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MAUNFBD

Muş Alparslan Üniversitesi Fen Bilimleri Dergisi
Mus Alparslan University Journal of Science

YIL/YEAR: 2024 ● CİLT/VOLUME: 12 ● SAYI/NUMBER: 1

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Muş Alparslan Üniversitesi Fen Bilimleri Dergisi

Mus Alparslan University Journal of Science

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Muş Alparslan Üniversitesi / Türkiye
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Amaç ve Kapsam

Muş Alparslan Üniversitesi Fen Bilimler Dergisi, temel bilimler, mühendislik bilimleri, çevre ve enerji alanlarında ulusal ve uluslararası düzeyde yapılan bilimsel nitelikli ve özgün çalışmaları bilimsel bir yaklaşımla ele almak amacıyla yayımlanan uluslararası hakemli bir dergidir. Muş Alparslan Üniversitesi Fen Bilimleri Dergisinin temel amacı; uluslararası alanda bilim ve teknolojideki yenilikler ve gelişmeler, güncel ortaya konulan bilimsel çalışmalar, tespit edilen sorunların ve çözüm önerilerinin tartışıldığı özgün ve nitelikli makaleler yayınlanan bilimsel bir dergi olmaktır. Ayrıca Muş Alparslan Üniversitesi Fen Bilimleri Dergisi, yükseköğretim kurumlarında görev alan akademisyenler, lisansüstü öğrenciler, sanayi ve endüstride çalışan kişilerin akademik ve mesleki gelişimlerine katkı sağlayan bilimsel, nitelikli akademik çalışmaların yaygınlaştırılmasına hizmet etmeyi hedeflenmektedir.

Muş Alparslan Üniversitesi Fen Bilimleri Dergisi; temel bilimleri, tarım ve uygulamalı bilimleri, doğa bilimleri ve mühendislik alanları ile alakalı konularda özgün ve nitelikli bilimsel çalışmaları kapsamaktadır. Dergide, yukarıda belirtilen alanlarda yapılmış deneysel ve teorik ilerlemeleri içeren bilimsel ve özgün araştırma makalesi türündeki bilimsel çalışmalara ve güncel içerikli derlemelere yer verilmektedir. Dergide yayımlanan tüm makalelere DOI numarası atanmakta ve yayımlanan makaleler için herhangi bir ücret talep edilmemektedir. Muş Alparslan Üniversitesi Fen Bilimleri Dergisinde yayımlanan yazıların bilimsel ve hukukî sorumluluğu, yazarlarına aittir. Yayımlanan yazıların bütün yayın hakları Muş Alparslan Üniversitesi'ne ait olup yayın, yayıncının izni olmadan kısmen veya tamamen elektronik ortama taşınamaz. Muş Alparslan Üniversitesi Fen Bilimleri Dergisi, özgün bilimsel araştırmalar ile uygulama çalışmalarına yer veren Haziran ve Aralık sayısı olmak üzere yılda iki defa düzenli olarak yayımlanan bir dergidir.

Muş Alparslan Üniversitesi Fen Bilimler Dergisi aşağıdaki indekslerce taranmaktadır:

- **TR Dizin**
- Academic keys
- CAB Abstract
- CiteFactor
- COSMOS IF
- CrossRef DOI
- DRJI (Directory of Research Journals Indexing)
- Eurasian Scientific Journal Index (ESJI)
- Index Copernicus
- Infobase index
- International Innovative Journal Impact Factor (IIJIF),
- ResearchBib
- ROAD
- Scientific Indexing Service
- Sindex
- SOBİAD

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Aims and Scope

Muş Alparslan University Journal of Science is an international refereed journal that is published with a scientific approach to handle scientific and original studies in the fields of basic sciences, engineering sciences, environment and energy. The main aim of Muş Alparslan University Science Journal is to become a scientific journal which published original and qualified articles, current scientific studies, their identified problems, and their solution suggestions, discussing innovations and developments in science and technology in the international surroundings. In addition, Muş Alparslan University Journal of Sciences is aimed to serve the dissemination of scientific and qualified academic studies which contributed to the academic and professional development of academicians, graduate students, working people in industry.

Muş Alparslan University Journal of Science covers original and qualified scientific studies in the fields of basic sciences, agriculture and applied sciences, natural sciences, and engineering. There are scientific, original research articles and current content reviews that include experimental and theoretical advances mentioned above in the fields in the journal. All published articles in the journal are assigned a DOI number and no fee is charged for the published articles. The authors belong to scientific and legal responsibility of the articles published in Muş Alparslan University Journal of Science. Muş Alparslan University belongs to all publishing rights of the published articles, and it cannot be published to the electronic medium partially or completely without the permission of the publisher. Muş Alparslan University Journal of Science including the June and December issues is a regular journal published twice a year that is included original scientific research and application studies.Mus Alparslan University Journal of Science is included in the following abstracting and indexing services:

- **TR Dizin**
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MAUNFBD Dergi Yayın Etiği ve Sorumluklar

MAUNFBD Dergisinde uygulanan yayın süreçlerinde yazarlar, hakemler ve editörler etik ilkelere yönelik standartlara uyması önem taşımaktadır. **MAUNFBD** Dergisinde yayın etiği kapsamında tüm yazarlar, hakemler ve editörler aşağıdaki etik sorumlulukları taşıması beklenmektedir. Aşağıda yer alan etik görev ve sorumluluklar oluşturulurken açık erişim olarak **Committee on Publication Ethics (COPE)** tarafından yayınlanan etik kurallara ve sorumluluklar dikkate alınarak hazırlanmıştır.

Yazarların Etik Sorumlulukları

Yazar(lar)ın gönderdikleri çalışmaların özgün olması beklenmektedir. Yazar(lar)ın başka çalışmalardan yararlanmaları veya başka çalışmaları kullanmaları durumunda eksiksiz ve doğru bir biçimde atıfta bulunmaları ve/veya alıntı yapmaları gerekmektedir. Çalışmanın oluşturulmasında içeriğe katkı sağlamayan kişiler, yazar olarak eklenmemelidir. Yazarlar çalışmalarını aynı anda birden fazla derginin başvuru sürecinde bulunduramaz. Her bir başvuru önceki başvurunun tamamlanmasını takiben başlatılabilir. Başka bir dergide yayınlanmış çalışma **MAUNFBD** Dergisine gönderilemez. Yayınlanmak üzere gönderilen tüm çalışmaların varsa çıkar çatışması teşkil edebilecek durumları ve ilişkileri açıklanmalıdır. Yazar(lar)dan değerlendirme süreçleri çerçevesinde makalelerine ilişkin ham veri talep edilebilir, böyle bir durumda yazar(lar) beklenen veri ve bilgileri yayın kurulu ve bilim kuruluna sunmaya hazır olmalıdır. Değerlendirme süreci başlamış bir çalışmanın yazar sorumluluklarının değiştirilmesi (Yazar ekleme, yazar sırası değiştirme, yazar çıkartma gibi) teklif edilemez. Yazar(lar) kullanılan verilerin kullanım haklarına, araştırma/analizlerle ilgili gerekli izinlere sahip olduklarını veya deney yapılan deneklerin rızasının alındığını gösteren belgeye sahip olmalıdır. Yazar(lar)ın yayınlanmış, erken görünüm veya değerlendirme aşamasındaki çalışmasıyla ilgili bir yanlış ya da hatayı fark etmesi durumunda, dergi editörünü veya yayıncıyı bilgilendirme, düzeltme veya geri çekme işlemlerinde editörle iş birliği yapma yükümlülüğü bulunmaktadır.

Editörlerin Etik Görev ve Sorumlulukları

MAUNFBD Dergisindeki editörler ve alan editörleri, açık erişim olarak Dergipark sayfasında yayınlanan **Committee on Publication Ethics (COPE)** tarafından belirtilen etik görev ve sorumluluklara sahip olmalıdır:

Genel Görev ve Sorumluluklar

Sürekli olarak derginin gelişimini sağlama, dergide yayınlanan çalışmaların kalitesini geliştirmeye yönelik süreçleri yürütme, okuyucuların ve yazarların bilgi ihtiyaçlarını karşılamaya yönelik çaba sarfetme, düzeltme, açıklama gerektiren konularda yayın açısından açıklık ve şeffaflık gösterme. Fikri mülkiyet hakları ve etik standartlardan taviz vermeden iş süreçlerini devam ettirme editörün görev ve sorumluluklarındandır.

Hakemlerin Etik Sorumlulukları

Sadece uzmanlık alanı ile ilgili çalışma değerlendirmeyi kabul etmelidir. Tarafsızlık ve gizlilik içerisinde değerlendirme yapmalıdır. Gizlilik ilkesi gereği inceledikleri çalışmaları değerlendirme sürecinden sonra imha etmelidir. Değerlendirme sürecinde çıkar çatışması ile karşı karşıya olduğunu düşünürse, çalışmayı incelemeyi reddederek, dergi editörünü bilgilendirmelidir. Değerlendirmeyi nesnel bir şekilde sadece çalışmanın içeriği ile ilgili olarak yapmalıdır. Değerlendirmeyi yapıcı ve nazik bir dille yapmalıdır. Düşmanlık, iftira ve hakaret içeren aşağılayıcı kişisel yorumlar yapmamalıdır. Değerlendirmeyi kabul ettikleri çalışmayı zamanında ve yukarıdaki etik sorumluluklarda gerçekleştirmelidir.

Yayıncının Etik Sorumlukları

MAUNFBD Dergisinde gönderilen çalışmaların tüm süreçlerinden editör sorumludur. Bağımsız editör kararı oluşturulmasını taahhüt eder. **MAUNFBD** Dergisinde ekonomik ya da politik kazançlar göz önüne alınmaksızın karar verici kişi editördür. **MAUNFBD** Dergisinde yayınlanmış her makalenin mülkiyet ve telif hakkını korumak zorundadır. Editöre ilişkin her türlü bilimsel suiistimal ve intihalle ilgili önlemleri alma sorumluluğuna sahiptir.

