

SCIENTIFIC STUDIES IN THE FIELD OF SCIENCES

EDITED BY
Dr. Neslihan BAL

AUTHORS
Prof. Dr. İlknur DAĞ
Assoc. Prof. Betül YILMAZ ÖZTÜRK
Assist. Prof. Dr. Elif AKSÖZ
Lecturer Dr. Bükay YENİCE GÜRSU
Lecturer Dr. Derviş ÖZTÜRK
Dr. Neslihan BAL
Büşra ASLAN
Nurbanu GÜRSOY



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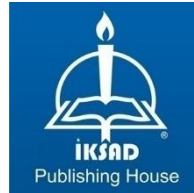
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TURKEY TR: +90 342 606 06 75
USA: +1 631 685 0 853
E mail: iksadyayinevi@gmail.com
www.iksadyayinevi.com

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PREFACE

Our aim in preparing this book for publication is to bring together academic studies in different fields of Science, to transfer the findings and results obtained from these studies to the world of science, to be a reference to future studies and to reveal new ideas. It is thought that the book, which consists of interdisciplinary fields, will contribute to the development and studies of students, academicians, researchers and experts. I would like to thank all our professors who contributed to our meticulously prepared book, and the valuable managers and all employees of the İKSAD publishing house family who provided the opportunity to convey this carefully collected information to our readers.

Dr. Neslihan BAL

CHAPTER 1

INVESTIGATION OF MORPHOLOGICAL AND ULTRASTRUCTURAL EFFECTS OF METFORMIN ON RAT KIDNEY TISSUES

Prof. Dr. İlknur DAĞ¹
Assoc. Prof. Dr. Betül YILMAZ ÖZTÜRK²
Lecturer Dr. Bükay YENİCE GÜRSU³
Büşra ASLAN⁴
Asts. Prof. Elif AKSÖZ⁵

¹Eskişehir Osmangazi University, Central Research Laboratory Application and Research Center (ARUM); Vocational Health Services High School, Eskişehir/Turkey. idag280@gmail.com/ ildag@ogu.edu.tr. Orcid no:0000-0002-7352-8653

²Eskişehir Osmangazi University, Central Research Laboratory Application and Research Center, Eskişehir/Turkey
byozturk@ogu.edu.tr. Orcid no 0000-0002-1817-8240

³Eskişehir Osmangazi University, Central Research Laboratory Application and Research Center, Eskişehir/Turkey
bukayyenice@gmail.com.Orcid no: 0000-0002-6822-3484

⁴Balıkesir University, Medical Faculty, Department of Pharmacology, Balıkesir/Turkey.Orcid no 0000-0002-4123-8408

⁵Balıkesir University, Medical Faculty, Department of Pharmacology, Balıkesir/Turkey. elifaksoz@hotmail.com.Orcid no 0000-0002-4827-804X

INTRODUCTION

Metformin, which is basically an insulin sensitizing drug, reduces hepatic glucose output from gluconeogenesis. It is a drug used both in the treatment of type 2 diabetes mellitus and metabolic syndrome since the 1960s. It increases insulin sensitivity, induces glycolysis and suppresses gluconeogenesis in the liver. Pleiotropically, it activates AMP-activated protein kinase (AMPK), reduces ATP synthesis and leads to an increase in cellular AMP: ATP ratio (Brunmair et al., 2004). In addition, mitochondrial respiratory chain complex 1 in various tissues inhibits depending on time and shows different effects on different tissues Lalau et al., 2015). In recent years, the effects of using metformin in various metabolic syndromes that shorten the life span, such as cardiovascular diseases, cancer or inflammatory disorders, have been extensively studied. It has been reported that metformin may have beneficial effects that reduce the risk of cancer or cardiovascular disease, and this is often attributed to calorie restriction mimetics (He et al., 2009; Martin-Montalvo et al., 2011; Martin-Montalvo et al., 2013). The most important side effect of metformin is lactic acidosis (LA) but this is a rare condition and it has a high mortality rate of 30-50% (Lalau et al., 1999). Therefore, metformin use is contraindicated in men with serum creatinine concentrations of 1.5 mg / dL or higher and in women with 1.4 mg / dL or higher (Hsu et al., 2018). It has been reported that ARF (acute renal failure) develops with LA in metformin poisoning (Rifkin et al., 2011). It is also believed that the use of metformin in patients with kidney failure may increase the risk of LA. In many case reports, the formation of metformin-related lactic acidosis

(MALA) is mentioned in ARF patients. The reason for MALA is thought to be the increased concentration of metformin due to acute intoxication or rapid impairment of kidney function. Protti et al. showed that in an experimental model, high dose metformin reduced oxygen consumption and increased lactic acid production by disrupting kidney mitochondrial function. This effect is commonly observed in all mitochondria throughout the body, including kidneys. If the lactic acid formed can be removed from the organism, there will be no problems. However, if the dose, concentration and accumulation increases, acute renal failure may be observed (Protti et al., 2012). In the retrospective study conducted by Cucchiari et al., in diabetic patients with ARF, plasma creatinine levels increased and kidney function decreased significantly as the dose of metformin in most patients with prerenal insufficiency increased. Researchers have suggested that this effect is independent of kidney function (Cucchiari et al., 2016). In another study, the efficacy of metformin and virgin olive oil on streptozotocin-induced diabetes in rats was investigated using biochemical and histopathological parameters. The serum levels of uric acid (URCA) and blood urea nitrogen (BUN) in the metformin-treated group were significantly higher than both the control and diabetes groups (Balamash et al., 2018). Serum BUN, CREA (creatinine) and URCA levels are indicators of nephrotoxicity in the diagnosis of kidney damage (Khan et al., 2004). At the same time, the relationship between continuous metformin treatment and deterioration in kidney function in patients with diabetes mellitus and moderate chronic kidney disease has been reported. All this suggests that metformin may

have dose-dependent nephrotoxic effects. However, studies on this topic have generally been done on the background of diabetes or kidney failure.

The aim of this study is to investigate whether the use of metformin in rats without diabetes or kidney impairment causes any ultrastructural changes in kidney tissues by light and transmission electron microscopic methods.

1. MATERIALS AND METHODS

Twenty Wistar Albino male rats were included in our study. During the experiments, the animals were housed in a 12-hour light, 12-hour dark cycle, and their feed and water intakes were released and the temperature was kept constant ($21 \pm 3^{\circ}\text{C}$). This study was carried out with the approval of Balıkesir University Animal Experiments Local Ethics Committee No 2020 / 3-9. Experimental animals were divided into two groups as control and metformin groups. Metformin (methyl) (METFULL 1000 mg effervescent tablets Vitalis Pharmaceutical, Turkey) were prepared by dissolving in 0.9% saline. The control groups were administered oral saline with gavage for 3 weeks, and the metformin group with the drug for three weeks with gavage oral 100 mg / kg / day. After three weeks of application, kidney samples were taken under ketamine / xylazine anesthesia and euthanasia was performed with cervical dislocation. Kidney samples taken after biopsy were reduced to 1 mm³ pieces quickly and with the help of a scalpel. Then, it was taken into the primary fixation solution containing 2.5% glutaraldehyde prepared in 0.1 M phosphate buffer.

After the samples were kept overnight at +4°C, they were washed 3 times with PBS buffer to remove excess fixative. While it binds strongly to carbohydrates and proteins in primary fixative tissue, its binding to lipid is weak. For this reason, samples should be taken to secondary fixation. For this purpose, samples were kept in room temperature and rotator for 2 hours and in the dark with 1% osmium tetroxide containing 0.1 M phosphate buffer. At the end of the period, the tissues were washed three times again with PBS buffer. In order to remove excess water in the tissue (dehydration process), the samples were passed through ethyl alcohol series at increasing concentrations and twice at 4°C for a certain period of time. (30%, 50%, 70%, 90%, 96%, 100%). Here, the first alcohol series were kept in the refrigerator, and the last stages were done at room temperature.

After dehydration, clarification steps were performed in propylene oxide for 30 minutes two times. After 2 hours rotator incubation with propylene oxide-araldite mixture, samples were taken in pure araldite and kept on rotator overnight. Then, the samples embedded in the prepared epoxy resin were polymerized at 60°C for 48 hours and tissue blocks were obtained.

1.1. Light Microscopy

Semi thin sections of the samples were taken with the help of an ultramicrotome (Leica Ultracut R) at a thickness of 700 nm. These sections were stained for 5 minutes with toluidine blue paint in a magnetic heater,

washed through distilled water series, dried by fixing on the slide and examined under a light microscope (Ozatik et al., 2016).

1.2. Transmission Electron Microscopy (TEM)

The data obtained from the semi-thin sections were evaluated and the important areas were determined for TEM. After trimming on the blocks, it was cut to 60 nm thickness with the help of ultramicrotome. The ultra thin sections were collected on 300 mesh copper grids and dried with uranyl acetate-lead citrate paint and then analyzed in TEM device (Hitachi HT 7800) (Kocman et al., 2020).

2. RESULTS

In this study, whether there is a change in rat kidney tissues as a result of metformin application, it was first examined at the light microscopic level; Afterwards, the ultrastructural findings in subcellular organelles such as nucleus, mitochondria, ER, vacuole and changes in tubule and basal lamina structure were evaluated with TEM.

2.1. Light Microscopic Findings

According to our light microscopic data, normal renal corpuscle structure was observed in semi-thin sections stained with toluidine blue.

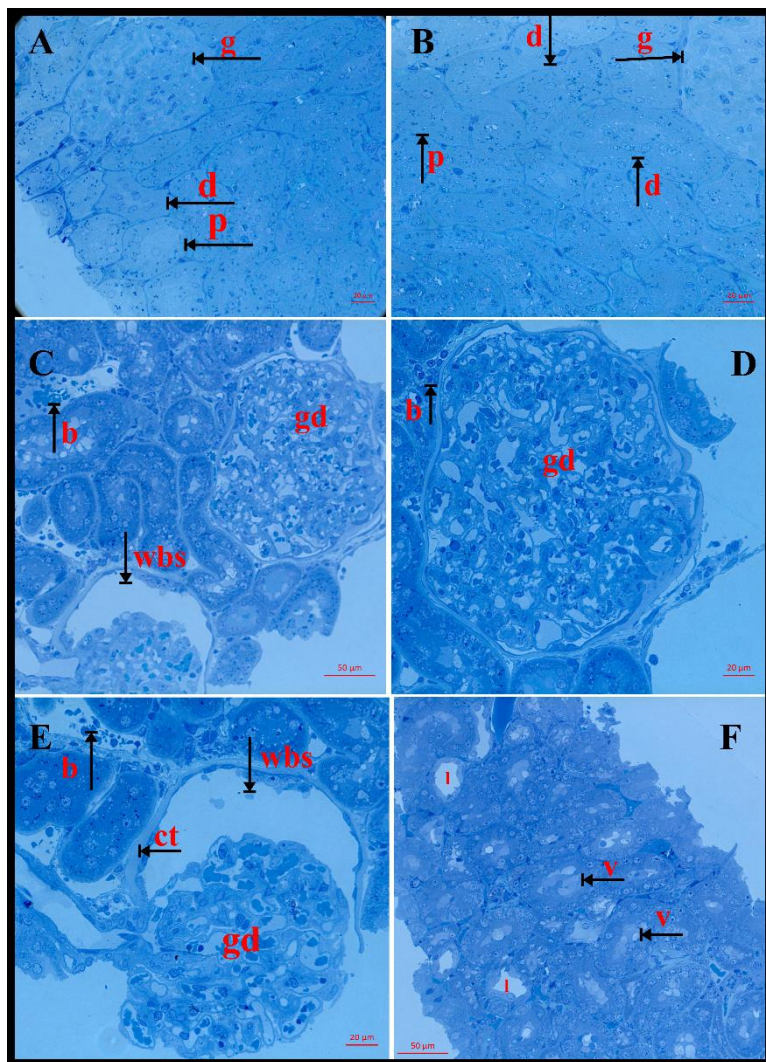


Figure 1: Light microscopic images of Toluidine blue stained sections in the rat kidney tissues. A and B: Control group showing regular glomerulus (g) with Bowman's capsule, intact and regular proximal (p) and distal (d) tubule structures. C, D, E and F: metformin group showing red blood cell (b), glomerular damage (gd), marked vacuol formations (v), widened Bowman's space (wbs), capsular thickening (ct) and lumen structures (l). (Scale bar is 20μm for A, B, D, E and 50 μm for C and F images).

The glomerular structure is dense and organized and is surrounded by a narrow Bowman's spaces. Proximal and distal tubule structures are in a regular organization and their diameters are in different sizes. In metformin-treated groups, the findings of hypertrophic appearance and separation from the renal corpuscle structure were observed in some glomeruli. Some tubules also have a degenerative appearance (Figure 1).

2.2. Transmission Electron Microscopic Findings

In order to investigate the ultrastructural effects of metformin on rat kidney tissues with TEM, at least 10 different areas were scanned for each preparation belonging to the control and experimental group during analysis, and the common findings obtained were determined and demonstrative images were selected. Accordingly, in the control group samples, the nucleus is regular and normal-appearance, large number of mitochondria, cristae structures are clearly visible and in regular form (Figure 2 A, B, C). In metformin applied samples, ruptures and melts in the nuclear membrane, openings resembling the appearance of the bracelet, and a heterochromatic appearance in some nucleus were detected. There are many vacuols and microvillus irregularities in some areas. Generally, indistinction was detected in mitochondria and crystal structures, and mitochondria are generally electron-dense appearance (Figure2 D,E,F,G,H).

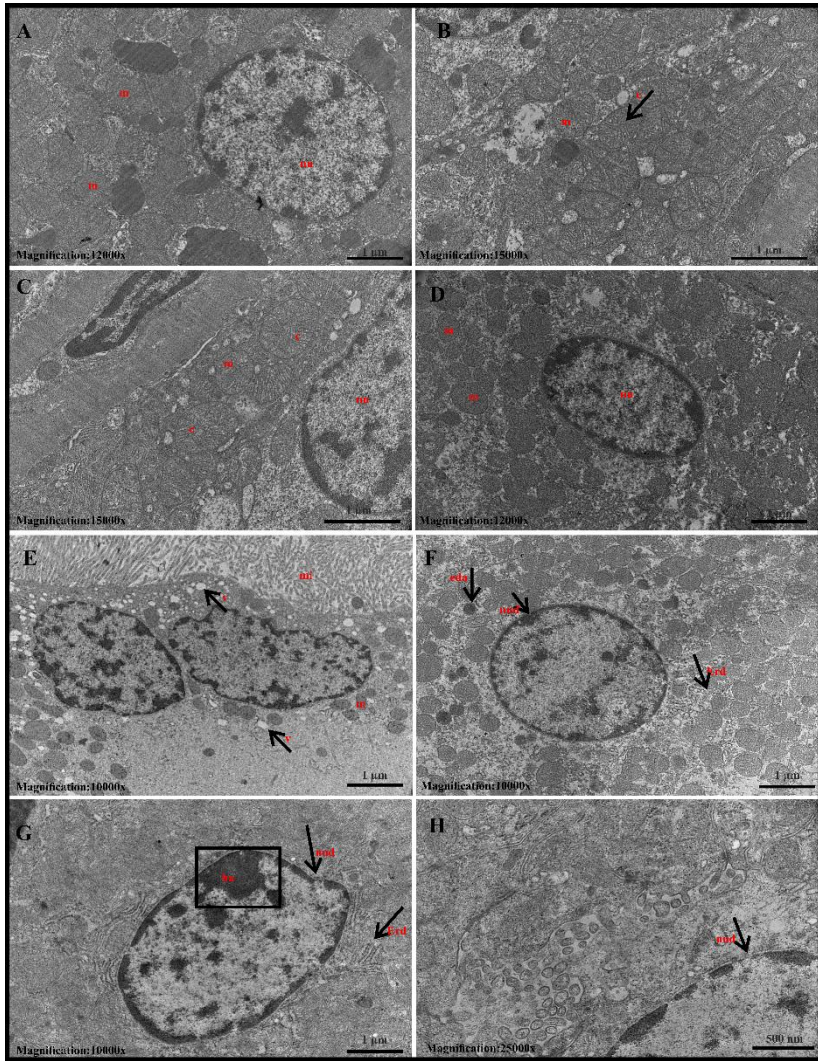


Figure 2: The transmission electron micrographs of rat kidney tissue in control and metformin groups. A, B, C: Control group renal tubule showing abundant mitochondria (m) in the spherical or elongated form, regular cristae (c) structure, round and euchromatic nucleus appearance (nu). D, E, F, G and H: Metformin treated groups showing microvillus irregularities (mi), vacuol formations (v), ER fractures (Erd), heterochromatic nucleus appearance (ha) and bracelet-like nuclear membrane structure (nud), electron-dense mitochondria appearance (eda) and cristae damages. (Scale bar is 1 μ m for A, B, C, D, E, F, and G images and 500 nm for H images).

Figure 3 A, B shows the integrity of the renal tubules and the regular structure of the basal lamina in the control group rat kidney tissue. The microtubule structures observed in renal tubular epithelial cells are abundant, thin and parallel. Mitochondria are in regular appearance.

