figure 6: *E. pubescens* (Koch, 1803) a. Habitus b. Spermatheca c. Aedegus (Warchalowski, 2010)

Key to species in Turkey

1. Upper side black, often with metalic reflex……...……..**4**

-Upper side at least partly pale coloured, yellow or Brown…....**2**

2. Pronotum black. Upper side at least partly pale coloured, yellow or brown. Legs and antennae pale, hind femora slightly but distinctly darkened. Thickening of anterior angles of pronotum short, transverse impression very shallow, longitudinal impressions very short.Length 1.5-2.0 mm. Variations: elytra entirely black (ab. nigritula WEISE, 1886), elytra entirely pale with traces of darker pattern (unnamed), elytra black with pale humeral and apical spots (ah. quadrimaculata WEISE, 1886) (Figure 1)…..……...***atropae* Foudras, 1860**

-Pronotum yellowish or Brown……...…….**3**

Thickenings at anterior angles of pronotum long. Here an aberration of*pubescens* (thesis 6).

-Thickenings at anterior angles of pronotum short. Scutellar rows of points not reaching to midlength of elytra. Upper side pale, elytra with darkened suture and with a darker, blurred transverse stripe. Length 1.6-2.0 mm. American species, pest of tobacco cultures, introduced to Greece, Italy and Asia Minor (Figure 5)...……***hirtipennis*** **(MELSHEIMER, 1847).**

3. Thickenings at anterior angles of pronotum long (fig. 3958), at anterior seta usually without a protruding, tooth-like angle. Upper side normally pure black, antennae and legs pale, hind femora darkened. Length i.5-2.0 mm (= lencorana Pre, 1903, suturalis BEDEL, 1897). Variation: upper side reddish brown, with darkened suture and lateral sides of elytra (ab.ferruginea WEISE, 1886). Broadly distributed in Europe and Mediterranean area (except for African part) from Azores, the British Isles and Sweden to W Siberia (Figure 6). ……...***pubescens*** **(KocH, 1803).**

-Thickenings at anterior angles of pronotum short (fig. 3957)…….....….**4**

4. Scutellar row short. Pronotum strongly and densely punctate, its ground reticulated. Thickenings of anterior angles of pronotum with a little, sharp tooth. Length 1.7-2.1 mm. Distributed in European part of Mediterranean area, Balkans, Asia Minor and Caucasian countries (Figure 6)…..*****intermedia*** **Founras, 1860.**

-Scutellar row long……...……**6**

5. Aedeagus short and broad, in dorsal view at most 3 x longer than broad. Length usually under 1.4-1.8 mm, primary punctures of upper side distint and sharply impressed, in male first tarsomere of anterior legs weakly or at most moderately widened. Body black, antennae, tibiae and tarsi pale, apex of last antennomere and all femora black. Last abdominal sternite with a great, shallow impression. Aedeagus as in figs 3981, 3982, spermatheca as in fig. 3977. Length i.4-1.8 mm (= paliji GRUEV, 1975). Distributed in Near East, Saudi Arabia, Iran and Turkmenia(Figure 4).....***dieckmanni* MOHR, 1968.**

-Aedeagus slender, at least 4 x longer than broad……..…….**7**

6. Apex of aedeagus sharp. Here black forms of ***atropae*** (thesis 3).

-Not as above……..…….**8**

7. Aedeagus in dorsal view slightly narrowed in the middle of tubular part, on underside the median concavity, somewhat spoon-shaped, is present only in apical part and near base. Length 1.6-2.0 mm. Broadly distributed from Egypt and Turkey to Mongolia and N China (Figure 1).....***abeillei* (BAUDUER, 1874).**

-Aedeagus in dorsal view subparallel, on underside the median concavity runs on whole length. Frontal furrows situated arcuately. Aedeagus as in figs 3999, 4000, Length 1.3- 1.6 mm. Distributed from Turkey, S Russia and Transcaspia to W Himalaya (Figure 3)…..***caucasica* (HEIKERTINGER, 1950).**

2.RESULTS

Epitrix is an important group due to its impact on cultural production area all over the world. Some species of *Epitrix* are major pests of potato, tomato, eggplant, tobacco and other plants in North America, Europe and Turkey. In addition, there are 4 species of *Epitrix* which are plant quarantine species in A1 and A2 lists of EPPO. These species have not been reported yet in Turkey. Due to difficulty in distinguishing practice even by specialists as their external morphology is very similar (Seeno & Andrews, 1972) a key and figures given in this study for the identification of Turkish *Epitrix* species is necessary for plant quarantine and protection services to be in alert for the quarantine species in Turkey.

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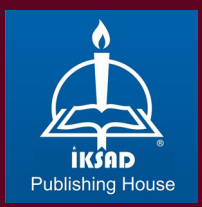

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