Yazarlar ile İlişkiler

Editör, çalışmaların önemi, özgün değeri, geçerliliği, anlatımın açıklığı ve derginin amaç ve hedeflerine dayanarak olumlu ya da oluMAUNz karar vermelidir. Yayın kapsamına uygun olan çalışmaların ciddi problemi olmadığı sürece ön değerlendirme aşamasına almalıdır. Editör, çalışma ile ilgili ciddi bir sorun olmadıkça, olumlu yöndeki hakem

önerilerini göz ardı etmemelidir. Yeni editör, çalışmalara yönelik olarak önceki editör tarafından verilen kararları ciddi bir sorun olmadıkça değiştirmemelidir. **MAUNFBD** Dergisinde bir Yazar Rehberi yayınlamalıdır. Yazarlara açıklayıcı ve bilgilendirici şekilde bildirim ve dönüş sağlanmalıdır.

Hakemler ile İlişkiler

Editör; dergi yayın politikalarında yer alan **Kör Hakemlik ve Değerlendirme Süreci** politikalarını uygulamakla yükümlüdür. Hakemleri yayının alan konusuna uygun olarak seçilmelidir. Yayının değerlendirme sürecinde gerekli tüm bilgileri hakemlere sağlamakla yükümlüdür. Yazarlar ve hakemler arasından çıkar çatışması olup olmadığını gözetmek durumundadır. Yayının değerlendirme sürecinde hakemlerin kimlik bilgilerini gizli tutmalıdır. Hakemleri tarafsız, bilimsel ve nesnel bir dille çalışmayı değerlendirmeleri için teşvik etmelidir. Hakem havuzunun geniş bir yelpazeden oluşması için adımlar atmalıdır. Hakemlerin performansını artırıcı uygulama ve politikalar belirlemelidir. Bilimsel olmayan değerlendirmeleri engellemelidir.

Okuyucu ile İlişkiler

Editör tüm okuyucuların ihtiyaç duydukları bilgi, beceri ve deneyim beklentilerini dikkate alarak karar vermelidir. Yayınlanan çalışmaların okuyucu, araştırmacı, uygulayıcı ve bilimsel literatüre katkı sağlamasına ve özgün nitelikte olmasına dikkat etmelidir. Editör okuyuculardan gelen geri bildirimleri dikkate almak, açıklayıcı ve bilgilendirici geri bildirim vermekle yükümlüdür.

Yayın Kurulu ile İlişkiler

Editör, tüm yayın kurulu üyelerinin süreçleri yayın politikaları ve yönergelere uygun ilerletmesini sağlamalıdır. Yayın kurulu üyelerini yayın politikaları hakkında bilgilendirmeli ve gelişmelerden haberdar etmelidir. Yeni yayın kurulu üyelerini yayın politikaları konusunda eğitmeli, ihtiyaç duydukları bilgileri sağlamalıdır.

Dergi Sahibi ve Yayıncı ile İlişkiler

Editör ile yayıncı arasında yapılan yazılı sözleşme gereği, editörün alacağı tüm kararlar yayıncı ve dergi sahibinden bağımsızdır. Yani editör ve yayıncı arasındaki ilişki bağımsızlık ilkesine dayanmaktadır.

Kişisel Verilerin Korunması

Editör; değerlendirilen çalışmalarda yer alan deneklere veya görsellere ilişkin kişisel verilerin korunmasını sağlamakla yükümlüdür. Çalışmalarda kullanılan bireylerin açık rızası belgeli olmadığı sürece çalışmayı reddetmekle görevlidir. Ayrıca editör; yazar, hakem ve okuyucuların bireysel verilerini korumaktan sorumludur.

Etik Kurul, İnsan ve Hayvan Hakları

Editör; değerlendirilen çalışmalarda insan ve hayvan haklarının korunmasını sağlamakla yükümlüdür. Çalışmalarda kullanılan deneklere ilişkin etik kurul onayı, deneysel araştırmalara ilişkin izinlerin olmadığı durumlarda çalışmayı reddetmekle sorumludur.

Olası Suiistimal ve Görevi Kötüye Kullanmaya Karşı Önlem

Editör; olası suiistimal ve görevi kötüye kullanma işlemlerine karşı önlem almakla yükümlüdür. Bu duruma yönelik şikayetlerin belirlenmesi ve değerlendirilmesi konusunda titiz ve nesnel bir soruşturma yapmanın yanı sıra, konuyla ilgili bulguların paylaşılması editörün sorumlulukları arasında yer almaktadır.

Fikri Mülkiyet Haklarının Korunması

Editör; yayınlanan tüm makalelerin fikri mülkiyet hakkını korumakla, olası ihlallerde derginin ve yazar(lar)ın haklarını savunmakla yükümlüdür. Ayrıca editör yayınlanan tüm makalelerdeki içeriklerin başka yayınların fikri mülkiyet haklarını ihlal etmemesi adına gerekli önlemleri almakla yükümlüdür. Bu aşamada yazarlardan makaleleri ile birlikte almış oldukları intihal raporu talep edilmektedir.

MAUNFBD Dergisinde Etik Olmayan Bir Durumla Karşılaşırsanız!

MAUNFBD Dergisinde yukarıda bahsedilen etik sorumluluklar ve dışında etik olmayan bir davranış veya içerikle karşılaşırsanız lütfen **h.onlu@alparslan.edu.tr** adresine e-posta yoluyla bildiriniz.

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MAUNFBD Journal Editorial Ethics and Responsibilities

It is important for authors, referees and editors to comply with the standards regarding ethical principles in the publication processes applied in the Journal of MAUNFBD. All authors, referees and editors are expected to have the following ethical responsibilities within the scope of publication ethics in MAUNFBD journal. The following ethical duties and responsibilities have been prepared as open access, taking into account the ethical rules and responsibilities published by the Committee on Publication Ethics (COPE).

Authors' Ethical Responsibilities

The works submitted by the author (s) are expected to be original. If the author (s) benefit from other studies or use other studies, they must cite and / or cite completely and accurately. People who do not contribute to the content of the study should not be added as author. The authors work in the application process can not contain more than one journal at a time. The work published in another journal cannot be sent to the MAUNFBD Journal. That might constitute a conflict of interest if all studies submitted for publication must be explained and relationships. Author (s) can be requested from the evaluation process raw data of the frame in the article, in such a case the author (s) must be ready to provide the expected data and information science committee and the editorial board. Replacing the responsibility of the author began a study of the evaluation process (authors add, modify order of authors, writers like stickers) cannot be offered. The author (s) must have a document showing that they have the right to use the data used, the necessary permissions for research / analysis, or that the subjects who have been experimented with have consent. Author (s) of the published case early view or assessment notice a wrong or error about her work on stage, to inform the journal editor or publisher has an obligation to carry editors to cooperate in the correction or retraction.

Editors' Ethical Duties and Responsibilities

Editors and field editors in the MAUNFBD Journal should have the ethical duties and responsibilities specified by the Committee on Publication Ethics (COPE) published on the Dergipark page as open access:

General Duties and Responsibilities

Continuously improving the quality of the journal, carrying out processes to improve the quality of the work published in the journal, striving to meet the information needs of readers and authors, correcting, showing publicity and transparency in matters requiring explanation, continuing business processes without compromising intellectual property rights and ethical standards is one of his duties and responsibilities.

Ethical Responsibilities of Referees

Only study related to the specialty should accept the assessment. It should evaluate in impartiality and confidentiality. The study examined the privacy policy should be destroyed after the evaluation process. If referee thinks that he/she faces a conflict of interest during the evaluation process, he should refuse to review the study and inform the journal editor. The referee should make the assessment objectively only in relation to the content of the study. Referee should make the assessment in a constructive and kind language. It should not make humiliating personal comments that include hostility, slander and insults. They should perform the work they accepted to evaluate on time and with the ethical responsibilities above.

Publisher's Ethical Responsibilities

The editor is responsible for all the processes submitted in the MAUNFBD Journal. The independent editor commits to the decision. The decision maker is the editor, regardless of economic or political gains in the Journal of MAUNFBD. It must protect the property and copyright of every article published in the MAUNFBD journal. It has the responsibility to take all sorts of scientific abuse and plagiarism related measures.

Relations with Authors

Editor, the importance of the work, the original value, validity, the openness of expression and should give a positive or negative decision based on the journal's goals and objectives. Unless there is a serious problem for the studies that are suitable for publication, they should take the preliminary evaluation stage. The editor should not ignore the positive reviews of the referees unless there is a serious problem with the work. The new editor should not change the decisions made by the previous editor for the work unless there is a serious problem. It should publish an Author Directory in the MAUNFBD journal. Authors should be provided with informative and informative feedback and feedback.

Relations with Referees

Editor is responsible for applying the Blind Review and Evaluation Process policies included in its publication policies. Referees should be selected in accordance with the subject of the publication. It is obliged to provide all necessary information to the referees during the evaluation process of the publication. It has to observe whether there is a conflict of interest among the authors and reviewers. The identity information of the referees must be kept confidential during the evaluation of the broadcast. Encourage referees to evaluate working in an objective, scientific and objective language. The referee should take steps to make the pool a broad spectrum. Implementations and policies that increase the performance of the referees should be determined. It should prevent unscientific evaluations.

Relations with Readers

The editor should make a decision by considering the knowledge, skills and experience expectations of all readers. The reader should pay attention to the fact that the published studies contribute to the reader, researcher, practitioner and scientific literature and be original. The editor is obliged to take into account the feedback from the readers and to provide explanatory and informative feedback.

Relations with the Editorial Board

The editor should ensure that all editorial board members advance the processes in accordance with editorial policies and guidelines. The editorial board should inform the members of the editorial policies and inform the developments. The new editorial board should educate its members on broadcast policies and provide the information they need.

Relations with the Owner of the Journal and the Publisher

In accordance with the written contract between the editor and the publisher, all decisions taken by the editor are independent of the publisher and magazine owner. In other words, the relationship between the editor and the publisher is based on the principle of independence.