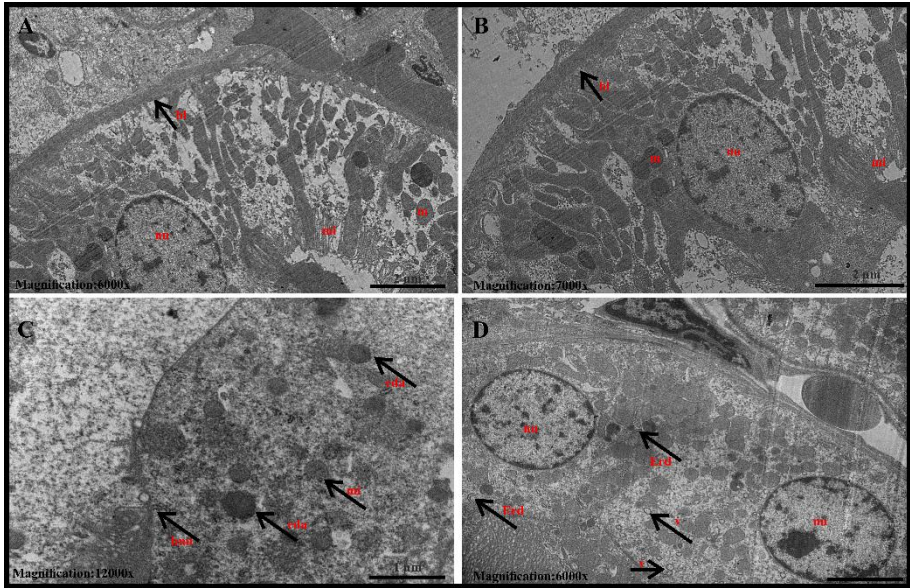


Figure 3: A and B: The control group renal tubules are intact, the basal lamina (bl) is ordered, the microvilli (mi) structures are regular and abundant, and the mitochondria (m) and nucleus (nu) structures are organized. C and D: There are some irregularities in the basal lamina of renal tubules (bmi) in metformin-treated groups, vacuol formations (v), ER fractures and swellings (Erd), melts in mitochondria (md), loss and some electron-dense appearance findings (eda) (Scale bar is 2 μm for A, B and D and 1 μm for C images).

In the metformin-treated group, the basal lamina structure in the renal tubules has an irregular appearance. Vacuol formations and endoplasmic reticulum fractures and swellings were observed in some regions. The most obvious damages were observed in mitochondria

and there are mitochondria losses, melting, shrinkage, and electron-dense appearance (Figure 3 C,D)

3. DISCUSSION

Metformin is a biguanide-grade oral drug, approved by the Food and Drug Administration (FDA). It decreases the level of glucose in the blood by reducing hepatic glucose production. It also improves insulin sensitivity of peripheral tissues by increasing peripheral glucose uptake and use. It is recommended as an initial pharmacological treatment against type 2 diabetes mellitus (DM) disease, since it is low cost, reliable and has a low risk of cardiovascular events (Hsu et al., 2018; Thomas and Bakris, 2013). Metformin also has benign pleiotropic effects on polycystic ovarian syndrome, cancer, heart and cardiovascular diseases, non-alcoholic fatty liver disease and early puberty. In recent years, studies have been conducted on the possible beneficial effects of metformin on kidney. It has been shown in various clinical trials that it may have beneficial effects on the kidney, especially in cases of acute kidney injury or chronic kidney disease. Metformin has protective effects on the development or progression of the disease in these kidney diseases according to the underlying etiological condition (Corremans et al., 2019). However, it has been reported that individuals with serum creatinine levels greater than 1.5 mg / dL may be associated with the risk of LA, but there is no clear evidence about this. It is reported in the literature that the ability of metformin to produce nephrotoxicity may possibly be related to the

glucose-lowering mechanism, and different mechanisms are proposed for this effect. Although it is reported that the use of metformin in appropriate dosage and manner does not cause any negative effects, the mechanisms underlying the high dose-induced nephrotoxicity should be well understood and dose adjustments should be made very carefully (Thomas and Bakris, 2013).

The mechanism of action of metformin is partially attributed to AMPK activation. This enzyme is essential for glucose and lipid metabolism, as well as at the cellular or whole organism level. AMPK activation is a process triggered by an increase in AMP / ATP ratio. It is reported in the literature that metformin partially inhibits the electron transport system complex 1 and can change the mitochondrial order. This situation can increase the AMP / ATP ratio by disrupting ATP production in mitochondria. As there is a state of energy depletion, glycolysis is induced to protect cellular metabolism. However, there is no evidence of metformin inducing the formation of reactive oxygen species or evidence of oxidative damage accumulation (Martin-Montalvo et al., 2013). On the other hand, contradictory results are obtained on the protective effects of metformin in non-diabetic cancer. The optimal dose, schedule, time and heterogeneity of disease-related genotypes are important factors in cancer treatment. Metformin is absorbed 1-3 hours after oral administration and 90% of it is metabolized by the kidneys. It is a relatively safe drug with a risk of lactic acidosis and mild toxicity due

to kidney function. The most common side effects include anorexia, nausea or diarrhea (Chen et al., 2020).

In this study, morphological and ultrastructural effects of metformin on kidney tissues were investigated at the light and electron microscopic levels. When the literature data are analyzed, it is seen that the effects of metformin in underlying conditions such as diabetes or kidney failure are investigated. In a study conducted by Zheng et al., The effects of metformin on renal medullar interstitial cell (RMIC) survival in mice with normal or Type 2 diabetes mellitus were investigated. Male mice were used in the study and metformin was administered for a week. The authors stated that there were signs of RMIC apoptosis in diabetic animals treated with metformin, but not in normal hydrated animals. In the study, it was stated that this was due to AMKP activation and it was stated that RMIC apoptosis was significantly induced in animals treated with an AMKP activator, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), to test this condition (Zheng et al., 2014). In our study, drug application was made and the findings obtained after 3 weeks of metformin application were evaluated. In our study, some differences were detected between the control and experimental groups at the ultrastructural level.

Kidney is an organ with very important functions such as preserving homeostasis in the body, regulating the extracellular environment, excretion of drugs and toxic metabolites (Ferguson et al., 2008). Kidney is also the essential target organ for exogenous toxicants.

Since the kidney is the main excretory route in the body, mitochondrial dysfunction may be observed in the filtration and secretion process for some drugs that may be mitochondrial toxins. This event also plays an important role in nephrotoxicity (Morales et al., 2010). The role of mitochondria in programmed cell death is associated with the release of apoptotic signal molecules. The production of reactive oxygen species (ROS) by mitochondria is also a contributing factor to the cell degradation process. It is mentioned in the literature that metformin has mitochondrial effects. Mitochondrial dysfunction, especially observed in various renal diseases, is of great importance for nephrotoxicity (Amini et al., 2012). In also our study, important mitochondrial damages were observed in metformin-treated groups. In addition, intense electron appearance in mitochondria, cristae damage, mitochondrial fracture and melting were observed.

Histology, immunohistology, electron microscopy, examination of various biomarkers or evaluation of metabolic responses are important in the examination of structural and functional changes in kidney tissue. In a study conducted by Chihanga et al., changes in nephron in ischemia reperfusion injury on mouse model were investigated. In addition to various analyzes, structural and cellular changes and nephron structures were analyzed by TEM. In the study, irregularities in the podocyte structures in the glomeruli, microvillus structures, the formation of microvesicular structures and mitochondrial damages were evaluated. Significant differences were observed compared to the control group (Chihanga et al., 2018). Similarly, Moreno et al.

performed ultrastructural analysis of kidney, liver and duodenum tissues of rats they applied with Ginkgo Biloba extract and investigated the effects of this plant on the biodistribution of radiopharmaceutical sodium pertechnetate. Differences in glomerular basal lamina, mitochondria, granular endoplasmic reticulum structures were evaluated in TEM examinations (Moreno et al., 2008). Kassab et al. investigated the effects of metformin and insulin on the development of 20-day fetal kidneys in streptozotocin-induced gestational diabetic albino rats. In the study, tissue samples were subjected to light, morphometric and electronmicroscopic examination. In light and microscopic evaluations, glomerular and tubule structures, Bowman's capsule structure, vacuol formations and hemorrhage findings were evaluated. With TEM analyses, glomerular capillaries, basement membrane structures, regularity in microvilli structures, mitochondria, lysosomes, pycnotic vesicles and nucleus structures were evaluated. The authors reported that metformin creates moderate protection, and the combination of metformin and insulin produces the best glycemic control and protects fetal kidneys (Kassab et al., 2019). In also our study, the differences in light microscopy and TEM level, glomerular structures, renal tubules, nucleus and organelle structures were investigated. Glomeruli and tubule degenerations were observed at the light microscopic level in the groups treated with metformin compared to the control group. In the TEM examinations, some irregularities in the basement membrane structure of the renal tubules and mitochondrial damage, vacuol formations, nuclear irregularities, ER fractures and swellings were detected in the

experimental group. Nephrotoxicity is a condition caused by the failure of the kidney to perform detoxification and excretion processes as a result of damage to kidney function of various exogenous or endogenous toxicants. This condition harms the kidneys and the body, which has a very important role in maintaining homeostasis in the body. In general, effects such as change in glomerular hemodynamics, tubular cell toxicity, inflammation or crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy are observed in drug induced nephrotoxicity. In fact, nephrotoxicity can be detected with a simple blood test and blood urea nitrogen, serum creatinine concentration, glomerular filtration rate and creatinine clearance measurements can be made with this test. However, these procedures can only be performed when the majority of kidney functions are damaged. For this reason, it is important to develop biomarkers that can detect kidney dysfunction at an early stage (Kim and Moon, 2012). Also, it is reported in the literature that various imaging techniques are used for diagnostic purposes related to nephrotoxicity (Perazella, 2018).

CONCLUSION

Our findings support that metformin has some degenerative effects on the rat kidney tissues at the ultrastructural level. This study includes a chronic application, but our findings do not show a long-term effect. In the literature, metformin is hydrophilic based and is located at physiological pH as a cationic species;

therefore, it is reported that the passage through the cell membrane through passive diffusion is very limited (Graham et al., 2011). Light microscopic and TEM findings alone are not sufficient for the defining of damages in the kidneys, but detailed studies are needed to elucidate the mechanisms leading to these ultrastructural changes. Metformin, which is a relatively safe drug in the literature, has been reported to have many protective effects, but various negative effects can be observed depending on variety factors. For this reason, the long-term effects of metformin need to be studied in detail.

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CHAPTER 2

GREEN SYNTHESIS OF MICROALGAE-BASED GOLD NANOPARTICLES WITH ANTIFUNGAL ACTIVITY AGAINST PATHOGENIC *CANDIDA* SPECIES¹

Assoc. Prof. Dr. Betül YILMAZ ÖZTÜRK¹
Nurbanu GÜRSOY²,
Prof. Dr. İlknur DAĞ³,

¹Eskişehir Osmangazi University, Central Research Laboratory Application and Research Center, Eskişehir/Turkey. byozturk@ogu.edu.tr.
Orcid no 0000-0002-1817-8240

²Eskişehir Osmangazi University, Institute of Science, Biotechnology and Biosafety Department, Eskişehir/Turkey. nurbanugurso@gmail.com.
<https://orcid.org/0000-0002-6694-2784>

³Eskişehir Osmangazi University, Central Research Laboratory Application and Research Center (ARUM); Vocational Health Services High School, Eskişehir/Turkey. idag280@gmail.com/ ildag@ogu.edu.tr.
Orcid no:0000-0002-7352-8653

INTRODUCTION

During the recent years, metal nanoparticle synthesis has been carried out with eco-friendly and green chemistry-based techniques and natural materials such as plants, fungi, bacteria, polysaccharides, biodegradable polymers or algae have been used for this purpose. Thus, nanoparticle production is possible using a simple, economical and clean technology (Ishak et al., 2019). In order for a reaction or material to be 'green', it must be safe, contain a single reaction step, not produce waste, use renewable raw materials, be environmentally acceptable, the product must be easily separated from the reaction medium and it should efficiency level 100%. However in many reactions, it is difficult to fulfill all of these conditions.

Algae are used as a biofabricate for the synthesis of metal nanoparticles. Their abundant and easy availability makes them an important source for metal nanoparticle synthesis. In addition, algal nanoparticle synthesis takes place in a shorter time than other biosynthesis methods. Algae also have significant advantages in nanoparticle synthesis due to their high metal uptake capacities, low costs and structures (Yılmaz-Öztürk & Dağlıoğlu, 2018; Dağlıoğlu & Öztürk, 2018; Dağlıoğlu & Ozturk, 2016). Microalgae in this group are prokaryotic or simple eukaryotic photosynthetic microorganisms and are widely found in aquatic and terrestrial ecosystems. They also need a minimum amount of sunlight, atmospheric CO₂, and very little mineral salt to rapidly increase their microalgae biomass. Considering the extraordinary diversity and chemical richness of algae, more

research is needed to obtain a detailed platform on their potential in nanoparticle production (Jena et al., 2014; Dağlıoğlu & Oztürk, 2019).

Green microalgae *Scenedesmus incrassatulus* belongs to the Scenedesmaceae family, and its main morphological characteristics are found in cells arranged in one or two rows, with a straight or slightly curved plate of coenobium. It is characteristic of some species having horn-like protrusions. Recently, studies on bioenergy production have been developed with the species belonging to this family. This is due to the lipids contained in this microalgae. Thus, they are reported to be alternative to fossil fuels (Wu et al, 2013). However, there are very limited studies on the nanoparticle biosynthesis capabilities of *Scenedesmus* species in the literature (Jena et al., 2014; Öztürk, 2019).

Gold is a precious, inert and less toxic metal and is used in the treatment of some diseases. They have a strong potential for use in nanobiotechnology, especially because of providing appropriate surface bioconjugation with biomolecular probes and their remarkable plasmon resonance optical properties (Rajeshkumar et al., 2013). AuNPs are also used in many commercial and industrial applications owing to their small size and unique properties (Li et al., 2011).

Nowadays, the rapid increase in the incidence of fungal infections, the limited number of current antifungal drugs, toxic effects of antifungal drugs on eukaryotic human cells, antimicrobial resistance threat and even increase of multi-drug resistant strains causes very important

health problems. Thus, there is an urgent need for new treatment choices. Among all fungal infections, *Candida* species are the most common fungal microorganisms that affect human health. For example, the vast majority of nosocomial *Candida* infections show high mortality rates (Gutiérrez et al., 2018). In many studies to date, strong fungicidal activities of various algae species on fungal pathogens have been reported (Mickymaray & Alturaiki, 2018; Pesando & Caram, 1984; Abedin & Taha, 2008). However, studies on the antifungal activities of algae-based metal nanoparticles are quite new and detailed research is needed in this regard.

In this study, it is aimed to provide the synthesis of AuNPs in a pure and stable way by reducing the aqueous gold ions (Au^{+3}) using *S. incrassatulus* algae. After detailed characterization tests, the antifungal activities of the *S. incrassatulus* mediated gold nanoparticles (S-AuNPs) on three different *Candida* isolates were investigated.

1. MATERIALS AND METHODS

1.1. Microorganisms

In the study, three *Candida* isolates (*C. albicans* ATCC 14053, *C. tropicalis* 1660 and *C. glabrata* 1744) were used and these isolates were obtained from Eskisehir Osmangazi University Faculty of Medicine, Department of Microbiology. For identifications studies; germ tube test, microscopic morphology examination in Cornmeal Tween 80 agar, carbohydrate fermentation tests and API 20C

(bioMerieux, Marcy l'Etoile-France) commercial assimilation test were used. Isolates stored in Yeast peptone Dextrose (YPD) containing 20% glycerol and during the study they were inoculated into RPMI 1640 medium and incubated at 37 ° C for 24 hours (CLSI M27-A2)

1.2. Algae Production and Preparation for Study

Various stone, plant and mud samples were taken from Musaözü Pond (39 ° 41 ' 51 " North 30 ° 19 ' 25 " east) located on Eskişehir-Kütahya road and 21 km away from the center. These samples were placed in glass bottles filled with lake water. They were developed on the BG-11 medium for one week with the streaking method. After 1 week, samples were taken into BG-11 broth. Samples kept at 25 ° C and 3000 lux white fluorescent light were kept until they reached the logarithmic phase within 15-20 day. Samples in mixed culture were diluted with sterile water. With the dilution method repeated several times, it was aimed to obtain the samples in pure culture as a single cell. After this procedure, the cells were isolated separately under the reverse microscope with the help of a pasteur pipette. Later, BG-11 was developed on the medium and diagnosed according to its morphological features.

1.3. Obtaining the Algal Extract

Scenedesmus incrassatulus isolates were centrifuged at 4500 rpm for 10 minutes and then washed with ultrapure water. It was then heated to 80 ° C for 20 minutes and filtered with Whatman No 1. They were stored until use in the refrigerator.

1.4. Synthesis of S-AuNPs

After the collection, development and pure culture of the algae samples, the samples were kept under 3000 lux fluorescent lamp (26 ± 2 ° C, 100 rpm, 12:12 hour bright-dark environment) with the help of a thermostatic controlled shaking incubator. After the cells reaching the logarithmic phase were collected and washed twice with distilled water, they were kept at 80 ° C for 40 minutes to release the water-soluble biomolecules; centrifugation was performed for 10 minutes at 20 ° C and 4500 rpm. The synthesis method was made by modifying the method of Swain et al. (Swain et al., 2016). 1 ml of *S.incrassatulus* extract was mixed with 2.5 ml of 1 mM HAuCl₄ at a magnetic heater. The color change observed from light green to pink-purple during the reaction indicates that *S.incrassatulus* mediated gold nanoparticles (S-AuNPs) occur.

1.5. Optimization Studies

1 ml *S.incrassatulus* extract and 2.5 ml HAuCl₄ are prepared at 80 °C, optimum pH value and molarities of 0.5 mM, 1 mM and 5 mM,

respectively and they were optimized according to the UV-vis measurement results.

The pH of the solution prepared with 1 ml *S.incrassatulus* isolate and 2.5 ml (1 mM) HAuCl₄ was adjusted to pH 4, 5, 6, 7, 8, 9 with the help of HCl / KOH (80 ° C), respectively. pH optimization was performed according to UV vis measurement results.

After preparing 1 ml of *S.incrassatulus* extract and 2.5 ml of HAuCl₄ (1 mM) solution at optimum pH and 80 ° C, at 1., 3., 5., 10., 15., 30., 45., 60. minutes and 24th hour, measurements were taken and time optimization was performed.

1.6. Characterization of AuNPs

1.6.1. UV-Vis Spectrophotometer Analysis

UV-Vis measurement processes were performed to determine the spectrum of optimization processes (AE-S90-2D Spectrophotometer, China). The measurements taken at the maximum absorbance wavelength were evaluated to determine the spectrum of the optimization processes. These scans were taken at a wavelength of 190-1100 nm.

1.6.2. Determination of Particle Size and Zeta Potential Values

Spectral analysis was performed to determine the optical properties of nanoparticles. To determine the absorbance spectrum, a spectrophotometer and 10 mm path length quartz cuvette were used. A zetapotentiometer device was used for the particle size, zeta potential, and electrical conductivity measurements of S-AuNPs. The values obtained from three consecutive measurements were recorded (25 ° C and the light scattering angle 90 °)

1.6.3. Electron Microscopy (FE-SEM, TEM)

FE-SEM (Field Emission scanning electron microscope) analysis was used to determine the surface characteristics of S-AuNPs. For this purpose, the samples were dried on Whatman paper (No 1) paper and then fixed on aluminum stubs. Analyzes were performed on the FE-SEM (Hitachi Regulus 8230) device and elemental analysis was determined with the Energy Distribution X-Ray Spectroscopy (EDS) detector. In order to determine the morphological characteristics of nanoparticles with TEM (Transmission electron microscope), samples were taken on copper grids and then analyzed in TEM device (Hitachi HT 7800).

1.6.4. Fourier-Transform Infrared Spectroscopy (FTIR)

For FTIR analysis, samples were washed three times with distilled water and organic components not bound to the nanoparticle surface were removed. After lyophilisation and solidification of the samples, Potassium Bromide (KBr) was heated at 100 °C for 1 hour. The powdered NPs were placed in the press machine after the ambient moisture was removed. Thus, a thin disc at 3000 bar pressure was obtained. This disk (Fourier-transform infrared spectroscopy) was examined on the FTIR device (PerkinElmer Spectrum Two; ranging 400-4000 cm^{-1}). The surface chemistry of the reduced nanoparticles and biofunctional parts in the extract structure were determined.

1.6.5. X Ray Diffraction Assay (XRD)

With XRD (Panalytical Empyrean X-Ray diffractometer), the crystal structure of nanoparticles was determined and powder diffraction pattern analysis was performed (the Cu K target tube (λ : 1.54 Å); 2 θ angle scanning; 45 kV voltage and 40 mA).

1.6.6. Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Thermo iCAP RQ ICP-MS device was used to determine the quantification of the nanoparticles. S-AuNPs ionized in the device are separated and mass / load (m / z) ratios are determined. Before the analysis, the sample was dissolved in the microwave to remove the

organic content and then burned with HNO_3 and H_2O_2 . After dilution, a calibration curve was created with the help of the stock standard solution (Redox-423A). Quantification was made by considering dilution factor and standard curve. These data were decisive for antifungal activity studies and determined the initial concentration.

1.7. Antifungal Activity Studies

1.7.1. Agar Diffusion Method

The agar diffusion method was used to determine the antifungal activities of the S-AuNPs on *Candida* isolates. Turbidity of activated cells was adjusted to 0.5 McFarland (1.5×10^8 CFU per ml) and they were inoculated into Yeast peptone dextrose (YPD) medium. The nanoparticle solution was absorbed into sterile discs at 10 μl each. The zone diameters formed around the discs were measured after 24 hours incubation at 37 ° C. The study was repeated 3 times (Jorgensen et al., 2015).

1.7.2. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) Determination

In our study, the antifungal effects of S-AuNPs were performed in accordance with the criteria determined by the Clinical and Laboratory Standard Institute (CLSI, M27-A2). Isolates buffered with 0.1654 M 3- (N-morpholin) -propane-sulfonic acid MOPS, with l-glutamine and 0.2% (w / v) d-glucose without sodium bicarbonate

(Sigma-Aldrich Co., St. Louis, MO (USA, RPMI 1640). For this purpose, fungal cultures were used in SDA medium at 37 ° C for 24 h. The initial concentration of the isolates was adjusted to $1-5 \times 10^6$ CFU / mL. Optical density was determined as 0.5 McFarland. Lastly, cells were suspended in RPMI 1640 broth to obtain final concentrations as 5×10^3 CFU/mL. The inhibitory effects of S-AuNPs at different concentrations against *C. albicans*, *C. glabrata* and *C. tropicalis* isolates in 96-well microplates were evaluated after 48 hours incubation at 35 ° C. Growth and sterility controls were included to study. The lowest nanoparticle concentration that inhibits yeast growth compared to the control group was defined as the MIC value. (Gómez-Sequeda et al., 2017)

In determining the MFC value, 50 µl was taken from clean wells below the MIC value and inoculated into YPD plates. The lowest concentration without yeast growth on the medium after 48 hours incubation at 37 ° C was determined as MFC.

1.7.3.Evaluation of the Ultrastructural Changes of AuNPs on *Candida* isolates

After the non treated control and S-AuNPs applied cells were adjusted to 10^5 CFU / ml, they were taken in 2.5% glutaraldehyde buffered with PBS for primary fixation. After waiting for 24 hours at +4 ° C, then they were washed three times with PBS by centrifugation (5000 rpm, 5 min). Afterwards, the samples were taken for secondary fixation in 1% Osmium tetroxide in the dark and with rotator for 2

hours. After washing with PBS, they were dehydrated by increasingly alcohol series. After polymerization with propylene oxide and embedding in araldite, they were polymerized at 60 ° C for 48 hours. Ultra thin sections (60 nm) taken with an ultramicrotome (Leica Ultracut R) from the blocks obtained were placed on copper grids; Later, they were stained with uranyl acetate-lead citrate, dried and examined in TEM device (Hitachi HT 7800) (Öztürk et al., 2020; Ayrim et al., 2017).

2. RESULTS

The reduction of gold ions to gold nanoparticles is determined by a visually traceable color change. Usually there is a conversion from yellow to dark pink, but the duration of the reaction or the phytochemicals in the extract can affect this color. Color change takes place by stimulating surface plasmon vibrations with gold nanoparticles (Rajeshkumar et al., 2013). Thus, in our study, the synthesis was monitored by both color change and UV-vis spectroscopy.

2.1. Optimization of S-AuNPs

In order to determine the effect of HAuCl_4 concentration in the formation of AuNPs, the reactions were established with molarity ratios of 0.5 mM, 1 mM and 5 mM, respectively. As a result of UV-

vis measurements, it is seen that 1 mM HAuCl_4 concentration gives the best result (Figure 1 A).

In order to determine the effect of the initial pH on the formation of AuNPs, the reactions were adjusted to pH 4, 5, 6, 7, 8, 9 and UV-Vis measurements were taken, respectively. The optimum pH value was determined as 5 (Figure 1 B).

To determine the effect of reaction time on AuNPS formation, UV-vis measurements were taken at the 1st, 3rd, 5th, 10th, 15th, 30th, 45th, 60th, 75th, 90th and 24th hours, respectively. When the UV-vis measurements were evaluated, the wavelengths were recorded as 553 nm for the 30th, 45th and 60th minutes; 552 nm for 75 min; 551 nm for 90 minutes and 549 nm for 24 hours. According to the results, it was found that the optimal value was obtained at 90 minutes (Figure 1 B).

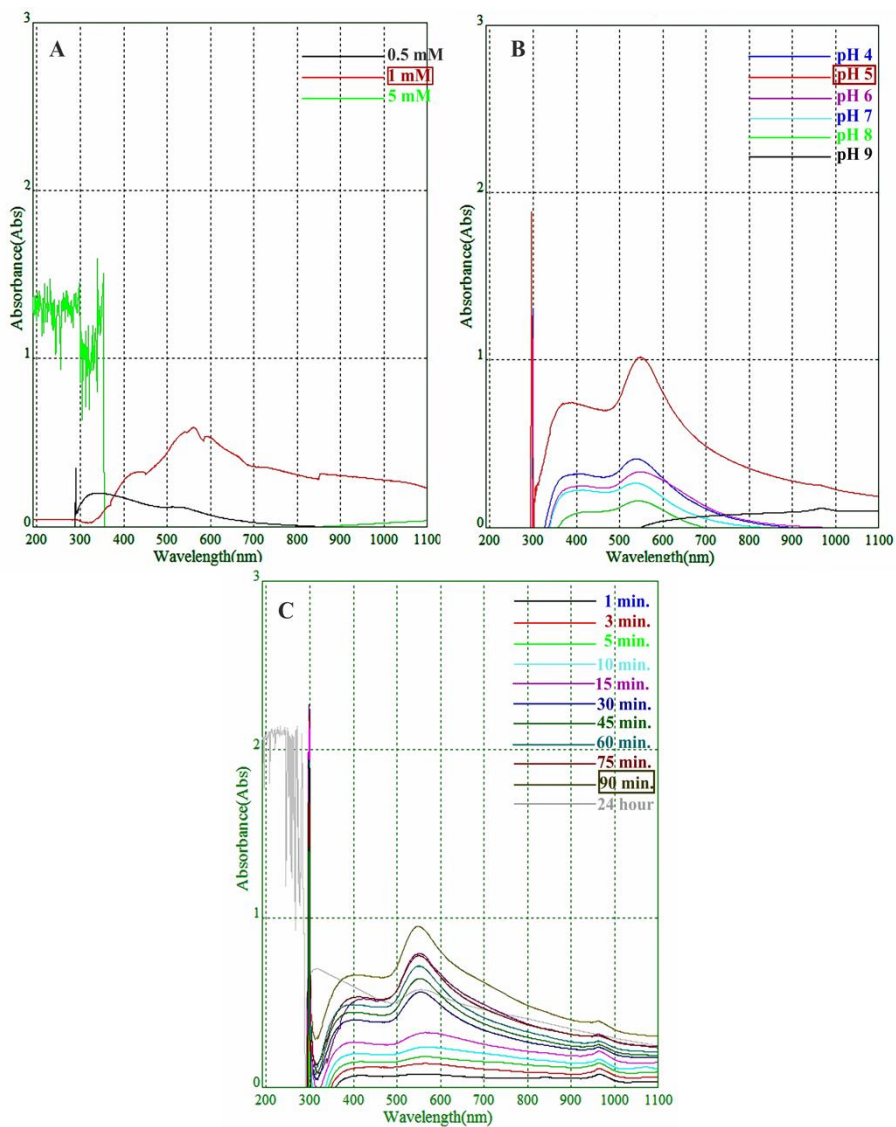


Figure 1: UV–Visible Spectrum Of Biosynthesized S-Aunps Saved For Optimization Of (A) Salt Concentration, (B) Ph, (C) Time Parameters

2.4. Characterization of AuNPs

2.4.1.Characterization of AuNPs with Zeta Potential and DLS

DLS analysis was performed to obtain information about the average particle size, distribution and polydispersity index (PDI) of gold nanoparticles. According to our data, DPI for S-AuNPs was determined as 0.46 (Figure 2 A). The zeta potential is used to determine both the stability and the total load of the gold nanoparticles, and is an important tool for predicting the long-term stability of the nanoparticles and also used to determine the surface charge in the solution (Swain et al., 2016). After three repeated analysis, the measurement of S-AuNPs was determined as -16.7 mV (Figure 2 B) in Malvern-Zetasizer (NanoZ590, UK) device.

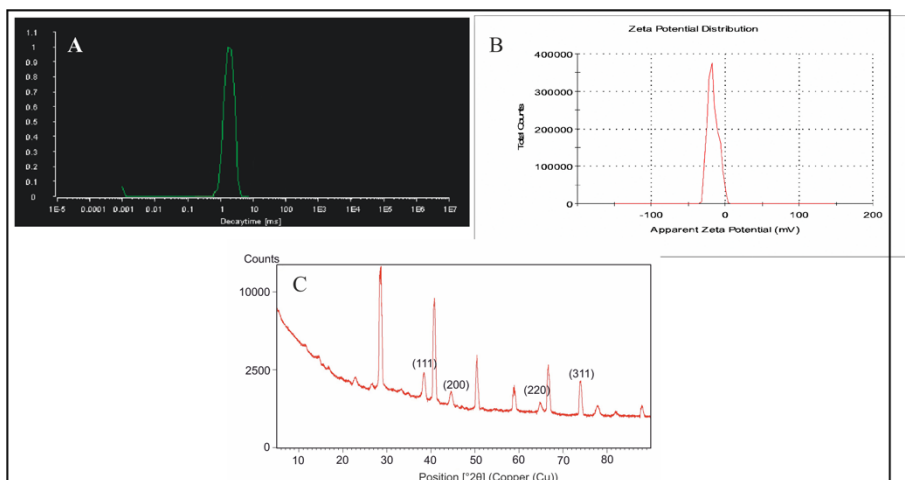


Figure 2: To Determine The Size, Stability And Crystal Structure Of The S-Aunps DLS, Zeta Potential And XRD Diagrams;(A) DLS,(B) Zeta Potential, (C) XRD

2.4.2. XRD Analysis

X-ray diffraction data determine the crystalline structure of AuNPs. The reported XRD pattern is reflections (111), (200), (220) and (311), respectively. According to our results, diffraction peaks of $2\theta = 38.4^\circ$, 44.6° , 64.8° and 77.8° were observed for S-AuNP (Figure 2 C). Dense peaks corresponding to nanoparticles showed matching in accordance with the reflections of Bragg's diffraction pattern (Shankar et al., 2003).

2.4.3. FE-SEM and TEM

FE-SEM analysis supported spherically distributed AuNPs (Figure 3 A) In our study, TEM was used to provide detailed information about the morphological structure and elemental analysis of S-AuNPs. Gold nanoparticles from *S. incrassatulus* extract were found to have an average size of 3-15 nm and homogeneously distributed (Figure 3 B and C). Nanoparticles analyzed elementally with the EDS detector (Oxford Instruments X-MaxN) were confirmed to be gold (Figure 3 D).

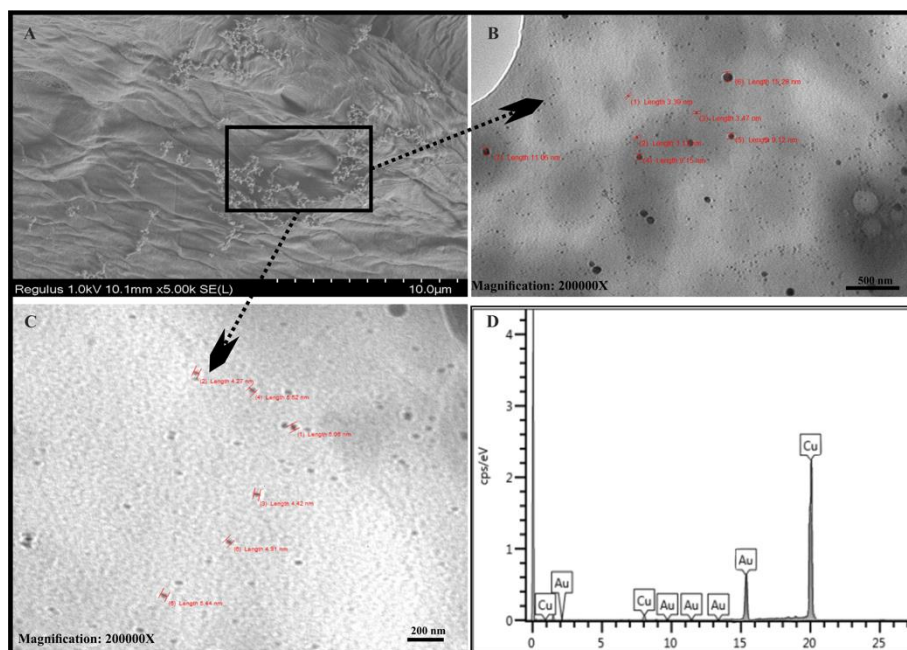


Figure 3: FE-SEM Images Of Biosynthesized S-Aunps (A), TEM Images (B, C), EDX Spectroscopy Showing The Chemical Composition Ofthe S-Aunps (D).

2.4.4. FTIR

For *S. incrassatus* extract, bands were observed at 3278.98, 2919.18, 2850.3, 1637.68, 1535.55, 1455.3, 1400.8, 1376, 1239.91, 1150.5, 1073.7, 1027.91, 880.44 cm^{-1} respectively (Figure 4 A). For S-AuNP, bands were determined at 3266.01, 2951.8, 2919.7, 2855.3, 1629.52, 1532.1, 1408.54, 1353.7, 1059.88, 858.14 cm^{-1} , respectively (Figure 4 B). The spectrum at 3278.98 cm^{-1} in *Scenedesmus* extract showed a large peak originating from polyphenols and polysaccharides, and this peak widened after reduction. (González-Ballesterosa et al., 2017). While the same spectrum at 3278.98 cm^{-1} shows N-H band, it also reveals that the weak carbonyl band is seen. This band expanded

during the reduction of gold ions. The spectrum of 1535.55 cm⁻¹ seen in the extract indicates N-H bending, while N = O stretching also shows that it is similar to carbonyl compounds. During reduction, the C-H out-of-plane tendency of these structures turned into SO₂ asymmetric stress. It was observed that the P-H bending at 1239.91 cm⁻¹ and the C-H in-plane symmetrical curve at 1150 cm⁻¹ disappeared completely (Erdik, 2008).