Protection of Personal Data

The editor is obliged to ensure the protection of personal data related to the subjects or images in the evaluated works. Unless the explicit consent of the individuals used in the studies is documented, they are responsible for refusing to work. Also, editor is responsible for protecting the individual data of the author, referee and readers.

Ethics Committee, Human and Animal Rights

The editor is obliged to ensure the protection of human and animal rights in the evaluated works. Ethics committee approval for subjects used in studies is responsible for refusing to work in cases where there is no permit for experimental research.

Measure Against Potential Abuse and Abuse

The editor is obliged to take measures against possible abuse and misconduct. In addition to conducting a rigorous and objective investigation about the identification and evaluation of complaints for this situation, sharing the findings on the subject is among the responsibilities of the editor.

Protection of Intellectual Property Rights

The editor is obliged to protect the intellectual property right of all published articles and to defend the rights of the journal and author (s) in possible violations. In addition, the editor is obliged to take necessary precautions so that the content in all published articles does not violate the intellectual property rights of other publications. At this stage, the plagiarism report they received with the articles is requested from the authors.

NOTE: If you encounter an unethical situation in MAUNFBD Journal!

If you encounter any ethical responsibilities and unethical behavior or content mentioned above in MAUNFBD Journal, please report it to **h.onlu@alparslan.edu.tr** by e-mail.

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h.onlu@alparslan.edu.tr

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Strength Values of Soil Stabilized with Fly Ash, Lime, and Seawater

Gamze BİLGEN [1], Zekeriya DOĞAN [2]
[1] İnşaat Mühendisliği, Mühendislik Fakültesi, Zonguldak Bülent Ecevit Üniversitesi, Zonguldak, Türkiye
[2] İnşaat Mühendisliği, Mühendislik Fakültesi, Zonguldak Bülent Ecevit Üniversitesi, Zonguldak, Türkiye

✉: gamzebilgen@beun.edu.tr [1] 0000-0002-2840-7369 [2] 0000-0002-2721-4450

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ABSTRACT

This study investigates the strength values of soil that was treated with fly ash (FA) and lime additives. Besides the effects of additives on the mechanical behavior of soil, feasibility of using seawater as kneading water was examined. For these purposes, a number of geotechnical tests were carried out on the samples, including sieve analysis, California bearing ratio (CBR), consistency limits, proctor, and unconfined compressive strength (UCS) tests. Fourier transform infrared (FT-IR) and scanning electron microscope (SEM) analyses were also conducted to describe the structural properties of FA. Test results showed that the UCS and CBR values of the soil were 134 kPa and 3.1 %, respectively. In the mixture where all additives were used together, the UCS and CBR values increased up to 846 kPa and 16.3 % after 28 days of the curing period.

Keywords: Fly ash, Lime, Seawater, Soil stabilization

Uçucu Kül, Kireç ve Deniz Suyu ile Stabilize Edilen Bir Zeminin Dayanım Değerleri

ÖZ

Sunulan bu çalışmada, uçucu kül (FA) ve kireç katkıları uygulanan bir zeminin dayanım değerleri, deneysel olarak incelenmiştir. Çalışmada katkıların etkilerinin yanında, yoğurma suyu olarak deniz suyu kullanılmasının etkisi de irdelenmiştir. Deneysel çalışmalarda, numuneler üzerinde elek analizi, kıvam limitleri, Proctor deneyi, serbest basınç (UCS) ve Kaliforniya taşıma oranı (CBR) deneyleri uygulanmıştır. Ayrıca FA'nın yapısal özelliklerini tanımlamak için Fourier dönüşümü kızılötesi (FT-IR) analizinin yanında taramalı elektron mikroskobu (SEM) görüntülerinden de faydalanılmıştır. Deney sonuçları, zeminin UCS ve CBR değerlerinin sırası ile 134 kPa ve % 3,1 olduğunu, 28 günlük kür sonucunda tüm katkıların birlikte kullanıldığı karışımda bu değerlerin sırası ile 846 kPa ve % 16,3 değerine yükseldiğini göstermiştir.

Anahtar Kelimeler: Deniz suyu, Kireç, Uçucu kül, Zemin iyileştirme

INTRODUCTION

During the implementation of additives as a means of soil stabilization, the use of seawater instead of fresh water has attracted significant attention in recent years [1–4]. Using seawater in construction works instead of fresh water will contribute to the sustainable conservation of dwindling clean water resources. It has been officially reported by the World Meteorological Organization (WMO) that the clean water resources in the world are rapidly decreasing [5–7]. In addition, using seawater, especially in construction works near the sea, will contribute to reducing undesirable costs (i.e., transportation, fuel consumption) and environmental effects such as exhaust emissions (i.e., from vehicles) since clean water will not be needed for transportation [8-9].

In addition to the decrease in clean water resources, the increase in waste volume and the damage they cause to the environment are also prominent issues [10–14].

Fly ash (FA), which is a waste generated as a result of coal combustion, is based on silicate and aluminate as the chemical composition [15,16]. FA has been researched on its use in different application areas such as ceramic industries and road construction applications. It is also interpreted as a potential raw material for the synthesis of materials such as porous silica. Moreover, FA has a pozzolanic effect depending on its fineness and the amount of free lime it contains, and therefore it is a waste material to be used in soil stabilization [16,17]. FA is already available in powder form, and the reuse of pulverized waste is handled in different ways in the published literature [10,17,18]. Previous studies have shown that the use of FA together with lime is more effective in improving geotechnical properties [13,19].

The effects of FA and lime on the mechanical properties of the soil are widely studied in the published literature. However, there is a lack of research examining the feasibility of using seawater in the soil stabilization process, and this study aims to fill this gap. In addition, available studies mostly focus on the effects of additives

on clayey soils, whereas this study places its main focus on silty sand (SM).
In the present work, the test samples were prepared by blending the additives and soil, namely, silty sand. Seawater was used as kneading water in the application of FA and lime which were used as additives during the soil stabilization process. Eventually, the mechanical and geotechnical properties of treated soil samples were determined to assess the geotechnical suitability of the additives with seawater.

MATERIAL and METHODS

Materials

The FA used as an additive is obtained from the chimneys of Çatalağzı Thermal Power Plant in Zonguldak province. The FA is an F-type fly ash. Lime, the other additive, is hydrated lime purchased from the market. The soil used was obtained from the vicinity of Alapli district, approximately 0.8-1.0 meters below the surface. Seawater was obtained from the shores of the town of Alapli, located on the Black Sea coast.

Methods

In the experimental study, the geotechnical properties of the soil were determined. In addition, Fourier transform infrared (FT-IR) analyses were applied and Scanning Electron Microscopy (SEM) images were utilized to describe the structural properties of FA used as an additive material. XRF analysis was performed with the Rigaku ZX Primus-2 instrument.

Table 1. Granulometric values of the soil and FA

Property	N	F
Gravel (%)	13	0
Sand (%)	47	0
Fine (%)	40	100
D_{10}	0.021	0.003
D_{30}	0.050	0.013
D_{60}	0.166	0.033
Coefficient of uniformity (C_u)	7.89	11.00
Coefficient of curvature (Cc)	0.72	1.71
Classification (USCS)	SM	ML
Classification (AASHTO)	A-2-6	A-4

The soil classification was made in accordance with both the Unified Soil Classification System (USCS) and the American Highways Soil Classification System (AASHTO). Then, mixtures were prepared by using 5 % FA and 5 % lime additives.
Two sets of samples were prepared in the same conditions. The only difference was the type of kneading water used, which was either tap water or seawater.
Specific gravity [20], organic matter determination [21], consistency limits [22], sieve analysis [23], and hydrometer tests [24] were conducted on the prepared samples for soil classification. This was followed by the modified Proctor tests [25], which were performed to identify the water-density relationships. The optimum water (moisture) content (OWC) and maximum dry density (MDD) parameters of mixtures were determined for all mixtures. These values were used in the preparation of the strength test samples.

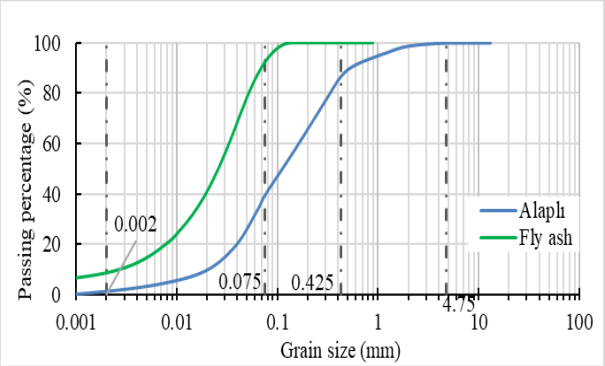

Figure 1. Granulometry curves of the soil and FA

The strength tests were performed on the blends prepared according to the OWC of the relevant mixture. The tests were performed 0-day (in an hour after blending), 7-day, and 28-day curing period. Samples were kept in a desiccator after being wrapped in airtight plastic bags and labeled for the test of 7 days and 28 days. Finally, the Unconfined Compressive Strength (UCS) test [26] and California Bearing Ratio (CBR) test [27] were performed to determine the strength values of the blends.
The materials were symbolized as follows: the soil is "N", FA is "F", lime is "L", tap water is "T" and sea water is "S". Thus, for example, the sample prepared with tap water was coded as "NT", and the sample prepared with seawater and lime was coded as "NLS". The codes and components of all mixtures are shown in Table 4.

Table 2: XRF analysis of FA

Oxide	%	Oxide	%
Al_2O_3	25.6586	Na_2O	1.0116
CaO	5.4701	P_2O_5	1.3392
Fe_2O_3	7.6509	SiO_2	51.9666
K_2O	2.3516	TiO_2	1.1865
MgO	1.7316	SO_3	1.5436
MnO	0.0679	Cl	0.0219

RESULTS and DISCUSSION

The physical properties and the grain distribution curves of the soil used, and FA are presented in Table 1 and Figure 1, respectively. The soil is classified as SM, and FA is classified as low plasticity silt (ML) according to the USCS. The specific gravity (Gs) is 2.65, the Atterberg limits are 29.3 % for liquid limit (LL) and 25 % for plastic limit (PL). The soil has 0.5 % organic matter (OMC). The OWC and MDD were determined as 17.2 % and 15.2 kN/m^3, respectively.
The chemical composition of FA can be seen in Figure 2, Figure 3, and Table 2. The FA were analyzed at the Düzce University Scientific and Technological Research Application and Research Center (DUBIT) using the

SEM Quanta FEG250 FEI. SEM is a device used to image the surface texture of each particle in detail as well as its morphology.

The 1410 cm^{-1} peak belongs to symmetric and asymmetric CH3–deformation vibrations. The absorption peak of the C–H bond, which belongs to the aromatic functional group, is at 777 cm^{-1}, corresponding to the out-of-plane bending vibration, and the Si-O-(Si) bending vibration peak is at 678 cm^{-1}. Therefore, the peak is thought to occur at 999 cm^{-1}. This vibration means stretching vibration consisting of silicon oxide or alumina such as Si-O-Si or Si-O-Al.

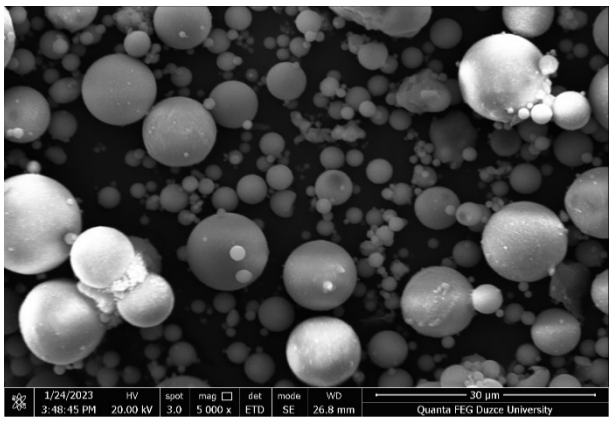

Figure 2. SEM image

While controlling the morphology of FA, combustion temperature and cooling rate were taken into account. SEM image, as seen in Figure 2, shows that fly ash is in spherical lumps.

The range of particle sizes is from less than 1 µm to greater than 8 µm in this study.

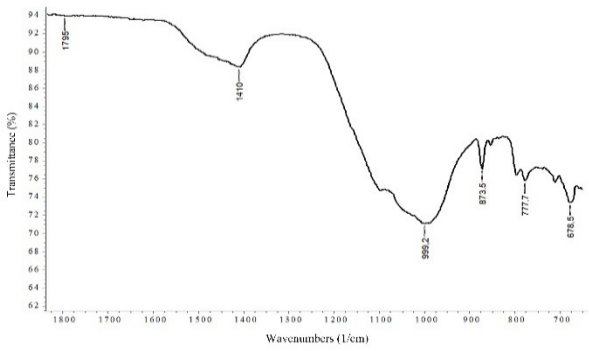

Figure 3. FT-IR spectrum of FA

Silicon oxide and aluminum oxide ratios in fly ash are in the order of 77.6252 % as seen in Table 2. Recordings of infrared spectra were carried out at room temperature and a Perkin-Elmer Spectrum 100 FT-IR Spectrophotometer instrument was used. The instrument features an attenuated total reflection (ATR) accessory containing a zinc selenide (ZnSe) crystal. The wavelength used in the analysis is in the range of 400–4000 cm^{-1} as seen in Figure 3.

The consistency of blends is seen in Table 3. Seawater causes an increase in LL from 29.3 % to 32 %, a decrease in PL from 25 % to 21.7 %, and an increase in PI from 4.3 % to 10.3. FA with tap water increases LL, PL, and PI to 31.5 %, 26.3 %, and 5.2 %, respectively. FA with seawater also increases LL, PL, and PI to 31.5 %, 23.2 %, and 8.3 %, respectively. The lime with tap water increases the LL and PI to 32 % and 9.1 %, while it decreases PL from 25 % to 22.9 %. Likewise, the lime with seawater increases the LL and PI to 30 % and 7 %, whereas it decreases PL from 25 % to 23 %. While all additives used together with tap water do not affect the PI, using them with seawater increases the PI to 7.2 %.

The UCS values of samples are presented in Table 4 and Figure 4. The UCS value (0 days) of the soil used was determined as 134 kPa. With the effect of compaction and aging, the 28-day UCS value reached 162 kPa. In the samples prepared with seawater, these values were determined as 141 kPa and 167 kPa, respectively.

Table 3. Consistency limits of blends

Code	LL (%)	PL (%)	PI (%)
NT	29.3	25	4.3
NS	32	21.7	10.3
NFT	31.5	26.3	5.2
NFS	31.5	23.2	8.3
NLT	32	22.9	9.1
NLS	30	23	7
NLFT	31	27	4
NLFS	31.2	24	7.2

The FA used with tap water at a rate of 5 % as an additive, increased the 28-day UCS value to 214 kPa. This value was determined to be 201 kPa in blending with seawater. When 5 % lime was mixed with tap water, the 28-day UCS value increased to 419 kPa.

In the case of using seawater in the same blend, the UCS value, 28 days cured, increased to 435 kPa. When the lime and FA were blended together with tap water, the UCS value of the samples (28 days cured) was determined as 845 kPa. On the other hand, the UCS value was 856 kPa for the samples blended with seawater.

Load-sink curve and CBR values of the samples, 28 days cured, are presented in Figure 5 and Figure 6. The CBR of the sample which was kneaded with tap water and cured for 28 days, is 3 % while this value is 5 % for the sample prepared with seawater. The CBR value was determined as 6 % in the samples of the fly ash blended with both tap water and seawater.

CBR value, 28 days cured, increases to 8 % in the sample prepared with 5 % lime additive and tap water. In the sample prepared with 5 % lime additive and seawater, the CBR value, cured for 28 days, was determined as 9 %. In the samples prepared by using fly ash and lime together, the CBR values of the samples, cured for 28 days, and prepared with either fountain or seawater were determined as 14 % and 16 %, respectively.

Table 4. The blending content and geotechnical properties

Code	N	F	L	Water	OWC	MDD	UCS (kPa)			CBR (%)	
	(%)	(%)	(%)		(%)	(kN/m^3)	0-day	7-day	28-day	0-day	28-day
NT	100	0	0	T	15.0	17.2	134	150	162	2	3
NS	100	0	0	S	14.5	17.3	141	160	167	2	5
NFT	95	5	0	T	14.8	17.3	154	207	214	4	6
NFS	95	5	0	S	14.4	17.5	151	200	210	2	6
NLT	95	0	5	T	15.5	17.0	153	276	419	6	8
NLS	95	0	5	S	15.3	17.1	151	362	435	5	9
NLFT	90	5	5	T	15.2	17.1	187	679	845	6	14
NLFS	90	5	5	S	15.1	17.2	206	758	856	6	16

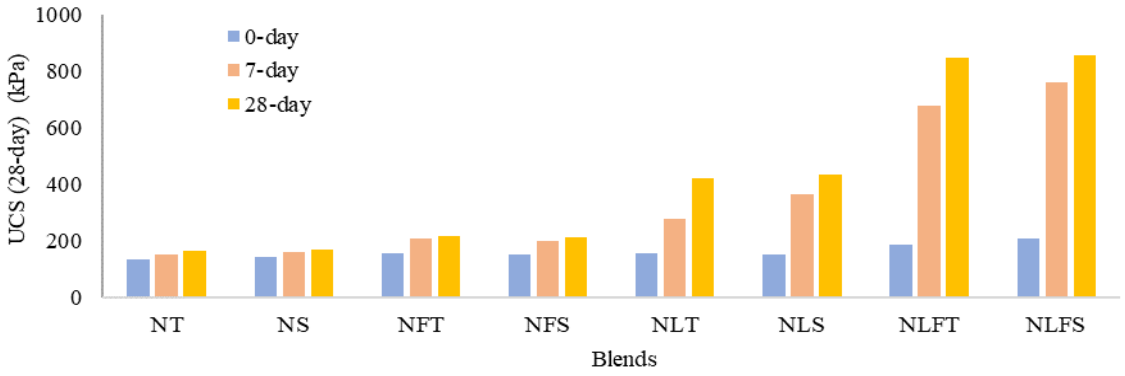

Figure 4. 0, 7, and 28 days UCS values of the blends

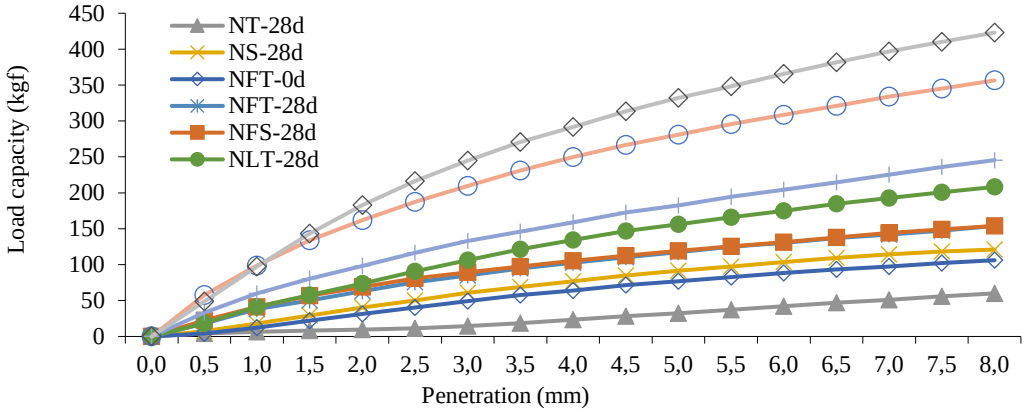

Figure 5. Load-sink curve of blends.