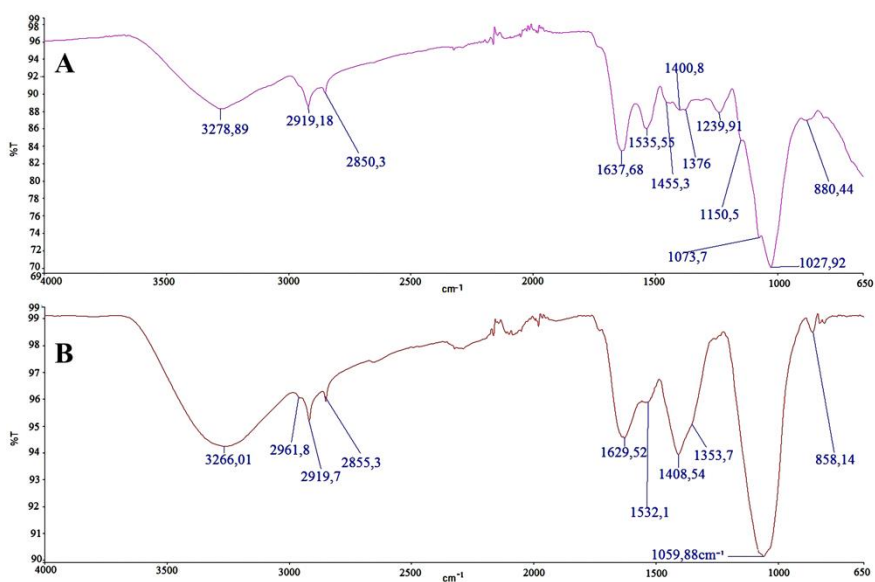


Figure 4:FTIR Spectra Of *S. Incrassatulus* (A); Synthesized AuNPs (B).

2.5. Antifungal activity

The zone diameters of S-AuNPs obtained by agar diffusion on *Candida* isolates are presented in Table 1. The results were compared with Amphotericin B control drug. According to the results, S-AuNPs showed a stronger zone diameter than Amphotericin B. A 10 mm zone

diameter was measured for *C. albicans* and *C. tropicalis*, while a 12 mm zone diameter was measured for *C. glabrata*.

MIC and MFK values provide quantitative data on the antifungal activity of the tested gold nanoparticles. Our MIC results reveal that S-AuNPs show the same MIC values in all isolates (Table 1). In order to determine the concentration range, quantitative analysis was performed on the ICP-MS device and the concentration rate was measured as 280 µg / ml. *C. albicans* ATCC 14053 showed the strongest result with a MIC of 4.31 µg / ml. In addition, there was a two-fold increase in the MFK values of the isolates compared to the MIC values. The antifungal effect of S-AuNPs is higher than *C. tropicalis* 1660 isolate on *C. glabrata* 1744 isolate.

Table 1:Antifungal Activity Of S-Aunps On *Candida* Isolates By Disc Diffusion And Broth Microdilution Tests: MIC (µg/ml), MFC (µg/ml) And The Diameters Of Zone Inhibition

Fungal Pathogens	Amphotericin B			Biosynthesized Gold Nanoparticle		
	Disc Diffusion Assay (mm dia)	MIC µg mL ⁻¹	MFC µg mL ⁻¹	Disc Diffusion Assay (mm dia)	MIC µg mL ⁻¹	MFC µg mL ⁻¹
<i>C. albicans</i>	8±0.2	3.13±0.8	6.25±1.2	10±0.3	2.19±1.3	4.37±0.8
<i>C. tropicalis</i>	8±0.2	6.25±0.7	12.5±0.9	10±0.2	8.75±1.2	17.5±0.8
<i>C. glabrata</i>	7±0.1	3.13±0.8	6.25±1.0	12±0.4	4.37±0.9	8.75±1.2

2.5.1. The Ultrastructural Changes of S-AuNPs on *Candida* isolates

TEM images obtained after exposure of S-AuNPs with control group cells for *C. albicans* ATCC 14053 isolate are presented in Figure 5. A. Control cells exhibit a characteristic morphology with regular and well-preserved cellular structures. After S.-AuNP exposure, ghost cells, cell wall and membrane damage, and cytoplasmic retraction were observed. As a general finding, folds in cell wall and membrane structures attract attention. Although few intact cells are seen in places, the signs of damage are more dominant. Although the nucleus structure is properly rounded, it is observed in some cells as peripheral or damaged (Figure 5 B, C, D).

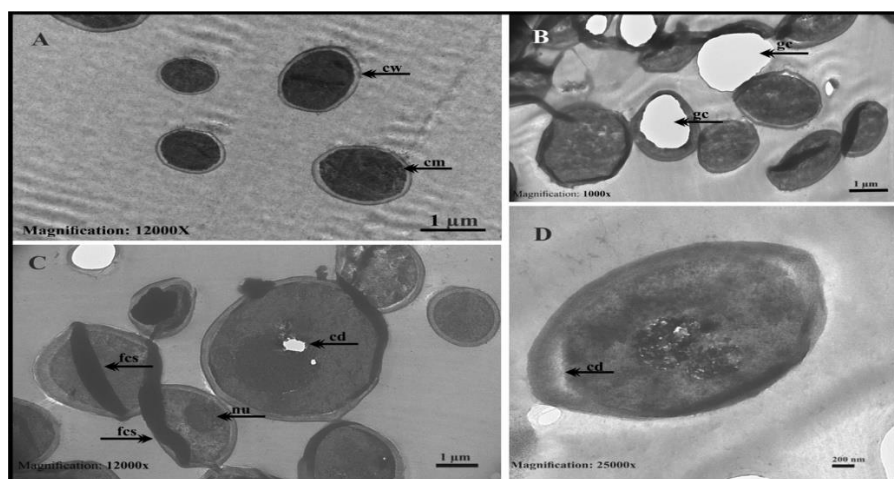


Figure 5: TEM Images Of *C. Albicans* Used With S-Aunps. (A) Untreated Control *Candida* Cells; B,C And D, Cells Used With C-Aunps. Cells Without Used Showed Regular And Well-Conserved Features, Homogenous Cytoplasm And Distinctive Membrane And Wall Structure: Cell Wall (Cw), Cell Membrane (Cm), Nucleus (Nu); After Used With Aunps, Cytoplasm Damage (Cd), Folded Cell Shapes (Fcs), Ghost Cells (Gc), Nanoparticles (Np), And Membrane Invagination (Mi) Were Prominent. (Scale Bars Is 1 µm For A-B-C Images And 200 Nm For D Image).

TEM images obtained from *C. tropicalis* 1660 isolate after S-AuNPs exposure with control group cells are presented in Figure. The control group TEM micrographs showed a well-preserved healthy morphology and whole cell structure. Significant damage findings were observed in cells treated with S-AuNP. Cells are fragmented or membrane, wall and cytoplasm structures are damaged (Figure 6).

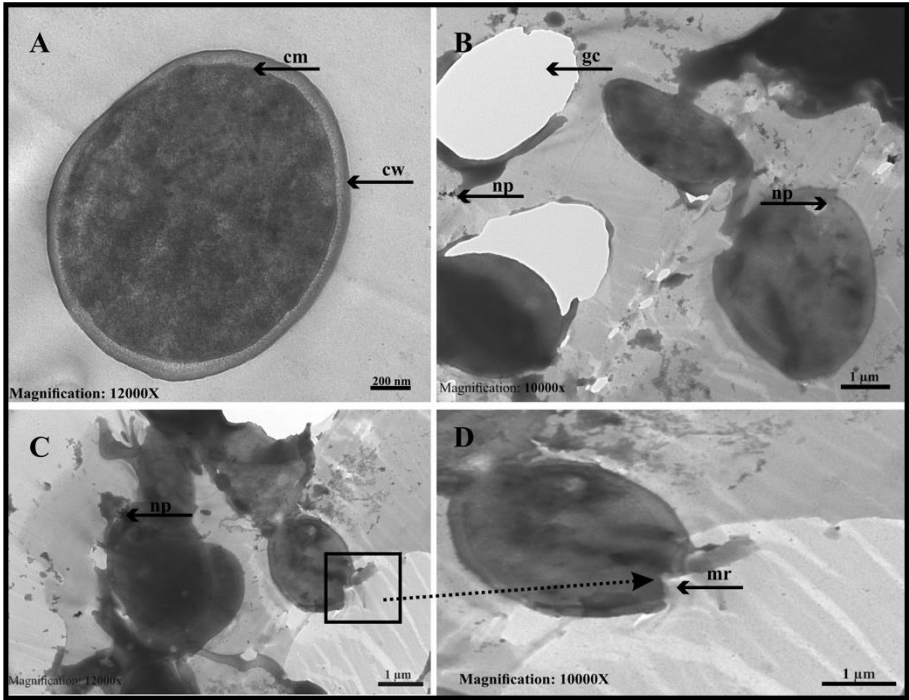


Figure 6: TEM Micrographs Of *C. Tropicalis* Used With S-Aunps. (A) Untreated Control *Candida* Cells; (B, C And D) Cells Used With C-Aunps. Cells Without Used Showed Well Preserved Cellular Morphology, And Entire Cell Structure: Cell Wall (Cw), Cell Membrane (Cm); After Treatment With C-Aunps, Ghost Cells (Gc), Membrane Invagination (Mi), Nanoparticles On The Cell Wall (Np), And Membrane Ruptures (Mr) Were Prominent. (Scale Bars Is 200 Nm For A; 1 μm For B-C-D Images).

TEM images obtained from *C. glabrata* 1744 isolate after S-AuNPs exposure with control group cells are presented in Figure 7 A. The control cells show integrity, healthy cell wall, membrane and stoplasma structures. After S-AuNP exposure, emptied structures called ghost cells, stoplasmic melts or stoplasma condensations in certain areas of cells, nanoparticles adhered outside the cell wall, and advanced damage, ruptures and structural deterioration were detected (Figure 7 B, C, D).

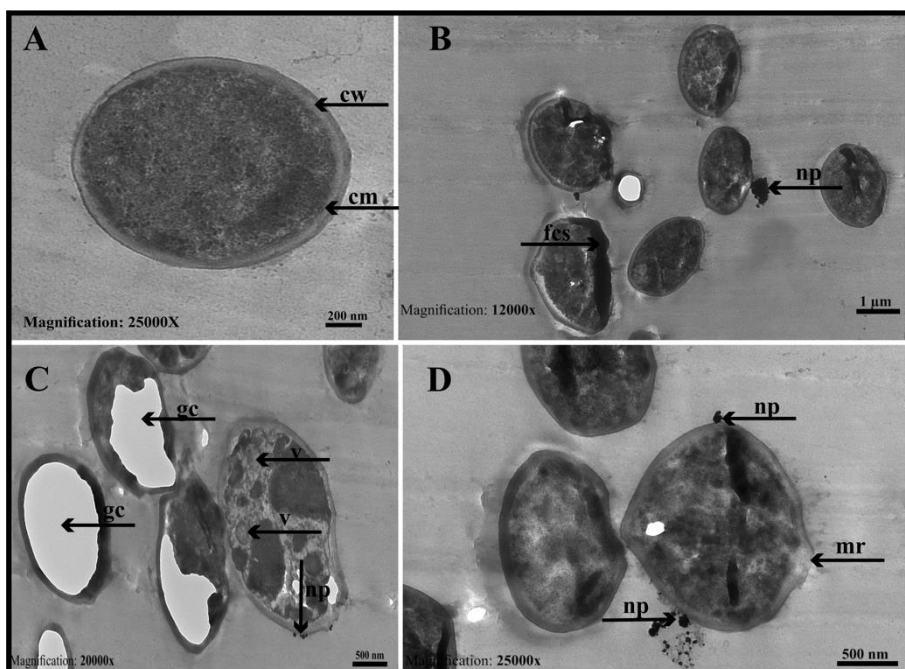


Figure 7: TEM Micrographs Of *C. Glabrata* 1744 Treated With S-Aunps. (A) Untreated Control *Candida* Cells; (B, C And D) Cells Used With C-Aunps. Control *Candida* Cells Show Homogenous Cytoplasm And Regular Morphological Features: Cell Wall (Cw), Cell Membrane (Cm); After Treatment With Aunps, Folded Cell Shapes (Fcs), Ghost Cells (Gc), Nanoparticles (Np), Vacuoles (V), And Membrane Invagination (Mi) Were Prominent. (Scale Bars Is 200 Nm For A Image; 500 Nm For B-C And D Images).

DISCUSSION

Bionanotechnology science has revolutionized at nanomaterial synthesis with the green synthesis method realized through biological systems. Especially microalgae have a huge potential among these systems with their ability to produce nanoparticles by the metal intake and detoxification process (Jena et al., 2014). However, the synthesis of metal nanoparticles using microalgae has not been adequately researched, but the use of algae for this purpose is a more biocompatible method than other biological methods. Synthesis is faster and takes place through a non-toxic process (Rajeshkumar et al., 2013). The use of chemicals as a reducing and stabilizing agent is eliminated. It is also reported in the literature that AuNPs obtained by green synthesis have a prospectively high potential in invivo research and regulation of algal metabolism (Pytlik et al., 2017). In addition, the gold nanoparticles obtained have the potential to be used in many fields of medicine and industrial.

In recent studies, algae species such as *Scenedesmus quadricauda* and *Scenedesmus platydiscusare* are reported to accumulate and degrade polycyclic aromatic hydrocarbons. Algal systems are frequently used in tertiary wastewater treatment processes. On the other hand, they also show wound healing, antifungal, anticancer, antibacterial activities. It is also reported that algal nanoparticles can be used as an antibiofilm agent against bacteria with multiple drug resistance, in the near future. Because these NPs can penetrate into EPS and into the

cell membrane. It is also recommended to investigate for nanocomposite and biosensor applications (Agarwal et al., 2019).

In our study, gold nanoparticle synthesis was performed as extracellular using green algae *S. incrassatulus*. The synthesized nanoparticles are spherical and average in size of 3-15 nm. Optimization studies have been carried out for pH, salt concentration and time to determine the most ideal reaction parameters. The antifungal activities of the AuNPs obtained were also evaluated. In the literature, there are some studies on green synthesis-mediated gold nanoparticle production with algae, but different algae types have been used in these studies and antibacterial and antitumor effect studies have been performed predominantly.

In the study of Isaac and Renitta, gold nanoparticles were synthesized by green synthesis using *Padina pavonica* brown algae and antimicrobial effects of the obtained nanoparticles against important bacterial pathogens were evaluated. In this study, extracellular synthesis method was applied and AuNPs occurred in 24 hours. The size of NPs was measured as 30-100 nm by particle size analysis. In our study, the size of AuNPs is about 3-15 nm in average. Nevertheless, gold nanoparticles in spherically morphology were confirmed by TEM and EDX analyzes, similar to our study. In the study of the authors, especially in *Bacillus subtilis*, a strong antibacterial effect was detected with a 15 mm zone diameter (Isaac and Renitta, 2015). Similarly, Rajeshkumar et al. synthesized gold nanoparticle with green synthesis using the algae extract of *Turbinaria*

conoïdes. In the TEM analysis, the sizes of nanoparticles were found between 6-10 nm. NPs are in small spherical, triangular and false spherical shapes. FTIR spectroscopy has supported the role of carboxylic, amine and polyphenolic compounds in algae-mediated synthesis (Rajeshkumar et al., 2013). In also our study, polyphenol and polysaccharides gave a high peak in FTIR analysis.

González-Ballesterosa et al. synthesized gold nanoparticle with green synthesis using brown microalga *Cystoseira baccata* (CB) extract. The final gold concentration used in the study is between 0.16 and 0.5 mM. However, 0.4 mM was chosen for optimum concentration. The characteristic surface plasmon resonance (SPR) absorption band formed for gold nanoparticles at 532 nm confirms the formation of nanoparticles. In this study, the synthesis took place at room temperature and in less than 15 minutes. The Zeta potential value of -30.7 ± 2.0 mV supported the formation of a stable colloidal suspension with negatively charged particles. The authors investigated the effects of CB extract on colon cancer cell lines HT-29 and Caco-2 and normal primary neonatal dermal fibroblast cell line PCS-201-010. The results reported that the effect of CB extract on HT-29 cell line was more cytotoxic than Caco-2. Interestingly, no toxic effect was found on PCS-201-010 (González-Ballesteros et al., 2017). On the other hand, Jena et al synthesized silver nanoparticles both as extracellular and intracellular using single cell green microalga *Scenedesmus* sp. These nanoparticles showed high antimicrobial

activity on gram positive and gram negative bacteria (Jena et al., 2014).

Abdel-Raoufa et al. synthesized gold nanoparticle using *Galaxaura elongata* powder or extract. With the TEM analysis, spherical, very few rod-shaped, triangular and hexagonal-shaped NPs were confirmed. FTIR analyzes have shown that nanoparticles are capping with algae compounds. In the study, gold nanoparticles showed a good antibacterial effect against *Escherichia coli*, *Klebsiella pneumoniae* and MRSA isolates (maximum 16-17 mm). They showed less effect on *Staphylococcus aureus* and *Pseudomonas aeruginosa* (13 mm). When using *G. elongata* powder, highly effective results were obtained on *E. coli* and *K. pneumoniae* (13-13.5 mm). The free ethanolic extract of *G. elongata* only showed high activity on MRSA (14 mm) (Abdel-Raoufa et al., 2017). Considering all these results, it is seen that algae-mediated gold nanoparticles have strong antibacterial effects.

Studies on the antifungal activity of gold nanoparticles are more limited and detailed studies are needed in this regard. Rahimi et al. have reported an antifungal activity of indolizide-linked gold nanoparticles against flucanazole resistant *C. albicans* strains isolated from burn infected patients (Rahimi et al., 2019). Nidhin et al. carried out a spherically shaped and 5 nm gold nanoparticle synthesis with green synthesis using starch and investigated the efficacy of the synthesized nanoparticles on *C. albicans* isolate. Researchers reported that the development of fungal cells at a concentration of 0.5 mM is

inhibited (Nidhin et al., 2019). In also another study, gold nanoparticle synthesis was performed from the leaf extract of *Annona muricata* and they investigated its antimicrobial activity on various fungi and bacteria. Researchers stated that gold nanoparticle activity is concentration dependent and better antimicrobial activity is seen as concentration increases (Folorunso et al., 2019).