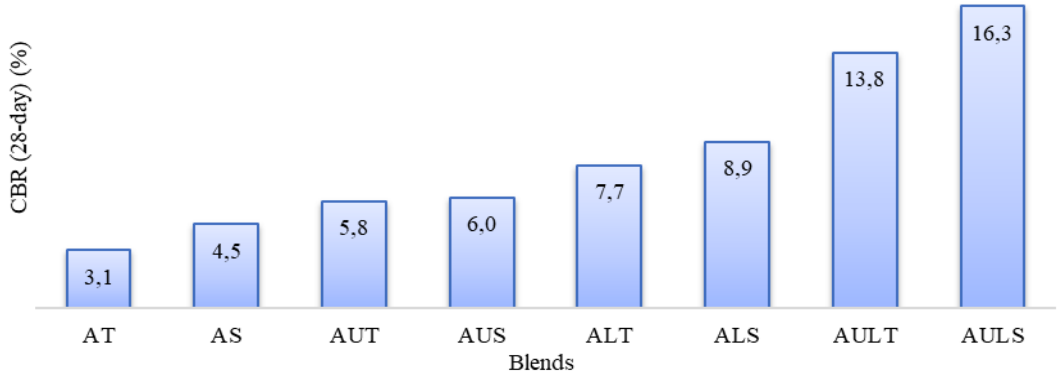

Figure 6. CBR values of blends

CONCLUSION

In the present study, the strength values of soil that was blended with seawater and additives such as FA, and lime, were investigated experimentally. While FA and lime were used as additives, seawater was used as kneading water. Accordingly, the effects of using seawater instead of tap water as the kneading water on treated soil were investigated.

The soil class used in the experimental work is SM (silty sand). Since the main research question in the study was to determine whether there was a difference between the effects of additives when using either seawater or tap water, the additive rates were limited to 5 % FA and 5 %. The detailed investigation of the experimental results showed that FA used at the rate of 5 % improves the strength values of SM-class soils. This improvement is slightly more effective when seawater is used as the kneading water. In addition, the use of FA together with lime ensures higher recovery rates. The mixture that provided the highest improvement in strength values was observed in mixtures where lime and FA were used together, and seawater was used as kneading water.

The test results suggest that the use of additives with seawater provides more desired results than tap water.

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Common Fixed Point Results for w-α-Distance

Gülcan ATICI TURAN[1], Fatma POLAT[2]
[1] Munzur University, Vocational School of Tunceli, Turkey
[2] Dicle University, Diyarbakır, Turkey

✉: gatici23@hotmail.com [1]0000-0002-1009-6072 [2]0009-0007-6100-2252

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ABSTRACT

In this study, we examined some fixed point theorems in non-full metric spaces. We define the notions of α-lower semi-continuous , w-α-distance, w_0-α– distance, w-α-rational contraction and generalized w-α-rational contraction mapping. We also give related theorem and example. Then, we prove Banach's fixed-point theorem thanks to the concept w-α- distance in metric spaces equipped with an abritrary binary relation. Also, w-α- rational contraction mapping and generalized w-α-rational contraction mapping are defined and by using these definitions, the theorem related fixed point is expressed and proved.

Anahtar Kelimeler: Binary Relation, Fixed Point, α-Complete Metric Space, w-Distance.

w-α-Uzaklık İçin Ortak Sabit Nokta Sonuçları

ÖZ

Bu çalışmada tam metrik olmayan uzaylarda bazı sabit nokta teoremleri incelenmiştir. α-alttan yarı-sürekli, w-α-uzaklık, w_0-α– uzaklık, w-α-rasyonel büzülme ve genelleştirilmiş w-α-rasyonel büzülme dönüşümü kavramları tanımlanmıştır. İlgili teorem ve örneği de verilmiştir. Daha sonra w-α- uzaklık kavramını kullanarak keyfi bir ikili bağıntı ile verilen metrik uzaylarda Banach sabit nokta teoremi ispatlanmıştır. Ayrıca w-α- rasyonel büzülme dönüşümü ve genelleştirilmiş w-α- rasyonel büzülme dönüşümü tanımları yapılmış ve bu tanımlar kullanılarak sabit nokta ile ilgili teorem ifade ve ispat edilmiştir.

Anahtar Kelimeler: İkili Bağıntı, Sabit Nokta, α-Geçişli Dönüşümü, α-Tam Metrik Uzay, w-Uzaklık.

INTRODUCTION

Kada et al [1] presented the idea of w-distance within a metric space. Considering (X,d) as a metric space, a function $\omega: X \times X \to [0,\infty)$ earns the designation of a w-distance on X when it meets these specified conditions for each $x,y,z \in X$,
(w1) $\omega(x,z) \leq \omega(x,y) + \omega(y,z)$;
(w2) a function $\omega(x,.): X \to [0,\infty)$ exhibits lower semicontinuous;
(w3) for any $\varepsilon > 0$, there exists $\delta > 0$ such that $\omega(z,x) \leq \delta$ and $\omega(z,y) \leq \delta$ imply $d(x,y) \leq \varepsilon$ [1].
Later on, they have achieved significant results using this definition in fixed point theory. In 2012, Samet et al [2] defined α-admissible mapping. On the other hand they have expressed and proved the theorems related to fixed point in complete metric spaces.
Hussain et al [3] have obtained fixed point results for rational contraction mapping in α-η-complete metric space. Kutbi and Sintunavarat [4] defined generalized w_α-multivalued contraction mapping and then they have proven fixed point theorems using this mapping in α -complete metric spaces. Many studies have been carried out on fixed points [5, 6, 7, 8].

Definition 1. Consider (X,d) as a metric space, and let $T: X \to Cl(X)$ represent a multivalued mapping. A point $x \in X$ is termed a fixed point of T if $x \in Tx$, and the collection of fixed points of T is symbolized as $F(T)$ [9].

Definition 2. Consider (X,d) as a metric space, and let $T: X \to Cl(X)$ represents a multivalued mapping. T is termed a contraction if there exists a constant $\lambda \in (0,1)$ such that, for every x and y in X, $H(Tx,Ty) \leq \lambda d(x,y)$ [9].

Definition 3. Suppose (X,d) represents a metric space, and $\alpha: X \times X \to [0,\infty)$ is a specified mapping. The multivalued mapping $T: X \to Cl(X)$ is termed a w_α-contraction if there exists a w_α-distance $\omega: X \times X \to [0,\infty)$ on X and a value $\lambda \in (0,1)$. This condition ensures that for any $x,y \in X$ and $u \in Tx$, there exists $v \in Ty$ such that

$$\alpha(u,v)\omega(u,v) \leq \lambda\omega(x,y)$$ [4].

Definition 4. In the context of (X,d) being a metric space and $\alpha: X \times X \to [0,\infty)$ a specified mapping, the multivalued mapping $T: X \to Cl(X)$ is referred to as a

generalized w_α-contraction if there exists a w_0-distance ω on X and a value $\lambda \in (0,1)$. This condition ensures that for any $x, y \in X$ and $u \in Tx$, there exists $v \in Ty$ such that

$$\alpha(u,v)\omega(u,v) \leq \lambda max\{\omega(x,y), \omega(x,Tx), \omega(y,Ty), \frac{1}{2}[\omega(x,Ty) + \omega(y,Tx)]\}$$ [4].

MAİN RESULTS

Definition 5. Let (X,d) be a metric space and $\alpha: X \times X \to [0,\infty)$. A function $f: X \to \mathbb{R} \cup \{-\infty, \infty\}$ is said to be α-lower semi-continuous at point x if for all sequence (x_n) which converges to $x \in X$ and $\alpha(x_n, x_{n+1}) \geq 1$ for all $n \in \mathbb{N}$, we have

$$\lim_{n\to\infty} \inf f(x_n) \geq f(x).$$

Definition 6. Let (X,d) be a metric space and $\alpha: X \times X \to [0,\infty)$. A function $\omega: X \times X \to [0,\infty)$ is said to be a w-α-distance on X if

(i) $\omega(x,z) \leq \omega(x,y) + \omega(y,z)$ for any $x, y, z \in X$,

(ii) For any $x \in X$, $\omega(x,.): X \to [0,\infty)$ is α-lower semi-continuous,

(iii) For any $\varepsilon > 0$, there exists $\delta > 0$ such that $\omega(z,x) \leq \delta$ and $\omega(z,y) \leq \delta$ imply $d(x,y) \leq \varepsilon$.

Definition 7. Let (X,d) be a metric space. The w-α-distance $\omega: X \times X \to [0,\infty)$ on X is said to be a w_0-α–distance if $\omega(x,x) = 0$ for all $x \in X$.

Example 8. Let $X = [0,\infty)$. Define $T: X \to X$ and $\alpha: X \times X \to [0,\infty)$ by

$$\alpha(x,y) = \begin{cases} 1, & x,y \in \left[0,\frac{1}{2}\right) \\ 0, & otherwise, \end{cases}$$

$$Tx = \begin{cases} \frac{3}{2}, & x \in \left[0,\frac{1}{2}\right) \\ \frac{4}{5}, & x = \frac{1}{2} \\ x, & x > \frac{1}{2} \end{cases}.$$

Clearly, T is neither α- continuous nor lower semi-continuous. However, it is α-lower semi-continuous.

In fact, let (x_n) be a sequence that not fixed convergent at point $x = \frac{1}{2}$. If $x_n \to \frac{1}{2}^-$, then $Tx_n = \frac{3}{2}$ for all $n \in \mathbb{N}$. If $x_n \to \frac{1}{2}^+$, then $Tx_n = x_n$ for all $n \in \mathbb{N}$ and so $\lim_{n\to\infty} Tx_n = \frac{1}{2}$. Therefore, it is not $\lim_{n\to\infty} inf\, Tx_n \geq T\frac{1}{2}$. Hence, T is not lower semi-continuous at point $x = \frac{1}{2}$. Now, let (x_n) be a sequence not fixed such that $\alpha(x_n, x_{n+1}) \geq 1$ and convergent at point $x = \frac{1}{2}$. Then $Tx_n = \frac{3}{2}$ where $(x_n) \subseteq \left[0,\frac{1}{2}\right)$. However, T is not α-continuous at point $\frac{1}{2}$ due to $Tx_n \to \frac{3}{2} \neq T\frac{1}{2} = \frac{4}{5}$. Also, T is α-lower semi-continuous at point $x = \frac{1}{2}$. Thus, $\frac{3}{2} = \lim_{n\to\infty} inf\, Tx_n \geq T\frac{1}{2} = \frac{4}{5}$.

Lemma 9. Consider (X,d) as a metric space, where $\alpha: X \times X \to [0,\infty)$ and $\omega: X \times X \to [0,\infty)$ are w-α-distances on X. Suppose (x_n) and (y_n) are sequences in X such that $\alpha(x_n, x_{n+1}) \geq 1$ and $(y_n, y_{n+1}) \geq 1$, respectively, with $x, y, z \in X$. Let (u_n) and (v_n) be sequences of positive real numbers approaching 0. Under these conditions, the following statements hold true:

(i) If $\omega(x_n, y) \leq u_n$ and $\omega(x_n, z) \leq v_n$ for all $n \in \mathbb{N}$, then $y = z$. Moreover, if $\omega(x,y) = 0$ and $\omega(x,z) = 0$, then $y = z$.

(ii) If $\omega(x_n, y_n) \leq u_n$ and $\omega(x_n, z) \leq v_n$ for all $n \in \mathbb{N}$, then $y_n \to z$.

(iii) If $\omega(x_n, x_m) \leq u_n$ for all $n, m \in \mathbb{N}$ such that $m > n$, then (x_n) be a Cauchy sequence such that $\alpha(x_n, x_{n+1}) \geq 1$ in X.

(iv) If $\omega(x_n, y) \leq u_n$ for all $n \in \mathbb{N}$, then (x_n) be a Cauchy sequence such that $\alpha(x_n, x_{n+1}) \geq 1$ in X.

Definition 10. Let (X,d) be a metric space and $\alpha: X \times X \to [0,\infty)$ and $T: X \to Cl(X)$ be given two mappings. T is said to be generalized multivalued w-α-rational contraction mapping if there exist $\lambda \in (0,1)$ and a w_0-α-distance $\omega: X \times X \to [0,\infty)$ on X such that for all $x, y \in X$ and $u \in Tx$ there is a $v \in Ty$ with

$$\alpha(u,v)\omega(u,v) \leq \lambda max\left\{\omega(x,y), \frac{\omega(x,Tx)}{1+\omega(x,Tx)}, \frac{\omega(y,Ty)}{1+\omega(y,Ty)}, \frac{1}{2}[\omega(x,Ty) + \omega(y,Tx)]\right\}.$$

Definition 11. Let (X,d) be a metric space, $\omega: X \times X \to [0,\infty)$ be a w_0-α-distance on X and $T: X \to Cl(X)$. Let

$$M(x,y) = max\left\{\omega(x,y), \frac{\omega(x,Tx)}{1+\omega(x,Tx)}, \frac{\omega(y,Ty)}{1+\omega(y,Ty)}, \frac{\omega(x,Ty)+\omega(y,Tx)}{2}\right\}.$$

Then T is said to be a multivalued w-α-rational contraction mapping if

$\alpha(x,y) \geq 1 \Rightarrow \omega(Tx,Ty) \leq \lambda M(x,y)$ for all $x, y \in X$ where $\lambda \in (0,1)$.

Theorem 12. Let (X,d) be a metric space and $\alpha: X \times X \to [0,\infty)$ be a function. Let $T: X \to Cl(X)$ be a generalized multivalued w-α-rational contraction mapping. Suppose that the following statements are indeed accurate:

(i) There exists $Y \subseteq X$ with $T(X) \subseteq Y$such that (Y,d) is α-complete;

(ii) T is a α-admissible mapping;

(iii) There exists $x_0 \in X$ and $x_1 \in Tx_0$ such that $\alpha(x_0, x_1) \geq 1$;

(iv) Either T is α- continuous or

(iv') (x_n) sequence such that $\alpha(x_n, x_{n+1}) \geq 1$ and $x_n \to x \in X$ for all $n \in \mathbb{N}$ has a (x_{n_k}) subsequence such that $\alpha(x_{n_k}, x) \geq 1$ for all $k \in \mathbb{N} \cup \{0\}$;

Then $F(T) \neq \emptyset$.

Proof. There exist $x_0 \in X$ and $x_1 \in Tx_0$ such that $\alpha(x_0, x_1) \geq 1$ from (ii). Since T is a generalized w-α-

rational contraction mapping, we obtain $x_2 \in Tx_1$ such that

$$\alpha(x_1,x_2)\omega(x_1,x_2) \leq \lambda max\left\{\omega(x_0,x_1), \frac{\omega(x_0,Tx_0)}{1+\omega(x_0,Tx_0)}, \frac{\omega(x_1,Tx_1)}{1+\omega(x_1,Tx_1)}, \frac{1}{2}[\omega(x_0,Tx_1)+\omega(x_1,Tx_0)]\right\} \quad (2.1)$$

Since T is a α-admissible mapping and $x_1 \in Tx_0$ such that $\alpha(x_0,x_1) \geq 1$, we have

$$\alpha(x_1,x_2) \geq 1. \quad (2.2)$$

Then by (2.1) and (2.2) we get

$$\omega(x_1,x_2) \leq \alpha(x_1,x_2)\omega(x_1,x_2) \leq \lambda max\left\{\omega(x_0,x_1), \frac{\omega(x_0,Tx_0)}{1+\omega(x_0,Tx_0)}, \frac{\omega(x_1,Tx_1)}{1+\omega(x_1,Tx_1)}, \frac{1}{2}[\omega(x_0,Tx_1)+\omega(x_1,Tx_0)]\right\}.$$

Again, since T is a generalized w-α-rational contraction, there exists $x_3 \in Tx_2$ such that

$$\alpha(x_2,x_3)\omega(x_2,x_3) \leq \lambda max\left\{\omega(x_1,x_2), \frac{\omega(x_1,Tx_1)}{1+\omega(x_1,Tx_1)}, \frac{\omega(x_2,Tx_2)}{1+\omega(x_2,Tx_2)}, \frac{1}{2}[\omega(x_1,Tx_2)+\omega(x_2,Tx_1)]\right\} \quad (2.3)$$

Since $\alpha(x_1,x_2) \geq 1$ and T be a α- admissible mapping, we have

$$\alpha(x_2,x_3) \geq 1. \quad (2.4)$$

Then we get

$$\omega(x_2,x_3) \leq \alpha(x_2,x_3)\omega(x_2,x_3) \leq \lambda max\left\{\omega(x_1,x_2), \frac{\omega(x_1,Tx_1)}{1+\omega(x_1,Tx_1)}, \frac{\omega(x_2,Tx_2)}{1+\omega(x_2,Tx_2)}, \frac{1}{2}[\omega(x_1,Tx_2)+\omega(x_2,Tx_1)]\right\}$$

by (2.3) and (2.4). Continuing this process, we get $x_n \in Tx_{n-1}$,

$$\alpha(x_n,x_{n+1}) \geq 1 \quad (2.5)$$

and

$$\omega(x_n,x_{n+1}) \leq \lambda max\left\{\omega(x_{n-1},x_n), \frac{\omega(x_{n-1},Tx_{n-1})}{1+\omega(x_{n-1},Tx_{n-1})}, \frac{\omega(x_n,Tx_n)}{1+\omega(x_n,Tx_n)}, \frac{1}{2}[\omega(x_{n-1},Tx_n)+\omega(x_n,Tx_{n-1})]\right\}$$

for all $n \in \mathbb{N}$. Now, we obtain

$$\omega(x_n,x_{n+1}) \leq \lambda max\left\{\omega(x_{n-1},x_n), \frac{\omega(x_{n-1},Tx_{n-1})}{1+\omega(x_{n-1},Tx_{n-1})}, \frac{\omega(x_n,Tx_n)}{1+\omega(x_n,Tx_n)}, \frac{1}{2}[\omega(x_{n-1},Tx_n)+\omega(x_n,Tx_{n-1})]\right\}$$

$$= \lambda max\left\{\omega(x_{n-1},x_n), \frac{\omega(x_{n-1},x_n)}{1+\omega(x_{n-1},x_n)}, \frac{\omega(x_n,x_{n+1})}{1+\omega(x_n,x_{n+1})}, \frac{1}{2}[\omega(x_{n-1},x_{n+1})+\omega(x_n,x_n)]\right\}$$

$$\leq \lambda max\left\{\omega(x_{n-1},x_n), \omega(x_n,x_{n+1}), \frac{1}{2}[\omega(x_{n-1},x_{n+1})]\right\}$$

$$\leq \lambda max\left\{\omega(x_{n-1},x_n), \omega(x_n,x_{n+1}), \frac{1}{2}[\omega(x_{n-1},x_n)+\omega(x_n,x_{n+1})]\right\}.$$

$$\leq \lambda max\{\omega(x_{n-1},x_n), \omega(x_n,x_{n+1})\} \quad (2.6)$$

for all $n \in \mathbb{N}$. In that case we get

$\omega(x_n,x_{n+1}) \leq \lambda max\{\omega(x_{n-1},x_n), \omega(x_n,x_{n+1})\}$. If $max\{\omega(x_{k-1},x_k), \omega(x_k,x_{k+1})\} = \omega(x_k,x_{k+1})$ for some $k \in \mathbb{N}$, then $\omega(x_k,x_{k+1}) = 0$ and so we have $\omega(x_{k-1},x_k) = 0$. We get

$\omega(x_{k-1},x_{k+1}) \leq \omega(x_{k-1},x_k) + \omega(x_k,x_{k+1}) = 0$

from the property of w-α-distance.