In the study of Annamalai and Nallamuthu, self-assembled gold nanoparticles (GNPs) biosynthesis was performed from the aqueous extract of green microalgae *Chlorella vulgaris*. The synthesized nanoparticle sizes were found to be 2-10 nm and FTIR analysis showed that the peptides, proteins, phenols and flavonoids in the environment were involved in the reduction of Au III. Antimicrobial effects of GNPs on human cogen, *E. coli*, *P. vulgaris*, *S. aureus*, *P. aeruginosa* and *C. albicans* isolates were investigated. *C. albicans* showed maximum inhibition with 16 mm zone diameter and *S. aureus* with 14 mm zone diameter. The other three pathogens were found to be moderately sensitive (Annamalai and Nallamuthu, 2015). In our study, a zone diameter of 10 mm was observed for *C. albicans* isolate in the agar diffusion method. This difference may have occurred depending on the type of algae used or the reaction conditions.

Omomowo et al. synthesized silver and gold nanoparticles via *Neodesmus pupukensis* algi and investigated their antimicrobial and antioxidant activities. Color change (from pale green to purple) was observed at the 2nd hour of incubation. The size of the synthesized nanoparticles was measured by TEM and was found between 5-34

nm. As a result of antimicrobial activity tests, gold nanoparticles showed antimicrobial activity with 27.5 mm zone diameter for *Pseudomonas sp* and 28.5 mm for *Serratia marcescens*. Mycelial inhibition percentages were evaluated in measuring antifungal potency. These values were 79.4% for *A. niger*, 44.3% for *A. fumigatus*, 75.4% for *A. flavus*, 54.9% for *F. solani* and 66.4% for *C. albicans* (Omomowo et al., 2020). In our study, S-AuNPs exhibited a strong antifungal effect.

Li et al. found that low reaction temperature helped control the nanoparticle formation rate and they reported that the pH value also affected the particle size distribution. The authors explained that with such environmentally friendly methods, gold nanoparticle production has a great potential in large-scale production in commercial and industrial scale (Li et al., 2011). By controlling various environmental factors in nanoparticle synthesis, the size of the nanoparticles can be manipulated. Similarly, for our study, optimizations were made in terms of time, pH and salt concentration factors and it was aimed to create the most ideal reaction conditions

Among various eukaryotic organisms, yeasts are important as model organisms and are used in many biochemical and physiochemical experiments. On the other hand, proteins and carbohydrates in the cell wall provide a suitable environment for the binding of metals (Sen et al., 2011). In the study of Jalal et al., ZnONPs were synthesized with the leaf extract of *Crinum latifolium*. TEM micrographs stated that ZnONPs penetrate into the cell and cause severe damage to the wall

and membrane structures (Jalal et al., 2018). In our TEM findings, S-AuNPs caused severe damage to the cell and it has been observed that it causes degeneration and loss of walls, membranes and stoplasma.

As a result, we performed an effective, short, environmentally friendly and easy synthesis of AuNPs was performed using *S. incrassatulus* algae extract. The pH, time and salt concentration conditions in the reaction were optimized and it was aimed to provide the most ideal conditions. S-AuNPs have strong antifungal effects on *Candida* isolates, and nanoparticles may have been impacted by multiple damage mechanisms in the cell, including the wall, membrane and stoplasm. Our findings are promising, but supportive studies are needed on the potential of using green synthesis-mediated gold nanoparticles in the treatment of *Candida* infections.

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CHAPTER 3
ANALYSIS OF MORPHOLOGICAL AND POLLEN
MICROMORPHOLOGY CHARACTERS OF THE
***Crocus antalyensis* B.MATHEW (IRIDACEAE)**

Dr. Lecturer Derviş ÖZTÜRK¹

¹ Eskişehir Osmangazi University, Mahmudiye Equine Vocational School,
Department of Plant and Animal Production, Eskişehir, Turkey, dozturk@ogu.edu.tr
Orcid no 0000-0001-7189-7407

INTRODUCTION

Crocus L. (Çiğdem) is a plant belonging to the ornamental family (Iridaceae). Iridaceae is a large and diverse family of about 92 genera and 1800 species, mainly distributed across the Southern hemisphere continents. *Crocus* L. (Çiğdem) genus consists of 40 endemic and 1 hybrid approximately 235 taxa. Species belonging to the genus *Crocus* L., from western Europe and northwestern Africa, western China, the Balkans and distributed the greatest diversity in Turkey. East Mediterranean elements, mostly in Western Anatolia in Turkey shows the spread. The main homeland of Cigdem is the Alps, Southern Europe and the Mediterranean. It is a plant that is sought and loved in gardening because of its flowers. 'Saffron' known as Judy's breed as eaten raw or cooked tubers ash in Turkey. It is also packed. Styles and stigmas are collected and dried, mainly for use as a spice and coloring agent in food. Saffron has long been among the most expensive spices in the world.

Crocus antalyensis B. Mathew is an endemic species unique to Turkey. Many cultures have been made, and are widely cultivated in gardens as ornamental plants in Europe. The onion, which spends the winter under the ground, opens dark blue-purple flowers between February and May with the melting of snow. The flowers close at night or in bad weather. In general, *Crocus* L. are perennial, tubular, yellow or blue-flowered herbaceous plants that appear at altitudes of 800-1600 m. There are two onions under the soil. The small onion at

the top is the bulbs of this year and the bottom of the previous year is the onion. The nutrients stored in the onions in the first year enable the onion to live the next year and create a new plant in the next year. The water and nutrients stored in the onion are protected by the cork layer, which covers the onion. It attracts nectar, insects and butterflies and ensures pollination to occur.

On the other hand, *Crocus L.* species are growing to refine parks and gardens. In terms of agricultural importance, this breed has a commercial significance in the world. Plant material for *Crocus antalyensis* B. Mathew was collected during flowering. The collected samples were transformed into herbarium. After the plants were collected, morphometric measurements were made on fresh material. Korm, leaf, brachteol, anther, filament, style and crests were measured from quantitative morphological characters. In this study, *Crocus antalyensis* pollen was examined for its micromorphology. It is aimed to use pollen micromorphological features of plant samples as an important taxonomic criterion. For this purpose, the crop structure of the pollen for samples was examined by scanning electron microscope (SEM). As a result of this research, the pollen decoration related to *Crocus antalyensis* has been observed to some extent differently and can be used as an aid for taxonomic features.

Our country has an important position in the world in terms of plant species diversity due to its geographical and climatic structure as well as its three gene centers at the intersection point. Turkey flora of 154

families in 1220 and 11707 species and genus is represented by taxa. The number of endemic species is 3500, the number of endemic subspecies is 497, the number of endemic variety is 390, the total number of endemic taxa is 4300, and 32% according to the total number of species.

When the location of Eskişehir in the country from the floristic point of view is examined, although it is located in the Iran-Turan phytogeographical region, the different habitats it possessed enabled it to contain many different phytogeographic elements. (Figure 1).

Crocus Linnaeus denominated (1753: 36), the Balkan Peninsula and western Western Europe and north-west Africa with the center of species diversity in Turkey consists of approximately 200 known species distributed in China (Mathew 1982, Harper et al. 2013, 2014, 2015, Rukšāns 2014, Harpke et al. 2016, Rukšāns 2017). It is the most important monographic study of the genus *Crocus* Linnaeus (1753: 36). It was first monographed by Maw (1886), who recognized 67 species. Approximately 96 years later, "A revision of the *Crocus* genus" was published, describing the comprehensive study of Mathew (1982) 80 species. The genus was divided into two subspecies, two parts and 15 series. The number of endemic taxa, indicate that an important distribution center for the breed in Turkey. Especially in Turkey in recent years it has identified a large number of new taxa. (Kerndorff et al. 2013a, 2013b, 2013c, Erol et al. 2012, 2014, 2015, Candan & Özhatay 2013, Harpke et al. 2013, Yüzbaşıoğlu 2012,

Rukšāns 2013, 2014, 2015, Yıldırım & Erol 2013, Harpke et al. 2014, Schneider 2014, Yüzbaşıoğlu & Özhatay 2014, Yüzbaşıoğlu et al. 2015) (Table 1).

Table 1: Floristic Summary of Turkey's Flora

	Natural	Endemic	%	Foreign	Agriculture	Total
Lycopodiophyta	13	1	8,00	0	0	13
Equisetaceae	73	2	2,74	0	0	73
Gymnosperm	37	6	16,00	4	1	42
Angiosperm	11343	3640	32,09	167	69	11579
Total	11466	3649	31,82	171	70	11707

Since 1984, Flora of Turkey (after Mathew 1984) publication, was added 140 includes many taxa and genera taxa currently in Turkey. Studies on the *Crocus* genus have increased rapidly in recent years (Erol et al. 2017, Kerndorrf et al. 2013a, b, c, Rukšāns 2013, 2014, 2015, 2016, 2017, Harpke et al. 2014, Schneider 2014, Yüzbaşıoğlu & Özhatay 2014, Yüzbaşıoğlu et al. 2015, Yüzbaşıoğlu & Celep 2016, Yüzbaşıoğlu 2017). The genus *Crocus* Linnaeus (1753: 36) occurs in the Mediterranean region and in the floristic area known as the Irano-Turanian region (Mathew 1982) in the east of the Mediterranean. (Erol et al. 2012, Harpke et al. 2013, Kerndorrf et al. 2013a, 2013b, 2013c, Peruzzi & Carta 2011, Peruzzi et al. 2013, Yıldırım & Erol 2013).

Crocus L. are small herbaceous, corm (hard onion) perennials. The lying corms are covered with a cover. A small number of leaves are all located at the base, green, thin and long. The upper surface is pale, the

middle part is striped and the bottom is surrounded by a membrane. There is no real body.

The flowers coming out of the ground are stems. The flowers are in the form of hermaphrodite, actinomorph or zygomorph symmetry, terminal simoses. The flower cover is white, yellow or lilac or dark purple; the long tube is slim; segments are similar; equal or almost equal. Androkeum has 3 stamens. Male organs are tubular and buried in the throat of the flower cover. The capsule is small, elliptical or rectangular-elliptical. The neck is thinly constructed, each neck consists of 3 branches united from the base that are far from each other. Ginekeum has 3 compound carpels, 3 loci, and ovarian sub-condition. Stilus has 3 parts. The leaves are simple, alternate, basal, equitant. Perigon is in 2 circles, 3 in each circle, tepals are petaloid, usually compound in the base. The fruit is in the form of loculusite capsules (Davis,1984).

Recent studies in the breed have shown that other than flower color, the number of ribs on the abaxial leaf surface is more important. They are perennial, tuberous, gypsy pink or herbaceous plants with blue flowers. The flowers open in spring or autumn, depending on the type. The species that bloom in spring have long flower tubes whose ovaries are under the ground. The flowers close at night or in bad weather. The main homeland of Cigdem is the Alps, Southern Europe and the Mediterranean. It is a plant that is sought and loved in gardening because of its flowers. Çiğdem is considered as an

ornamental plant as in colors. Due to these features, *Crocus* species can survive when they are grown in parks and gardens (Kandemir 2010). It is found in soil samples in different ecologically tube. (Sik 2009).

Flowering: February-March

Habitat and life form: Sparse oak forest or thickets, 800-1200 m, Geofit.

General and regional distribution: Turkey, endemic

B3 Esk .: İnönü, Göktepe, oaklares, 1210 m

Danger situation: Least Concern (LC)

Consumption as human food

Having a composition rich in sugar and starch, onions are consumed in Anatolia by being raw or cooked. It is also packed. Among the dishes are Çiğdem pilaf, Çiğdem vaccine, Çiğdem milk pudding. In the Black Sea Region, it is filled with flour and fried.

The only type of Çiğdem not known as Çiğdem is saffron of economic value known as the sultan of Eastern cuisines (Turkish, Arabian, Iranian, Indian ...). *Crocus* L. (Safran), saffron is grown in Turkey. The origin of the Latin word *crocus* is based on the words *kunkumam*

in Sanskrit, which means "saffron". From there, the Greek crocodile turned into a *Crocus L.* in the late 14th century.

How is Çiğdem Grown?

- Crocus bulbs should be planted at the beginning of winter in late summer.
- They do not need special care.
- In the preparation of the soil to be made in the garden for planting, shrub soil should be added to the processed place with a depth of 15-20 cm.
- The soil where my crocus is grown should be loose, well-watered, high organic content, clayey and calcareous soil.
- Onion planting should be done by adjusting at a depth of 7-14 cm with 5-10 cm intervals.
- It may not reflect the visual effect expected from the plant when planting individually by sprinkling or forming lines.
- If it stays in the soil with onions, it will bloom earlier than the previous year.

How to Care and Water the Plant?

- Although water stress efficiency affects growth and development, it is a plant with low irrigation requirement in crocus.

- The plant can survive the cold winters, it can be under the snow for a short time, and it can withstand the climatic conditions of - 10 C.
- It prefers semi-shade and bright places.

Towards the summer, the leaves of the plant dry up and the onions stay dormant under the ground throughout the summer. Thus, no replanting is carried out in the next year.

How is Çiğdem Reproduced?

- A small onion pulls out a shoot; each shoot will bloom one or two, sometimes three, better-developed onions can give more shoots.
- Onions, which take out shoots or shoots, disappear in a few months, instead, new onions are formed as many as the number of shoots.
- An onion that does not grow well produces a single shoot next year and forms a single onion.
- Crocuses can also be produced with seeds, but the production process with onions is a method that is preferred more in terms of time, labor and cost and provides better results.

Matters needing attention

Colchicum species, which are very similar to chewing but are not eaten but poisonous, are also called bitter crocus (coyote crocus,

poison crocus). Since the tubers of this plant (Colchicum), which should not be eaten, are mixed with crocus (Crocus) species in the Eastern Anatolia Region, there may be cases of severe poisoning among children who collect and eat.



Figure 1: Locality where *Crocus antalyensis* B Mathew species is collected.

1. MATERIALS AND METHODS

The morphology, pollen structure and habitat of *Crocus antalyensis* B. Mathew (Iridaceae) species were investigated. The distribution area of this species was determined from various localities in Eskişehir and data were collected in terms of habitat characteristics.

1.1. Morphological studies

Crocus antalyensis B. Mathew taxa belonging to the *Crocus* L. genus which spread in Eskişehir was collected and dried according to herbarium techniques and pictures were taken. Flora of Turkey and

the samples were made using diagnosis related resources. Species determinations were made based on Flora of Turkey and The East Aegean Islands.

1.2. Alynological studies

Pollen of *Crocus antalyensis* B. Mathew was photographed with a Transmission Electron Microscope (Cryo-TEM) Hitachi HT7800) microscope. Pollen samples were obtained from freshly collected herbarium samples. For SEM examination, the pollen particles were sprayed directly with gold plates glued with double-sided tape. The photographs of the samples were taken in the electron microscope in Eskişehir Osmangazi University, Central Research Laboratory Application and Research Center.

2. RESULTS

2.1. Morphological Studies

In the study, *Crocus antalyensis* B. Mathew (Antalya crocus), cormus tunica membrane, a long neck with a permanent end and split into parallel fibers. Leaves 3-8 occur at the same time as flowers, 1-2.5 mm wide, no protective tissue. Perianth throat is yellow, with short soft hair; segments 2-3.5 cm, lilac-blue, the outer ones are sometimes brownish-yellow or purple-stained or blue near the bottom and white outward. Filaments 3-5 mm; anthers 1-1.2 cm. The stylus is orange

oryellow and has 6-12 branches. (Akan ve Eker 2004, Davis 1984)
(Figure 2, Table 2).

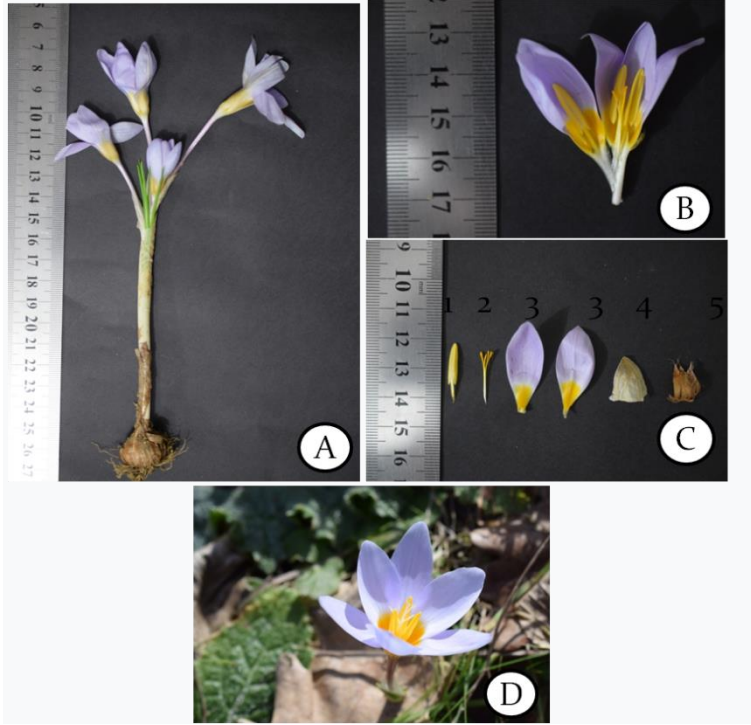


Figure 2: *Crocus antalyensis* B. Mathew (Antalya crocus), general view and various parts of the flowers of its kind. A. General view, D. Habitat, B. Çiçek, C1. Stamen, C2. Stilus, C3. Tepal, C4. Cataphylls, C5. Tunic.

Table 2: Morphological Measurements of *Crocus antalyensis* B. Mathew (Antalya crocus) Species.

Morphological Characters	Flora of Turkey	Findings in the study
Plant height (cm)	Unknown	12-25
Corm (cm)	Unknown	0.8-2 x 1-2.6
Tunica rings	Unknown	No ring on the base
Leaves	3-8	4-7
Leaf width (mm)	1-2.5	1-3
Scap length (cm)	Unknown	8-14
Perianth tube length (cm)	Unknown	4-10
Perianth parts (cm)	2-3.5 x 0.6-1.1	2.2-4.6 x 0.6-1.2
Filament	3-5	2-4

2.2. Palynological Studies

Pollen types are variable and are in the form of polirugoidate, polycolpat or nanoperturate (Figure 3).

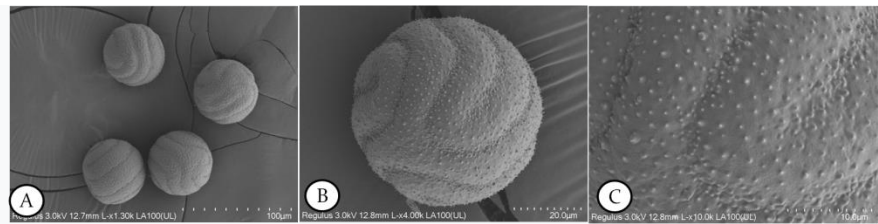


Figure 3:*Crocus antalyensis* B.Mathew (Antalya çiğdemi) A- B-Pollen, C- Pollen surface

Table 2: Palynological Measurements of *Crocus antalyensis* B. Mathew (Antalya çiğdemi) Species

Pollen Shape	Aperture	Ornamentation	Structure
Spiraperturate	corrugated	Scabrate	Tectate

3. DISCUSSION

In this study, *Crocus antalyensis* B. Mathew (Antalya çiğdemi) blooming in spring was investigated. This taxon some morphological characters Flora of Turkey 'not in the photo. This character is made into tables by making measurements in this study.

In palynological study, *Crocus antalyensis* B. Mathew pollen diameter is 45.62-58.14 μm , its shape is Spiraperturate, its structure is corrugated, ornamentation type is scabrate and its structure is tektat.

The overall findings of this study are consistent with Flora of Turkey and other flora studies (Mathew 1984 1988 2000).

Crocus antalyensis B. Mathew (Antalya çiğdemi) in our study varies according to the localities collected and the weather in the measurements taken as the morphological characters and varies between 12-25 cm. Plant height morphological characters as Flora of Turkey 'in the verilmemiştir. korm measurement ranges between 0.8-2 x 1-2.6. Kormi as the Flora of Turkey 'not in the photo. *Crocus antalyensis* B. Mathew (Antalya çiğdemi) tunica rings are found in some taxa in the genus and appear to be an important character for the determination. No ring was found in the observations made in the type we examined. Flora of Turkey 'was not given information about the tunic rings. Flora of Turkey, while the number of sheets specified in the number of sheets varies from 3 to 8 present species range from 4

to 7. This change is not very important. Because, there can be a difference in number of leaves in the species. When we examine the terms of the kind we have seen in Turkey Leaves genişliğ can show changes between 1 and 2.5 mm was measured at the Flora from 1 to 3 mm. Skapa as the size was not given any information on the Flora of Turkey. According to the habitat structure localities varying between 8 and 14 cm long tube of the type we have examined differences göstermektedirperianth to the dimensions given in the range of 2-3.5 x 0.6-1.1 cm Flora of Turkey. It was measured between 2.2-4.6 x 0.6-1.2 cm in the type we examined. While the number of filaments varies between 3 and 5 in the Flora of Turkey, it varies between 2 and 4 in the type we investigate.

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CHAPTER 4

***PACHYBRACHIS* SPECIES AND HOST PLANTS IN
TURKEY (CHRYSMELIDAE:
CRYPTOCEPHALINAE)**

Neslihan BAL¹

¹ Gazi University Faculty of Science Department of Biology Ankara Turkey,
neslihansilkin@gmail.com. <https://orcid.org/0000-0002-8122-7914>

INTRODUCTION

Turkey is one of the richest countries in Europe and Middle East with biodiversity, is ranked ninth in terms of biological diversity on the European continent. 65 million years ago, when Anatolia began to form, the mountain ranges formed by the compression of continents had a physical effect on rapidly spreading flowering plants and insects, allowing them to diversify into different species that separated their communities. In addition, in various earth movements and geological processes, especially during glacial periods in Central and Northern Europe, living things migrated from unfavorable regions and took refuge in Anatolia and diversified in their environment. Thus, Anatolian lands, which are also shelters for African and Asian species, have become quite rich in terms of species. Our country, which is a transit point between North and south; west and east, contains 3 different regions important for biodiversity, including Europe-Siberia, Iran-Turan and the Mediterranean from 37 flora regions of the world. Turkey, where three separate regions meet in an area covering a very small part of the world, shows a small continental feature in terms of biodiversity. In addition to the presence of species belonging to three different flora, the transition areas between these regions constitute quite rich areas in terms of endemic and hybrid species. Turkey is the country with the richest flora in the temperate climate zone, with about 10,000 species of natural flowering plants and ferns. A third (34.4%) of its flora consists of endemic species. Since plants form the first step of the food chain and the spread of insect species

depends on plants, this information about the plant geography of the region is also very important and guiding in terms of insect biodiversity (Özhatay et al., 2003).

Chrysomelidae or leaf beetles, as they are usually called, phytophagous among insects, 19 sub-family genera and more than 2000 around the world in approximately 37.000 (possibly up to 50,000) with defined types consists of a very diverse family. The Palaearctic Chrysomelidae fauna is represented by about 3500 species described up to now (Jolivet and Verma 2002; Gruev and Tomov 2007; Konstantinov et al. 2009). Although the Chrysomelidae family is taxonomically important in terms of containing a large number of species, it is an important group in terms of containing harmful and beneficial groups in terms of Agriculture. Larvae and adults of most species are among the most important pests of agricultural products, tree and shrub nurseries, medicinal plants and forage plants (Mirzoeva, 2001).

According to Jolivet ve Verma 2002, adult insects feed on leaves, flowers, pollen and young shoots; Larvae mostly feed on leaves and roots. A large proportion of leaf beetles are monophagous or oligophagous, while some groups are polyphagous. Many Chrysomelids feed on both adults and larvae from the same main source (Raupp ve Denno, 1983). therefore, host-use patterns of leaf beetles may have a large influence on the distribution of the family (Strauss, 1988).

Insects and plants have been developing together for over 300 million years, during which time they manage to establish a mutually beneficial biological partnership (Schoonhoven, 2005). In recent years, identifying relationships between plants and insects, including geographic, physiological, chemical, and evolutionary patterns of host use, has been a central area of interest (Becerra ve Venable, 1999). Chrysomelidae itself is a natural subject for the study of plant-insect interactions (Flowers ve Janzen, 1997).

500 species belonging to six genera have been identified in the Palearctic Region of the Cryptocephalinae subfamily, which has a wide distribution area in the world (Sassi ve Kışmal, 2000). Cryptocephalinae subfamily species feed on 64 plant families and some species carry plant viruses (Jolivet et al., 1988; Booth et al., 1990). The larvae and ergins of several species co-live with ants (Lopatin, 1977; Sassi ve Kışmal, 2000).

Approximately 150 species found in the region Palaeartik *Pachybrachis* (s. str.) Chevrolat, 1837 genus represented in Turkey with 27 species, seven of which are endemic to Turkey [Sassi and Kışmal, 2000; Warchałowski, 2008; Lopatin *et al.*, 2010; Schöller, 2010]. The seven endemic species are: *P. adaliensis* Weise, 1886, *P. anatolicus* Lopatin, 1985; *P. bodemeyeri* (Weise, 1906); *P. humeralis* Burlini, 1956; *P. pentheri* Ganglbauer, 1905; *P. velarum* Warchałowski, 1998 and *P. warchalowskii* Lopatin and Nesterova, 2010 (Şen ve Gök, 2011).





In the Palaearctic region, the genus *Pachybrachis* was studied by Warchalowski (2008). Almost all of the *Pachybrachis* species feed on the leaves of Fagaceae, Salicaceae and Betulaceae trees and shrubs. *Quercus*, *Salix*, *Populus*, *Corylus* and *Betula* are the genera in these families that eat the leaves of *Pachybrachis* (Mohr 1966; Sassi and Kışmalı, 2000). In addition, Şen and Gök found a large number of *Pachybrachis pentheri* species on the Poaceae plant with their study in 2011, but they thought that it could happen on this plant by chance due to the condition of the habitat.




1. MATERIAL METHOD

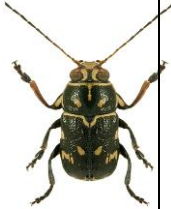
Pictures of male individuals of the species Dr. Lech Borowiec campus web site from the home page, provinces and states in Turkey, where they found the species in Turkey is benefiting from Ozdikmen & Cihan 2014 written publications. Host plant selection of species: Aslan, 1997; Atay & Çam, 2006; Gök & Çilbıroğlu, 2005, Butcher, 1980; Selmi, 1982; Şen & Gök 2009 was written using sources.




2. RESULTS





Pachybrachis species found in Turkey, pictures of male individuals of the species, provinces where the species is spreading in Turkey, the status of the species in Turkey and host plants for what is known are given below in the table;




<i>Pachybrachis</i> species in Turkey	Pictures of male individuals of the species	Provinces where the species is spread in Turkey	Status of species in Turkey	Host Plants
<i>P. adaliensis</i> (Weise, 1886)	-	Antalya	Endemic	-
<i>P. albicans</i> (Weise, 1882)		Artvin, Erzurum, Tokat	-	<i>Quercus</i> spp. (Atay & Çam, 2006)
<i>P. anatolicus</i> Lopatin, 1985	-	Van	Endemic	
<i>P. bodemeyeri</i> (Weise, 1906)		Bilecik, Bursa, Erzurum	Endemic	<i>Salix ssp.</i> (Aslan, 1997)
<i>P. cordatus</i> Sassi & Schöller, 2003		Amasya, Mersin	-	-
<i>P. excisus</i> (Weise, 1897)		Aksaray, Ankara, Antalya, Isparta, Niğde, Osmaniye ve Türkiye'nin Avrupa yakası	-	<i>Quercus</i> <i>pubescens</i> (Şen & Gök, 2009)


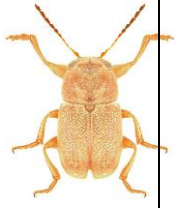
<i>Pachybrachis</i> species in Turkey	Pictures of male individuals of the species	Provinces where the species is spread in Turkey	Status of species in Turkey	Host Plants
<i>P. fimbriolatus</i> (Suffrian, 1848)		Adana, Afyon, Aksaray, Antalya, Ankara, Bayburt, Bilecik, Bingöl, Bitlis, Bolu, Çankırı, Diyarbakır, Erzincan, Eskişehir, Erzurum, Gümüşhane, Iğdır, Isparta, İstanbul, Kahramanmaraş, Karaman, Konya, Kars, Manisa, Nevşehir, Niğde, Osmaniye, Sivas, Tokat, Tunceli, Van	-	<i>Quercus</i> spp. (Atay & Çam, 2006)
<i>P. glycyrrhizae</i> (Olivier, 1808)		Gaziantep, Iğdır, Kars, Mersin, Urfa	-	-
<i>P. hieroglyphicus</i> (Laicharting, 1781)		Bilecik, Konya, Mersin	-	-

<i>Pachybrachis</i> species in Turkey	Pictures of male individuals of the species	Provinces where the species is spread in Turkey	Status of species in Turkey	Host Plants
<i>P. humeralis</i> Burlini, 1956	-	Türkiye'nin Avrupa yakası	Endemic	-
<i>P. instabilis</i> Weise, 1887	-	Balıkesir, Bursa, Sakarya	-	<i>Quercus</i> <i>pentraca</i> and <i>Q. robus</i> (Selmi, 1982).
<i>P. laticollis</i> (Suffrian, 1860)	-	Çorum, Diyarbakır, Manisa, Mardin, Sivas, Van	-	-
<i>P. leonardii</i> Sassi & Schöller, 2003		Antalya, Burdur, İzmir, Konya, Mersin, Muğla	-	-

<i>Pachybrachis</i> species in Turkey	Pictures of male individuals of the species	Provinces where the species is spread in Turkey	Status of species in Turkey	Host Plants
<i>P. limbatus</i> (Ménétriés, 1836)		Adana, Adıyaman, Ankara, Antalya, Balıkesir, Bilecik, Bolu, Bursa, Çanakkale, Eskişehir, Erzurum, Isparta, İzmir, Kayseri, Kütahya, Mardin, Mersin, Niğde, Samsun, Sivas, Yalova, Türkiye'nin Avrupa yakası	-	<i>Quercus</i> <i>coccifera</i> (Şen & Gök, 2009, Selmi, 1982) <i>Quercus</i> <i>infectoria</i> (Şen & Gök, 2009) <i>Quercus</i> <i>ssp.</i> (Gök & Çilbıroğlu, 2005) <i>Quercus</i> <i>cerris</i> , <i>Quercus</i> <i>fratnetto</i> Selmi, 1982)
<i>P. mardinensis</i> (Weise, 1900)		Adana, Adıyaman, Amasya, Bitlis, Denizli, Elazığ, Gaziantep, Hakkari, Hatay, Kahramanmaraş, Mardin, Mersin, Osmaniye, Muş, Van	-	-
<i>P. m. mendax</i> Suffrian, 1860		Ankara, Bayburt, Çanakkale, Denizli, Erzincan, Erzurum, Gümüşhane, Kars, Konya, Mersin, Niğde, Sivas, Van	-	-

<i>Pachybrachis</i> species in Turkey	Pictures of male individuals of the species	Provinces where the species is spread in Turkey	Status of species in Turkey	Host Plants
<i>P. nigropunctatus</i> Suffrian, 1854		Adana	-	-
<i>P. nitidicollis</i> (Weise, 1894)	-	Erzurum	-	-
<i>P. pentheri</i> (Ganglbauer, 1905)		Isparta, Kayseri	Endemic	-
<i>P. picus</i> (Weise, 1882)		Erzurum	-	-
<i>P. scripticollis</i> Faldermann, 1837		Diyarbakır, Gaziantep, Hakkari, Mardin, Urfa	-	-

<i>Pachybrachis</i> species in Turkey	Pictures of male individuals of the species	Provinces where the species is spread in Turkey	Status of species in Turkey	Host Plants
<i>P. scriptidorsum</i> Marseul, 1875		Artvin, Bolu, Diyarbakır, Erzurum, Gümüşhane	-	<i>Salix ssp.</i> (Aslan, 1997)
<i>P. sinuatus</i> (Mulsant & Rey, 1859)		Ankara, Bolu, Erzurum, Isparta	-	<i>Salix caprea</i> (Şen & Gök, 2009) and <i>Salix ssp.</i> (Aslan, 1997, Gök & Çilbiroğlu, 2005, Aslan & Özbek, 1997)
<i>P. tesellatus tauricus</i> Suffrian, 1848		Adana, Afyon, Ağrı, Aksaray, Amasya, Ankara, Antalya, Bayburt, Bolu, Bursa, Çankırı, Çorum, Erzincan, Erzurum, Gaziantep, Gümüşhane, Isparta, İzmir, Kahramanmaraş, Karaman, Kayseri, Konya, Kars, Kütahya, Mardin, Mersin, Nevşehir, Niğde, Sivas, Tokat, Van, Yozgat	-	<i>Quercus coccifera</i> (Şen & Gök, 2009; Gök & Çilbiroğlu, 2005) <i>Quercus pubescens</i> (Kasap, 1980) <i>Quercus ssp.</i> (Aslan & Özbek, 1997)

<i>Pachybrachis</i> species in Turkey	Pictures of male individuals of the species	Provinces where the species is spread in Turkey	Status of species in Turkey	Host Plants
<i>P. velarum</i> Warchałowski, 1998		Ankara, Bolu, Erzincan, Erzurum, Gümüşhane, Kars, Sivas	-	-
<i>P. vermicularis</i> Suffrian, 1854		Erzurum	-	-
<i>P. warchalowskii</i> Lopatin & Nesterova, 2010	-	Mardin	Endemic	-

Pachybrachis species found 150 species of Palaearctic is represented to the species 27 in Turkey. Turkey is very rich in *Pachybrachis* species. *Quercus*, *Salix*, *Populus*, *Corylus*, *Betula* species, which are members of the Fagaceae, Salicaceae and Betulaceae family, are generally host plants.

The majority of species choose *Quercus* species as host plants. The rest of them were seen to choose *Salix* species as hosts. *Quercus* spp. of *P. albicans* (Weise, 1882); *Quercus pubescens* of the species *P. excisus* (Weise, 1897); *Quercus* spp of the species *P. fimbriolatus* (Suffrian, 1848); *Quercus pentraca* and *Q.robis* of the species *P.*

instabilis Weise, 1887; *Quercus coccifera*, *Quercus infectoria*, *Quercus cerris*, *Quercus fraxinifolia* and *Quercus ssp* of *P. limbatus* (Ménétriés, 1836); *Salix ssp* of the species *P. scriptidorsum* Marseul, 1875; *Salix caprea* and *Salix ssp.* of *P. sinuatus* (Mulsant & Rey, 1859) species; *P. tessellatus tauricus* Suffrian, *Quercus coccifera*, *Quercus pubescens* and *Quercus ssp.* are host plants.

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CHAPTER 5

**RESEARCHERS CONTRIBUTING TO THE FAUNA
OF TURKEY'S CANTHARIDAE**

Neslihan BAL¹

¹ Gazi University Faculty of Science Department of Biology Ankara Turkey,
neslihansilkin@gmail.com. <https://orcid.org/0000-0002-8122-7914>

INTRODUCTION

The root of the family name Cantharidae is from the Greek word *Cantharis*, meaning a species of Coleopter. In English they are referred to as leather-winged beetles or soldier beetles, while in German they are referred to as Weichkafer or Schusterkafer. They are known as soft-bodied in Turkish (Lodos, 1991).