Since $\omega(x_{k-1},x_k) = 0$ and $\omega(x_{k-1},x_{k+1}) = 0$, then we get $x_k = x_{k+1}$ using Lemma 9. This is $x_k \in Tx_k$ and so it means that x_k is a fixed point of T. Now, let's consider the assumption that

$max\{\omega(x_{n-1},x_n), \omega(x_n,x_{n+1})\} = \omega(x_{n-1},x_n)$

for all $n \in \mathbb{N}$. We get

$$\omega(x_n,x_{n+1}) \leq \lambda\omega(x_{n-1},x_n) \quad (2.7)$$

for all $n \in \mathbb{N}$ from (2.6). By induction, we have

$$\omega(x_n,x_{n+1}) \leq \lambda\omega(x_{n-1},x_n) \leq \lambda^2\omega(x_{n-2},x_{n-1}) \vdots \leq \lambda^n\omega(x_0,x_1)$$

for all $n \in \mathbb{N}$.

Let $m > n$ for all $n,m \in \mathbb{N}$. Then we have

$$\omega(x_n,x_m) \leq \omega(x_n,x_{n+1}) + \omega(x_{n+1},x_{n+2}) + \cdots + \omega(x_{m-1},x_m) \leq \lambda^n\omega(x_0,x_1) + \lambda^{n+1}\omega(x_0,x_1) + \cdots + \lambda^{m-1}\omega(x_0,x_1) \leq \frac{\lambda^n}{1-\lambda}\omega(x_0,x_1).$$

Since $0 < \lambda < 1$, then we get $\frac{\lambda^n}{1-\lambda}\omega(x_0,x_1) \to 0$ as $n \to \infty$. It is found that (x_n) is a Cauchy sequence in Y satisfying $\alpha(x_n,x_{n+1}) \geq 1$ from Lemma 9 We know that $\alpha(x_n,x_{n+1}) \geq 1$ for all $n \in \mathbb{N}$ from (2.5). Since (Y,d) is α-complete, then we obtain $x_n \to z$ as $n \to \infty$ for some $z \in Y$. We now show that z is a fixed point of T. First, we consider that T is α-continuous. Then we obtain

$$d(z,Tz) = \lim_{n\to\infty} d(x_{n+1},Tz) = \lim_{n\to\infty} d(Tx_n,Tz) = d(Tz,Tz) = 0$$

Here, z is a fixed point of T.

Now, let's consider the existence of (iv'). So there exist a subsequence (x_{n_k}) of (x_n) such that $\alpha(x_{n_k},z) \geq 1$ for all $k \in \mathbb{N} \cup \{0\}$. In this case, we write

$$\omega(x_{n_k+1},z) \leq \lim_{k\to\infty} inf\ \omega(x_{n_k+1},x_{n_k+m}) \leq \lim_{k\to\infty} inf\ \frac{\lambda^{n_k-1}}{1-\lambda}\omega(x_0,x_1) = 0 \quad (2.8)$$

using w-α-distance lower semi-continuous from inequality $\omega(x_n,x_m) \leq \frac{\lambda^n}{1-\lambda}\omega(x_0,x_1)$. Also, since T be generalized w-α-rational contraction mapping and $\alpha(x_{n_k},z) \geq 1$, we have

$$\omega(x_{n_k+1},Tz) = \omega(Tx_{n_k},Tz) \leq \lambda max\left\{\omega(x_{n_k},z), \frac{\omega(x_{n_k},x_{n_k+1})}{1+\omega(x_{n_k},x_{n_k+1})}, \frac{\omega(z,Tz)}{1+\omega(z,Tz)}, \frac{1}{2}[\omega(x_{n_k},Tz)+\omega(z,x_{n_k+1})]\right\}$$

$$\leq \lambda max\{\omega(x_{n_k},z), \omega(x_{n_k},x_{n_k+1}), \omega(z,Tz), \frac{1}{2}[\omega(x_{n_k},Tz)+\omega(z,x_{n_k+1})]\right\}$$

$$\leq \lambda max\{\omega(x_{n_k}, z), \omega(x_{n_k}, x_{n_k+1}), \omega(z, x_{n_k+1}) + \omega(x_{n_k+1}, Tz)\} \leq \omega(x_{n_k+1}, Tz)\}.$$

$$\leq \lambda max\left\{\lim_{k\to\infty} inf \frac{\lambda^{n_k}}{1-\lambda}\omega(x_0, x_1), \lim_{k\to\infty} inf \lambda^{n_k}\omega(x_0, x_1), \lim_{k\to\infty} inf \frac{\lambda^{n_k}}{1-\lambda}\omega(x_0, x_1) + \omega(x_{n_k+1}, Tz)\right\}$$

If $\omega(x_{n_k+1}, Tz) > 0$, then

$$\omega(x_{n_k+1}, Tz) \leq \lambda\omega(x_{n_k+1}, Tz)$$

which is a contradiction. Hence, we have

$$\omega(x_{n_k+1}, Tz) = 0. \quad (2.9)$$

If (2.8) and (2.9) are combined, then we obtain $z = Tz$ from Lemma 9.

Teorem 13. In a metric space (X, d), considering the mapping $\alpha: X \times X \to [0, \infty)$ and $T: X \to Cl(X)$ as a multi-valued w-α-rational contraction mapping, assuming the validity of the following statements:
(i) $Y \subseteq X$ with $T(X) \subseteq Y$ such that (Y, d) is α-complete;
(ii) T is a α-admissible mapping;
(iii) There exists $x_0 \in X$ and $x_1 \in Tx_0$ such that $\alpha(x_0, x_1) \geq 1$;
(iv) Either T is α- continuous or
(iv') (x_n) sequence such that $\alpha(x_n, x_{n+1}) \geq 1$ and $x_n \to x \in X$ for all $n \in \mathbb{N}$ has a (x_{n_k}) subsequence such that $\alpha(x_{n_k}, x) \geq 1$ for all $k \in \mathbb{N} \cup \{0\}$;
Then $F(T) \neq \emptyset$.
Proof. The proof shares resemblance with the one in Theorem 12.

Result 14. Suppose (X, d) represents a metric space equipped with w-$\mathcal{R}$-distance, and $\mathcal{R}$ is any arbitrary binary relation on X. If $T: X \to Cl(X)$ fulfills these conditions, then it implies $F(T)$ is non-empty.
(i) There exists $Y \subseteq X$ with $T(X) \subseteq Y$, such that (Y, d) is $\mathcal{R}$-complete;
(ii) $X(T, \mathcal{R}) \neq \emptyset$ and $\mathcal{R}$ is T-closed;
(iii) Either T is $\mathcal{R}$-continuous
or
(iii') (x_n) such that $(x_n, x_{n+1}) \in \mathcal{R}$ and $x_n \to x \in X$ for all $n \in \mathbb{N}$ has a subsequence (x_{n_k}) such that $(x_{n_k}, x) \in \mathcal{R}$ for all $k \in \mathbb{N} \cup \{0\}$.
(iv) There exists a $\lambda \in [0,1)$ for all $x, y \in X$ such that $x, y \in \mathcal{R}$, then $\omega(Tx, Ty) \leq \lambda M(x, y)$.
There exists

$$M(x, y) = max\left\{\omega(x, y), \frac{\omega(x, Tx)}{1 + \omega(x, Tx)}, \frac{\omega(y, Ty)}{1 + \omega(y, Ty)}, \frac{\omega(x, Ty) + \omega(y, Tx)}{2}\right\}.$$

Proof. Let

$$\alpha(x, y) = \begin{cases} 1, & (x, y) \in \mathcal{R} \\ 0, & otherwise \end{cases}.$$

If there exists $x_0 \in X$ and $x_1 \in Tx_0$ such that $\alpha(x_0, x_1) \geq 1$, then since $X(T, \mathcal{R}) \neq \emptyset$, there exists a point $x_0 \in X(T, \mathcal{R})$ such that $(x_0, Tx_0) \in \mathcal{R}$. Since $(x_0, x_1) \in \mathcal{R}$ and $\mathcal{R}$ is T-closed, there exists a $x_2 \in Tx_1$ such that $(x_1, x_2) \in \mathcal{R}$. $\alpha(x_1, x_2) \geq 1$ due to the definition of α.
Continuing this process, we get $\alpha(x_n, x_{n+1}) \geq 1$ such that $x_n = Tx_{n-1}$. That is, T is a α-admissible. Since the definition of α and (Y, d) is $\mathcal{R}$-complete, then (Y, d) is α-complete. (iii) and (iii') conditions requires (iv) and (iv') hypotheses of Theorem 12. Now let $\alpha(x, y) \geq 1$. Then $(x, y) \in \mathcal{R}$. Because of the hypothesis (iv) there exists a $\lambda \in [0,1)$ such that
$\omega(Tx, Ty) \leq \lambda M(x, y)$.
Therefore, since it is provide all conditions of Theorem 12, then T has a fixed point. Also, w-$\mathcal{R}$-distance requires w-α-distance.

Result 15. Suppose (X, d) represents a metric space, $\alpha: X \times X \to [0, \infty)$ is a mapping, and $T: X \to Cl(X)$ is a multi-valued w-α -rational contraction mapping, given that the following conditions are satisfied:
(i) T is a α-contraction mapping;
(ii) There exist $x_0 \in X$ and $x_1 \in Tx_0$ such that $\alpha(x_0, x_1) \geq 1$;
(iii) Either T is α-continuous or (x_n) sequence such that $\alpha(x_n, x_{n+1}) \geq 1$ ve $x_n \to x \in X$ for all $n \in \mathbb{N}$ has a (x_{n_k}) subsequence such that $\alpha(x_{n_k}, x) \geq 1$ for all $k \in \mathbb{N} \cup \{0\}$;
Then $F(T) \neq \emptyset$.
Proof. As (X, d) constitutes a complete metric space, ensuring α-complete, the intended outcome is achieved by employing the proof outlined in Theorem 12.