Cantharidae is the most developed and most diverse family of the Cantharoidea superfamily. Although Cantharidae species spread almost all parts of the world except for polar regions, they are dominantly found in tropical and subtropical areas. As of today, the Cantharidae family contains 5100 species of 130 genera. The number of species in the Western Palearctic Region is about 870, of which 206 are located endemically in Turkey. Comparing number of species identified from Turkey with those from European countries enables us to recognize the richness of Turkish fauna with respect to Cantharidae. For example, *Dichelotarsus*, *Boveycanthis*, *Islamocanthis*, *Micropodaprus*, *Occathemus*, and *Sinometa* are only found in Turkey, while *Ancistronycha*, *Canthis*, *Cordicanthis*, *Metacanthis*, *Rhagonycha*, *Malthinus*, *Malthodes*, *Macrocerus* and *Trypherus* species appear to be more numerous in Turkey than in Poland and Germany (Lodos 1991; Brancucci 1980; Brancucci ve Kazantsev 2007).

According to Brancucci (1980), the Cantharidae family is divided into five subfamilies: (i) Malthininae, (ii) Cantharinae, (iii) Silinae, (iv) Chauliognathininae, (v) Dysmorphocerinae. Cantharinae species are found mainly in the Holarctic and Oriental region. Dysmorphocerinae species are usually found in the Southern Hemisphere. Malthininae species are found in the region from Canada to Brazil, in the Palearctic region, in the Oriental Region, in East Africa, India and the East Indies. Malthininae species are not found on the Australian continent. Silinae species are mostly found in tropical regions. Chauliognathini species are found in the region from Southern Canada to Chile, Australia and New Guinea. There are only 3 subfamily species in the Western Palearctic Region (Brancucci 1980; Kuska 1995; Ramsdale, 2002).

Studies focusing on Cantharidae in Turkey are few in number and their scope is somewhat narrow. The vast majority of the known species were recorded by non-Turkish researchers based on samples collected during excursions of scientific purposes (Wittmer, 1969, 1971, 1972, 1993; Svihla, 1993, 1994, 1998, 1999, 2002, 2009; Michael Geiser, 2017). For example, Walter Wittmer and Vladimir Svihla added new records and new species to the fauna of Turkey and Michael Geiser added new record species for some provinces. There are very few studies of local researchers (Wittmer, 1969, 1971, 1972, 1993; Svihla, 1993, 1994, 1998, 1999, 2002, 2009; Michael Geiser, 2017).

1. FOREIGN RESEARCHERS CONTRIBUTING TO THE FAUNA OF TURKEY'S CANTHARIDAE

Walter Wittmer and Vladimir Svihla added new records and new species to the fauna of Turkey and Michael Geiser added new record species for some provinces.

1.1. Walter Wittmer

1.1.1. Walter Wittmer 1969

Walter Wittmer worked on Palearctic Cantharids. Turkey was his the first study into the field. In his study, definitions of the *Metacantharis*, *Boveycantharis* and *Sinometa* genera in the Palearctic region, the genus key and the species key of the defined species of these genera are given. In particular, specimens have been collected from Lebanon, Caucasus and Turkey. A total of five new species, one new subspecies of the *Boveycantharis* genus and new record species in some provinces; one new species of the genus *Sinometa* have been identified (Wittmer, 1969).

Table 1: Species and subspecies found from Turkey by study

Name of the species or subspecies	Location of the collected thespecies or subspecies	The current status of the species or subspecies in the World or Turkey
<i>Boveycanthis rufimana</i>	Rize yol üstünden, Hopa, Artvin and Almus Tokat	New record for Rize, Artvin and Tokat in Turkey
<i>Boveycanthis tokatensis</i>	Abant Bolu, Uludağ, Sultan Dağları Tokat	New record for Bolu and Tokat in Turkey
<i>Boveycanthis dimidiatipes ssp. malatiensis</i>	Malatya	New subspecies
<i>Boveycanthis rufimanoides</i>	Soğuk Su Doğal Milli Parkı Kızılcahamam and Abant Bolu	New species
<i>Boveycanthis hetitica</i>	Nur Dağları Osmaniye and Dalaktersi Mersin	New species
<i>Boveycanthis phoeniciensis</i>	Amanos	New species
<i>Boveycanthis akshehirensis</i>	Akşehir, Sultan dağları and Ereğli	New species
<i>Sinometa besucheti</i>	Abant Bolu, Almus Tokat and Soğuk Su Doğal Milli Parkı Kızılcahamam	New species

1.1.2. Walter Wittmer 1971

In his study, he gave the distribution of some species belonging to the genus *Cantharis* in the world and Turkey (Wittmer, 1971).

Table 2: Species and subspecies found from Turkey by study

Name of the species or subspecies	Location of the collected thespecies or subspecies	The current status of the species or subspecies in the World or Turkey
<i>Cantharis livida</i> v. <i>adusta</i> Bourg. Variety has been upgraded to <i>Cantharis quadripunctata</i> .	Asia Minor (Tekir: Kilik)	<i>Cantharis livida</i> v. <i>adusta</i> Bourg has been upgraded to the genre.
<i>Telephorus fissicollis</i> Fairm regulated to <i>Cantharis fissicollis</i>	Tekir, Kilik, Taurus Bolu and Denizli Honaz dağı	<i>Telephorus fissicollis</i> species is regulated
<i>Cantharis livida</i> v. <i>ciliciensis</i> Bourg.its variation has been upgraded to the species <i>Cantharis ciliciensis</i> .	Gölbaşı Malatya	<i>Cantharis livida</i> v. <i>ciliciensis</i> Bourg. has been upgraded to the genre.
<i>Cantharis cedricola</i>	Tekir Kilik; Serik Alanya; Taurus and Namrun Mersin; Osmaniye, Gaziantep and Maraş	New species
<i>Cantharis atrofoveolata</i> Pis has fallen into synonym and so It has been <i>Cantharis melaspis</i> Chevr.	Erekli Amasya; Gölbaşı- Maraş and Gölbaşı-Malatya arası	<i>Cantharis atrofoveolata</i> has fallen into synonymy
<i>Cantharis pamphylica</i>	Mardin	New species
<i>Cantharis mülleri</i>	Akşehir	New record for Turkey
<i>Cantharis ziganadagensis</i>	Zigana Dağı	New species
<i>Cantharis anatolica</i> Bourg. has fallen into synonym and has become <i>Cantharis prusiensis</i> Mars.	Pamukkale Denizli; Eskişehir; Soğuk Su Milli Parkı Kızılcacahamam	<i>Cantharis anatolica</i> Bourg. has fallen into synonymy

1.1.3. Walter Wittmer 1972

He worked on Palearctic Region Cantharids. He studied the regions of Turkey, Israel, the Caucasus and Transcaucasia in particular. He has made important contribution to the fauna of Turkey with his works (Wittmer, 1972).

Table 3:Species and subspecies found from Turkey by study

Name of the species or subspecies	Location of the collected thespecies or subspecies	The current status of the species or subspecies in the World or Turkey
<i>Rhagonycha dahlgreni</i>	Kızılcahamam Doğal parkı soğuk Su milli and Bolu	New species
<i>Rhagonycha intermedia</i>	ÇamlıbelGeçidi and Celbıram Geçidi Sivas	New species
<i>Rhagonycha tridentata</i>	Akşehir	New species
<i>Rhagonycha nurdagensis</i>	Nur dağları Osmaniye	New species
<i>Rhagonycha holzschuhi</i>	Kızılcahamam Ankara	New species
<i>Rhagonycha hetitica</i>	Misis Adana	New species
<i>Rhagonycha turcica</i>	Belgrad ormanı, İstanbul	New species
<i>Rhagonycha robusticornis</i>	Nur dağları Osmaniye	New species
<i>Rhagonycha zwicki</i>	Kızılcahamam Doğal soğuk su milli parkı, Civcan Dağı Gerede	New species
<i>Rhagonycha elongatipes</i>	Karatepe Adana, Nurdağı Osmaniye	New species
<i>Rhagonycha marginithorax</i>	Antalya	New species
<i>Rhagonycha pamphylica</i>	Efes Aydın, Honaz Dağı Pamukkale-Denizli	New species
<i>Rhagonycha bernhaueri</i>	Rize İkizdere, Sümela Trabzon	New species
<i>Cratosilis osmana</i>	Belgrad Ormanı (İstanbul), Bolu Abant, Düzce	New species

1.1.4. Walter Wittmer 1993

He studied Vietnam, China, Taiwan, Turkey, Greece and Tajikistan in the Palearctic region and added four new species to Turkey's Cantharidae fauna, including two *Malthinus* and two belonging to the genus *Malthodes* (Wittmer, 1993).

Table 4: Species and subspecies found from Turkey by study

Name of the species or subspecies	Location of the collected thespecies or subspecies	The current status of the species or subspecies in the World or Turkey
<i>Malthinus rydhi</i>	Elmalı, Antalya	New species
<i>Malthinus complexus</i>	Yarpuz, Antalya	New species
<i>Malthodes gillerforsi</i>	AntalyaYarpuz and Manavgat	New species
<i>Malthodes pergensis</i>	Perge, Antalya	New species

1.2. Vladimir Svihla

1.2.1. Vladimir Sihla 1993

He has done studies on the family Cantharidae in Turkey, Bulgaria, Macedonia, Syria and the Caucasus and has made significant contributions to the fauna of the Eastern Mediterranean. For Turkey, nine new species and three new records have been described belonging to the genus *Rhagoncyha* (Svihla, 1993).

Table 5: Species and subspecies found from Turkey by study

Name of the species or subspecies	Location of the collected thespecies or subspecies	The current status of the species or subspecies in the World or Turkey
<i>Rhagonycha lundbergi</i>	Yarpuz Antalya	New species
<i>Rhagonycha osellai</i>	Sümela Trabzon	New species
<i>Rhagonycha walteri</i>	Yarpuz Antalya	New species
<i>Rhagonycha gillerforsii</i>	Elmalı Antalya	New species
<i>Rhagonycha kronbladi</i>	Elmalı Antalya	New species
<i>Rhagonycha rydhi</i>	Gündoğmuş and YarpuzAntalya	New species
<i>Rhagonycha carousi</i>	HakkariUludere	New species
<i>Rhagonycha catei</i>	Abant Bolu and Yıldız Dağlarından(Demirköy) Kırklareli	New species
<i>Rhagonycha Brancuccii</i>	Yazır Antalya	New species
<i>Rhagonycha gruziana</i>	Zigana Gümüşhane	New record for Turkey
<i>Rhagonycha helleni</i>	Demiköy Kırklareli, Düzce, Yalova İzmit	New record for Turkey
<i>Rhagonycha chevrolati</i>	Düzce	New record for Turkey

1.2.2. Vladimir Svihla 1994

He recorded a new breed from Algeria with his work in Algeria, Turkey, Syria, Israel, Sicily. The genus *Sidabia* and the type species belonging to this genus have been described. He has also described two new species and one new subspecies to the fauna of Turkey's Cantharidae (Svihla, 1994).

Table 6: Species and subspecies found from Turkey by study

Name of the species or subspecies	Location of the collected thespecies or subspecies	The current status of the species or subspecies in the World or Turkey
<i>Malthinus lundbergi</i>	-	New species
<i>Malthodes rydhi</i>	Elmalı Antalya	New species
<i>Malthodes seleucianus euphraticus</i>	Şanlıurfa Halfeti	New subspecies

1.2.3. Vladimir Svihla 1998

As a very important contribution to the literature, four new species belonging to the subfamily Malthininae have been described from Turkey in his study. Accordingly, a total of four new species have been recorded in the fauna of Turkey, one in the genus *Malthodes* and three in the genus *Malthinus* (Svihla, 1998).

Table 7: Species and subspecies found from Turkey by study

Name of the species or subspecies	Location of the collected thespecies or subspecies	The current status of the species or subspecies in the World or Turkey
<i>Malthinus wittmeri</i>	Güzeloluk Mersin	New species
<i>Malthodes rolciki</i>	Ağlı Kastamonu, Zonguldak, Safranbolu Karabük and Bolu	New species
<i>Malthodes vavrai</i>	Abant Bolu	New species
<i>Malthodes zahradniki</i>	Elmalı Antalya	New species

1.2.4. Vladimir Svihla 1999

His work contributed to the fauna of Turkey, Iran, Armenia and Lebanon. Two species belonging to *Metacantharis* genus, three species belonging to *Boveycantharis* genus and one species belonging to *Cordicantharis* genus were defined as new species from samples collected in Turkey. Svihla also gave the species keys of the genera *Metacantharis* and *Cordicantharis* in this study (Svihla, 1999).

Table 8: Species and subspecies found from Turkey by study

Name of the species or subspecies	Location of the collected thespecies or subspecies	The current status of the species or subspecies in the World or Turkey
<i>Metacantharis walteri</i>	Ak dağlar, Bozdağlar İzmir and Antalya	New species
<i>Metacantharis torosensis</i>	Taşkent Konya, Toros Dağları Sertavul Geçidi	New species
<i>Boveycantharis tauricola</i>	Ak Dağlar; Akseki, Yarpuz and Elmalı Antalya; Aslanköy Mersin; Seydişehir	New species
<i>Boveycantharis mersinensis</i>	Aslanlı Mersin, Güzeloluk İçel, Silifke kırobası- Aslanlı erdemli arası 15. Km	New species
<i>Boveycantharis holzschuhi</i>	Uludere Hakkari	New species
<i>Cordicantharis similis</i>	Karlıova Bingöl	New species

1.2.5. Vladimir Svihla 2002

He studied species belonging to the subfamily Malthininae collected from Turkey, Cyprus, Syria, Georgia, Iraq, Spain and Morocco. New species and new subspecies belonging to the genera *Malthinus* and *Malthodes* have been described (Svihla, 2002).

Table 9: Species and subspecies found from Turkey by study

Name of the species or subspecies	Location of the collected thespecies or subspecies	The current status of the species or subspecies in the World or Turkey
<i>Malthinus bezdeki</i>	Göktepe Dağı, ErdemliMersin	New species
<i>Malthodes prudeki</i>	Maçka Trabzon	New species
<i>Malthinus zahradni</i>	Erdemli Mersin	New species
<i>Malthinus walteri</i>	Borçka Artvin	New species
<i>Malthodes ruzickai</i>	Halfeti Şanlıurfa	New species
<i>Malthodes kopeckyi</i>	Isparta	New species
<i>Malthodes kopetzi</i>	Bey Dağları Antalya	New species
<i>Malthodes walteri</i>	Toros Dağları Mersin	New species
<i>Malthinus dimorphus cilicius</i>	Göktepe Dağı, ErdemliMersin	New subspecies
<i>Malthinus dimorphus phrygius</i>	Eskişehir and Gümele	New subspecies
<i>Malthodes denizlianus bergamensis</i>	Bergama İzmir	New subspecies
<i>Malthodes klapperichi assyrius</i>	Halfeti Şanlıurfa and Birecik	New subspecies
<i>Malthodes denizlianus denizlianus</i>	Pamukkale Denizli and Yatağan	New record for Turkey
<i>Malthinus anatolicus</i> Wittmer, 1974 specieshas become <i>Malthinus tauri anatolicus</i> subspecies.	Gölbaşı Maraş	Status of <i>Malthinus anatolicus</i> Wittmer,1974 is changed to <i>Malthinus tauri anatolicus</i> stat. n.

1.2.6. Vladimir Svihla 2009

Cyprus, Turkey, Greece and Iran have been studied from the Western Palearctic region and six new species have been described for Turkey belonging to the Malthininae subfamily (Svihla, 2009).

Table 10: Species and subspecies found from Turkey by study

Name of the species or subspecies	Location of the collected thespecies or subspecies	The current status of the species or subspecies in the World or Turkey
<i>Malthinus hulai</i>	Göynük Antalya	New species
<i>Malthinus ciraliensis</i>	Çıralı Antalya	New species
<i>Malthinus malinkai</i>	Hop Geçidi Mardin	New species
<i>Malthodes malinkorum</i>	Hop Geçidi Mardin	New species
<i>Malthodes zdeneki</i>	Güzeloluk, İçel	New species
<i>Malthodes flagellatus</i>	Haberli Şırnak, Midyat	New species
<i>Malthodes andreasi</i>	Belen Antalya	New species
<i>Malthodes lycicus</i>	Belen Antalya	New species
<i>Malthodes besucheti</i> <i>bucakensis</i> wittmer, 1970alttürü <i>Malthodes</i> <i>bucakensis</i> Wittmer, 1970 tür seviyesine çıkarılmıştır.	Gülükdağı and yazır Antalya Pazar Tokat	<i>Malthodes besucheti</i> <i>bucakensis</i> wittmer, 1970taken to species level

1.3. Michael Geiser

1.3.1. Michel Geiser 2017

The genus *Rhagonycha* is represented by 300 species in the Western Palaearctic region and has wide diversity in Turkey. About 60 species are found in Turkey, with most of the species described by Vladimir Svihla. In 2017, Michael Geiser gave the species *Rhagonycha bythinica* as the new record from some provinces (Geiser, 2017).

Table 11: Species and subspecies found from Turkey by study

Name of the species or subspecies	Location of the collected thespecies or subspecies	The current status of the species or subspecies in the World or Turkey
<i>Rhagonycha bythinica</i>	Bilecik, Bursa, Balıkesir, Çanakkale and İzmir	New record forBilecik, Bursa, Balıkesir, Çanakkale and İzmir in Turkey.

2. TURKISH RESEARCHERS CONTRIBUTING TO THE FAUNA OF TURKEY'S CANTHARIDAE

Tuatay (1972), Gül-Zümreoğlu (1972), Silkin (2008), Sayan (2010), Ertop and Özpınar (2011), Yildirim et. all (2011), Sert and Kabalak (2013), Demirözer and Karaca (2014) and Sezer (2018) have contributed to the fauna of Turkey by adding new species recordsfor some provinces.

2.1. Nazife Tuatay, Ayla Kalkandelen and Neş'e Aysev

2.1.1. Nazife Tuatay, Ayla Kalkandelen and Neş'e Aysev

1972

They made the museum catalog of Flora Protection in Turkey and gave new species records for some provinces (Tuatay et. all, 1972).

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Table 12: Species and subspecies found from Turkey by study

Name of the species or subspecies	Location of the collected thespecies or subspecies	The current status of the species or subspecies in the World or Turkey
<i>Cantharis annularis</i>	Keçiören Ankara and İzmir	New record for Ankara and İzmir in Turkey.
<i>Cantharis bilunatus</i>	Osmaniye Adana and Çınar Diyarbakır	New record for Adana and Diyarbakır in Turkey.
<i>Cantharis livida</i>	Aksaray, Diyarbakır, Isparta, Bornova İzmir, Konya and Nevşehir	New record for Aksaray, Diyarbakır, Isparta, İzmir, Konya and Nevşehir in Turkey.
<i>Rhagonycha chevrolati</i>	Diyarbakır, Nevşehir and Niğde	New record for Diyarbakır, Nevşehir and Niğde in Turkey.

2.2. Süheyla Gül-Zümreoğlu

2.2.1. Süeyla Gül-Zümreoğlu (1972)

İzmir Regional Agricultural pest control Research Institute has recorded two species belonging to the family Cantharidae in the catalogue of insects and general pests. This species is the new record for the given provinces (Gül-Zümreoğlu, 1972).

Table 13:Species and subspecies found from Turkey by study

Name of the species or subspecies	Location of the collected thespecies or subspecies	The current status of the species or subspecies in the World or Turkey
<i>Cantharis funebris</i>	Bornova, İzmir	New record forİzmir in Turkey.
<i>Cantharis marginiventris</i>	Bornova, İzmir	New record forİzmir in Turkey.

2.3 Neslihan Silkin

2.3.1. Neslian Silkin 2008

In her 2008 thesis, Neslihan Silkin examined the Cantharid specimens collected from various regions of Turkey between 2001 and 2008 and stored in the Zoological Museum of Gazi University. As a result of the evaluation of the sample, eight genera and 23 species belonging to two subfamilies were identified. They are new records for some provinces in Turkey (Silkin, 2008).

Table 14: Species and subspecies found from Turkey by study

Name of the species or subspecies	Location of the collected the species or subspecies	The current status of the species or subspecies in the World or Turkey
<i>Ancistronycha erichsonii</i>	Adana, Artvin, Kayseri, Konya, Mersin and Niğde	New record for Adana, Artvin, Kayseri, Konya, Mersin and Niğde in Turkey.
<i>Boveycanthis rufimana</i>	Gümüşhane	New record for Gümüşhane in Turkey.
<i>Boveycanthis tokatensis</i>	Isparta	New record for Isparta in Turkey.
<i>Canthis annularis</i>	Isparta, Kayseri, Mersin, Niğde and Yozgat	New record for Isparta, Kayseri, Mersin, Niğde and Yozgat in Turkey.
<i>Canthis flavilabris</i>	Adana, Kayseri, Mersin and Niğde	New record for Adana, Kayseri, Mersin and Niğde in Turkey.
<i>Canthis lateralis</i>	Adana, Karaman, Kayseri, Mersin, Niğde, Sivas and Yozgat	New record for Adana, Karaman, Kayseri, Mersin, Niğde, Sivas and Yozgat in Turkey.
<i>Canthis livida</i>	Adana, Ankara, Antalya, Artvin, Erzincan, Erzurum,	New record for Adana, Ankara, Antalya, Artvin,

	, İzmit, Karaman, Kocaeli, Kayseri, Konya, Mersin, Niğde and Yozgat	Erzincan, Erzurum, İzmit, Karaman, Kocaeli, Kayseri, Konya, Mersin, Niğde and Yozgat in Turkey.
<i>Cantharis longicollis</i>	Mersin	New record for Mersin in Turkey.
<i>Cantharis nigra</i>	Erzurum, İzmit, Sivas and Yozgat	New record for Erzurum, İzmit, Sivas and Yozgat in Turkey.
<i>Cantharis pulicaria</i>	Konya	New record for Konya in Turkey.
<i>Cantharis rufa</i>	Isparta, Konya, Mersin and Niğde	New record for Isparta, Konya, Mersin and Niğde in Turkey.
<i>Cantharis rustica</i>	Çankırı	New record for Çankırı in Turkey.
<i>Cantharis symrnensis</i>	Karaman, Mersin and Niğde	New record for Karaman, Mersin and Niğde in Turkey.
<i>Cordicantharis iliaca</i>	Antalya, Isparta and İzmit	New record for Antalya, Isparta and İzmit in Turkey.
<i>Metacantharis araxicola</i>	Konya	New record for Konya in Turkey.
<i>Metacantharis clypeata</i>	Ankara	New record for Ankara in Turkey.
<i>Metacantharis taurigrada</i>	Adana, Hatay, Isparta and Mersin	New record for Adana, Hatay, Isparta and Mersin in Turkey.
<i>Occathemus tarsalis</i>	Antalya, Isparta and İzmit	New record for Antalya, Isparta and İzmit in Turkey.
<i>Rhagonycha duplicata</i>	Adana, Antalya, Isparta, Konya, Karaman, Mersin and Niğde	New record for Adana, Antalya, Isparta, Konya, Karaman, Mersin and Niğde in Turkey.
<i>Rhagonycha fulva</i>	Adana, Aksaray, Çorum, Gaziantep, Gümüşhane, Hatay, İzmit, Niğde, Kahramanmaraş, Karaman, Kayseri, Kırklareli, Kocaeli, Niğde, Mersin, Osmaniye and Yozgat	New record for Adana, Aksaray, Çorum, Gaziantep, Gümüşhane, Hatay, İzmit, Niğde, Kahramanmaraş, Karaman, Kayseri, Kırklareli, Kocaeli, Niğde, Mersin, Osmaniye and Yozgat in Turkey.
<i>Rhagonycha kiesentwetteri</i>	Adana, Antalya, Konya, Karaman, Mersin and	New record for Adana, Antalya, Konya, Karaman,

	Niğde	Mersin and Niğde in Turkey.
<i>Rhagonycha lutea</i>	Adana	New record for Adana in Turkey
<i>Malthinus conspicuus</i>	Adana, Karaman, Kayseri, Manisa, Mersin and Niğde	Adana, Karaman, Kayseri, Manisa, Mersin and Niğde in Turkey.

2.4. Mustafa Sayan

2.4.1. Mustafa Sayan 2010

In his study, he identified Three species and one subspecies belonging to the family Cantharidae. One species and one subspecies are new records for Adana province (Sayan, 2008).

Table 15: Species and subspecies found from Turkey by study

Name of the species or subspecies	Location of the collected thespecies or subspecies	The current status of the species or subspecies in the World or Turkey
<i>Cantharis marginiventris marginiventris</i>	Ceyhan, İmamoğlu, Karaisalı and Kozan (Adana)	New record for Adana in Turkey
<i>Occathemus tarsalis</i>	Ceyhan, İmamoğlu, Karaisalı and Kozan (Adana)	New record for Adana inTurkey

2.5. Serkan Ertop and Ali Özpınar

2.5.1. Serkan Ertop and Ali Özpınar 2011

Cantharis decipiens is a new record for Çanakkale in their work (Ertop and Özpınar, 2011).

Table 16: Species and subspecies found from Turkey by study

Name of the species or subspecies	Location of the collected thespecies or subspecies	The current status of the species or subspecies in the World or Turkey
<i>Cantharis decipiens</i>	Çanakkale	New record forÇanakkale in Turkey

2.6. Erol Yıldırım, Andreas Kopetz and Yeşim Bulak

2.6.1. Erol Yıldırım, Andreas Kopetz and Yeşim Bulak 2011

In their study, the species belonging to *Boveycanthis*, *Cantharis*, *Cordicanthis*, *Islamocanthis*, *Metacanthis* and *Rhagonycha* have been given as new records for some Turkish provinces (Yıldırım et al., 2011).

Table 17: Species and subspecies found from Turkey by study

Name of the species or subspecies	Location of the collected thespecies or subspecies	The current status of the species or subspecies in the World or Turkey
<i>Boveycanthis rufimana</i>	Posof, Ardahan and Güngörmez Erzurum	New record forArdahan and Erzurum in Turkey
<i>Boveycanthis tokatensis</i>	Demirözü Bayburt and Çat yolu, Güngörmez, İspir, Narman (Erzurum)	New record forBayburt and Erzurum in Turkey
<i>Cantharis (Cantharis) bilunata</i>	Ilıcalar Bingöl and Mazgirt Tunceli	New record forBingöl and Tunceli in Turkey

<i>Cantharis (Cantharis) ciliciensis</i>	Posof Ardahan; Kopdağı Bayburt and İspir Erzurum	New record for Ardahan and Bayburt and Erzurum in Turkey
<i>Cantharis (Cantharis) livida</i>	Amasya; Posof (Ardahan); Ardanoç, Yusufeli (Artvin); Çalidere, Kopdağı, Aydıntepe, Demirözü (Bayburt); Bitlis; Osmancık (Çorum); Silvan (Diyarbakır); Akyazı, Geyikli, Kabataş, Refahiye, Tercan (Erzincan); Dört Yol (Hatay); Tuzluca (İğdır); Kağızman, Sarıkamış (Kars); Malatya; Pazar (Rize); Tokat; Mazgirt (Tunceli)	New record for Amasya, Ardahan, Artvin, Bayburt, Bitlis, Çorum, Erzincan, Hatay, İğdır, Kars, Malatya, Rize Tokat and Tunceli in Turkey.
<i>Cantharis (Cantharis) melaspis</i>	Çalidere, Aydıntepe, Demirözü (Bayburt); Çat yolu, Kırkgözeler, Yeşilyayla, Aşkale, Aziziye, Kayapa, Paşayurdu, Horasan, Köprüköy, Pasinler, Büyükdere, Tortum, Uzundere (Erzurum); Akyazı, Refahiye (Erzincan) and Tuzluca (İğdır)	New record for Bayburt, Erzurum, Erzincan and İğdır in Turkey
<i>Cantharis (Cantharis) terminata</i>	Kopdağı Bayburt	New record for Bayburt in Turkey
<i>Cantharis (Cyrtomoptila) lateralis</i>	Bayburt; Güzelova, Aşkale, Çayköy, Olur, Tortum, Kireçdağı (Erzurum)	New record for Bayburt and Erzurum in Turkey
<i>Cordicantharis bodemeyeri</i>	Mazgirt Tunceli	New record for Tunceli in Turkey
<i>Islamocantharis orientalis</i>	Tortum, Erzurum	New record for Erzurum in Turkey
<i>Metacantharis araxicola</i>	Çat yolu Erzurum	New record for Erzurum in Turkey
<i>Metacantharis clypeata</i>	Kopdağı (Bayburt) and Güngörmez (Erzurum)	New record for Bayburt and Erzurum in Turkey

<i>Metacantharis rosinae</i>	Bayburt; Çat yolu, Aziziye, Aşkale, Tortum (Erzurum) and Tuzluca Iğdır	New record for Bayburt, Erzurum and Iğdır in Turkey
<i>Metacantharis taurigrada</i>	Mazgirt (Tunceli)	New record for Tunceli in Turkey
<i>Metacantharis walteri</i>	Tortum Erzurum	New record for Erzurum in Turkey.
<i>Rhagonycha aliena</i>	Bayburt; Gölpazarı (Bilecik); Akyazı, Mercan, Refahiye (Erzincan); İspir, Oltu (Erzurum); Dört Yol (Hatay); Halkapınar (Konya) and Suluç (Şanlıurfa)	New record for Bayburt, Bilecik, Erzincan, Erzurum, Hatay, Konya and Şanlıurfa in Turkey.
<i>Rhagonycha delagrangei</i>	Güngörmez, Kabaköbek, Yeşilyayla (Erzurum)	New record for Erzurum in Turkey.
<i>Rhagonycha fulva</i>	Oltu Erzurum	New record for Erzurum in Turkey.
<i>Rhagonycha fulvaliena</i>	Antalya, Eskişehir, Giresun and Beyşehir (Konya)	New record for Antalya, Eskişehir, Giresun and Konya in Turkey.
<i>Rhagonycha kronbladi</i>	Güngörmez (Erzurum)	New record for Erzurum in Turkey.
<i>Rhagonycha ruzickai</i>	Güngörmez (Erzurum)	New record for Erzurum in Turkey.

2.7. Osman Sert and Mahmut Kabalak

2.7.1. Osmn Sert and Mahmut Kabalak 2013

Their study identified the insect fauna of Inkumu Bartın and gave *Cantharis livida* as a new record for Bartın province (Sert and Kabalak, 2013).

Table 17: Species and subspecies found from Turkey by study

Name of the species or subspecies	Location of the collected thespecies or subspecies	The current status of the species or subspecies in the World or Turkey
<i>Cantharis livida</i>	İnkumu, Bartın	New record forBartın in Turkey

2.8. Ozan Demirözer and İsmail Karaca

2.8.1.Ozan Demirözer and İsmail Karaca 2014

In their studie, they reported four species belonging to the family Cantharidae. Three of them are new records for the province of Isparta (Demirözer and Karaca, 2014).

Table 18: Species and subspecies found from Turkey by study

Name of the species or subspecies	Location of the collected thespecies or subspecies	The current status of the species or subspecies in the World or Turkey
<i>Cantharis prusiensis</i>	Gölcük, Yakaören and Keçiborlu (Isparta)	New record for Isparta in Turkey
<i>Cantharis marginiventris</i>	Gölcük, Yakaören and Gönen (Isparta)	New record for Isparta in Turkey
<i>Rhagonycha fulva</i>	Gölcük, Yakaören, Eğirdir and Gönen (Isparta)	New record for Isparta in Turkey

2.9. Deniz Sezer

2.9.1 Deniz Sezer 2018

She recorded species *Cantharis livida* from Eşelek Village in Gökçeada in her master thesis "Faunistic studies on species belonging to the order of Gökçeada and Bozcaada Coleoptera" (Sezer, 2018).

Table 19: Species and subspecies found from Turkey by study

Name of the species or subspecies	Location of the collected thespecies or subspecies	The current status of the species or subspecies in the World or Turkey
<i>Cantharis livida</i>	Gökçeada, Eşelek Köyü Çanakkale	New record forÇanakkale in Turkey

3. RESULTS

The contribution to the Cantharidae fauna of Turkey has been made mostly by non-Turkish researchers asthe number of Turkish scientists working on Cantharidae family remained limited. Walter Wittmer gave a total of 26 new species, one new subspecies and one new record to the fauna of Turkey. The table below summarizes Wittmer's work.

Name of the Genus	Total contribution to the fauna of Turkey
<i>Boveycantharis sp.</i>	4 new species, 1 subspecies
<i>Cantharis sp.</i>	3 new species, 1 new record for Turkey
<i>Cratosilis sp.</i>	1 new species

<i>Malthinus sp.</i>	2 new species
<i>Malthodes sp.</i>	2 new species
<i>Rhagonycha sp.</i>	13 new species
<i>Sinometa sp.</i>	1 new species

Vladimir Svihla has given a total of 35 new species, six new subspecies and three new records to the fauna of Turkey. The table below summarizes Svihla's work.

Name of the Genus	Total contribution to the fauna of Turkey
<i>Boveycantharis sp.</i>	3 new species
<i>Cordicantharis sp.</i>	1 new species
<i>Malthinus sp.</i>	8 new species, 2 new subspecies
<i>Malthodes sp.</i>	14 new species, 4 new subspecies
<i>Rhagonycha sp.</i>	9 new species, 3 new record for Turkey

According to the catalogue created by Brancucci and Kazantsev (2007), the total number of genera in Turkey consist of 12 species of *Boveycantharis*, 28 species of *Cantharis*, seven species of *Cordicantharis*, 51 species of *Malthinus*, 64 species of *Malthodes*, seven species of *Metacantharis* and 59 species of *Rhagonycha*. A study by Svihla in 2009 recorded three new species belonging to the genus *Malthinus* and five new species belonging to the genus *Malthodes*, contributing to the fauna of Turkey's Cantharidae. Thus, a total of 54 species belonging to the genus *Malthinus* and a total of 69 species belonging to the genus *Malthodes* have been recorded in Turkey. Walter Wittmer and Vladimir Svihla (Wittmer, 1969, 1971, 1972, 1993; Svihla, 1993, 1994, 1998, 1999, 2002, 2009) about 58%

of the species belonging to the genus *Boveycantharis*, about 19% of the species belonging to the genus *Malthinus*, 23% of the species belonging to the genus *Malthodes* and 37% of the species belonging to the genus *Rhagonycha* were included in the fauna of Turkey.

Turkish researchers have often given new record species for the provinces of Turkey. As a result of this study, it is understood that not enough studies have been done to reveal the biological richness of the Turkish fauna. In comparison, researchers from developed countries have not only been working hard to protect their biological wealth, but also attempt to contribute to the biological exploration of other countries such as Turkey as well. Although the number and scope of the studies conducted by Turkish researchers on Cantharidae of Turkey increases and expands beginning especially with Silkin's work in 2008, Turkish fauna has not been fully identified yet. Knowing that benefitting from the biological diversity depends on the exploration and conservation of the species, Turkish researchers' need to allocate more resources to this end becomes more important than ever.

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