Example 16. Let $X = (-1, \infty)$ and $d: X \times X \to [0, \infty)$ with the metric $d(x, y) = |x - y|$ for all $x, y \in X$. Define $\alpha: X \times X \to [0, \infty)$ by

$$\alpha(x, y) = \begin{cases} x^2 + y^2, & x, y \in [0,1] \\ 0, & otherwise. \end{cases}$$

$T: X \to Cl(X)$ multivalued mapping define by

$$Tx = \begin{cases} \left\{\frac{1}{4}x^2\right\}, & x \in [0,1] \\ \{|x|, |x+2|\}, & otherwise. \end{cases}$$

Now, we show that this is T a multivalued w-α-rational contraction mapping with $\lambda = \frac{1}{2}$ and w-α-distance $\omega: X \times X \to [0, \infty)$, defined as $\omega(x, y) = \max\{|x|, |y|\}$ for all $x, y \in X$. Let $u \in Tx = \left\{\frac{1}{4}x^2\right\}$ for $x, y \in [0,1]$. That is, we can found in a $v = \frac{1}{4}y^2 \in Ty$ such that
$u = \frac{1}{4}x^2$ and

$$\alpha(u, v)\omega(u, v) = \alpha\left(\frac{x^2}{4}, \frac{y^2}{4}\right)\omega\left(\frac{x^2}{4}, \frac{y^2}{4}\right)$$
$$= \left(\frac{x^4}{16} + \frac{y^4}{16}\right)\left(\frac{1}{4}\max\{x^2, y^2\}\right)$$
$$\leq (1+1)\frac{1}{4}\max\{x^2, y^2\}$$
$$\leq \frac{1}{2}\max\{|x|, |y|\}$$
$$= \lambda\omega(x, y)$$
$$\leq \lambda M(x, y).$$

That is, $\alpha(u, v)\omega(u, v) \leq \lambda M(x, y)$. Therefore T multivalued w-α-rational contraction mapping.
While (Y, d) may not qualify as a complete metric space, it does fulfill the criteria for being an α-complete metric space. Consider (x_n) as a Cauchy sequence within Y, with $\alpha(x_n, x_{n+1}) \geq 1$ for all n in the natural numbers.

Consequently, $x_n \in [0,1]$ for all $n \in \mathbb{N}$. Given that $([0,1], d)$ stands as a complete metric space, there exists $z \in [0,1]$ such that $x_n \to z$ as $n \to \infty$. Therefore, (Y, d) qualifies as an α-complete metric space.

If $\alpha(x, y) \geq 1$, it implies that $x, y \in [0,1]$. Concurrently, $Tc \in [0,1]$ for all $c \in [0,1]$. Consequently, $\alpha(Tx, Ty) \geq 1$, signifying that T qualifies as an α-admissible mapping. There exists $x_0 = 1$ such that $x_1 = \frac{1}{4} \in T1$ and $\alpha(x_0, x_1) = \alpha\left(1, \frac{1}{4}\right) \geq 1$.

$x_n \to x$ as $n \to \infty$ and (x_n) sequence provide $\alpha(x_n, x_{n+1}) \geq 1$ inequality for all $n \in \mathbb{N}$. Hence, $(x_n) \subseteq [0,1]$ for all $n \in \mathbb{N}$ and so $(Tx_n) \subseteq [0,1]$. Since T is continuous on $[0,1]$, then $Tx_n \to Tx$ as $n \to \infty$.

This implies that T is a mapping that maintains α-continuity.

Alternatively, let $\alpha(x_n, x_{n+1}) \geq 1$ and $x_n \to z \in X$. In this case, there exists a subset $\left(x_{n_k}\right)$ such that $x_n \in [0,1]$ and $x_{n_k} \to z$. Thus, $\alpha\left(x_{n_k}, z\right) \geq 1$.

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Investigation of the Physiological and Histopathological Effects of Omega Acids (3, 6, 9) and Stearic Acid on Rats in Ischemia Reperfusion

Tugba KARAHAN GÜR[1]✉, Okan ARIHAN[2], Gokhan OTO[3], Serkan YILDIRIM[4], Ahmet Ufuk KOMUROGLU[1], Fatih KARAHAN[5]

[1] Vocational School of Health Services, Van Yuzuncu Yil University, Van, Turkey
[2] Department of Physiology, Hacettepe University Faculty of Medicine, Ankara, Turkey
[3] Department of Pharmacology, Faculty of Medicine, Van Yuzuncu Yil University, Van, Turkey
[4] Department of Pathology, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Turkey
[5] Department of Chemistry, Faculty of Science, Van Yuzuncu Yil University, Van, Turkey

✉: tugbakarahan@yyu.edu.tr [1]0000-0001-7220-0210

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ABSTRACT

Ischemia causes reversible or irreversible cell or tissue damage due to insufficient blood flow to the organ or tissue. In this study, our aim is to investigate the protective effect of omega 3, 6 9 and stearic acid application before ischemia reperfusion injury in the leg muscles. For this purpose, 70 female albino rats were divided into 10 groups. The study continued at the same dose for 14 days. In addition, these fatty acids were given to other groups without ischemia-reperfusion. After the application different faty acid, blood biochemical parameters of different fatty acids, oxidative stress parameters and histopathology of tissues (liver, kidney, muscle) were examined in rats. As a result, it was observed that omega 9 fatty acid has better protective properties compared to other omega fatty acids and stearic acid in terms of histopathological properties and oxidative stress index. Additionally, other fatty acids and stearic acid provided some degree of protection against the deleterious effects of ischemia-reperfusion.

Keywords: Ischemia-reperfusion, total antioxidant status, total oxidant status, omega fatty acids, stearic acid, oxidative stress

Omega Asitleri (3, 6, 9) ve Stearik Asidin Sıçanlarda İskemi Reperfüzyon Hasarında Fizyolojik ve Histopatolojik Etkilerinin Araştırılması

ÖZ

İskemi, organ veya dokuya yetersiz kan akışı nedeniyle geri dönüşümlü veya geri dönüşümsüz hücre veya doku hasarına neden olur. Bu çalışmada 10 gruba ayrılan 70 adet dişi Wistar-albino sıçanın bacak kaslarına (kuadriseps) iskemi-reperfüzyon hasarı öncesi uygulanan omega 3, 6, 9 ve stearik asit yağ asitlerinin etkinliği araştırıldı. Çalışma 14 gün boyunca aynı dozda devam etti. Ayrıca bu yağ asitleri iskemi-reperfüzyon yapılmadan diğer gruplara da verildi. Uygulama sonrasında sıçanlarda farklı yağ asitlerinin kan biyokimyasal parametreleri, oksidatif stres parametreleri ve histopatoloji incelendi. Sonuç olarak omega 9 yağ asidinin histopatolojik özellikler ve oksidatif stres indeksi açısından diğer omega yağ asitleri ve stearik asit ile karşılaştırıldığında daha iyi koruyucu özelliklere sahip olduğu gözlendi. Ayrıca diğer yağ asitleri ve stearik asit, iskemi-reperfüzyonun zararlı etkilerine karşı bir dereceye kadar koruma sağlamıştır.

Anahtar Kelimeler: İskemi-reperfüzyon, toplam antioksidan durumu, toplam oksidan durumu, omega yağ asitleri, stearik asit, oksidatif stres.

INTRUDUCTION

Ischemia is defined as the hampered blood flow to an organ due to various reasons. This type of blood flow decrease and ischemia-reperfusion (IR) damage in tissues may occur in clinical conditions such as vascular and transplantation surgery, tourniquet application, tissue transfers and surgery of the amputated limb [1,2]. Depending on the ischemia, the tissue remains in hypoxia, resulting in tissue damage. Ischemia causes a decrease in the energy level in the cell and accumulation of toxic metabolites in the tissue, initiating a series of biochemical reactions that can lead to cell dysfunction and subsequent cell death [3].

Reperfusion is the reviving of tissue blood supply. In the case of blood flow in an ischemic tissue (reperfusion), free oxygen radicals released by the polymorphonuclear leukocytes, which come and settle in the tissue, have more enhancing effects on tissue destruction. The severity of the damage varies depending on the duration of ischemia, temperature of the tissue, and tissue-specific factors [4].

During ischemia, toxic oxygen radicals are produced in ischemic tissue. Free oxygen radicals and superoxide radicals after reperfusion cause endothelial damage and increased vascular permeability. In addition, activated adhesion molecules and cytokines initiate a systemic inflammatory response [5]. In order to prevent potential damages of free oxygen radicals, antioxidant substances counter with many cell protective enzymes and molecules in the body. In a healthy body, cellular antioxidant enzymes, antioxidant substances and free radicals have a balanced relationship between oxidants [6,7].

Free oxygen radicals can disrupt the structural elements of tissue in an organism and cause harmful effects. Oxidative stress is closely related to complications such as myocardial damage, pulmonary edema, kidney and liver failure, and increased mortality [8]. There are many antioxidant defense mechanisms in the cell to remove free radicals. Free radicals are not stationary and they often attenuate their radical feature in a short time period. In addition, many enzymatic and nonenzymatic systems cause inactivation of free radicals. With the catalytic effect of superoxide dismutases (SOD) found in most cells, scavenging of the radicals is significantly accelerated. Enzymes such as glutathione peroxidase are protective against free radicals. Catalase in peroxysomes enzymatically breaks down hydrogen peroxide. In addition, sulfhydryls such as cysteine, glutathione, ceruloplasmin, and vitamins A, C and E are endogenous and exogenous antioxidants that prevent or inactivate free radicals. [9].

Omega fatty acids are important fatty acids that play an important role in human nutrition. Since omega 3 (O3) and omega 6 (O6) fatty acids cannot be produced in the organism, they must be taken with diets. Omega 3 has anti-inflammatory properties and is a fatty acid used to explain why Eskimos are better for cardiovascular health than populations fed other diets [10]. Omega 6 fatty acid metabolites have pro-inflammatory properties [11]. Omega 9 (O9) fatty acid can be produced from these two fatty acids in the human body, and its external intake is especially recommended for cardiovascular and intestinal health [12]. Stearic acid (SA) is a saturated fatty acid found in butter, and saturated fatty acids are associated with cardiovascular health problems, such as atherosclerosis. It has been demonstrated that O3 fatty acids can be incorporated into breast cancer and lung cancer treatment [13,14]. Consuming foods containing O3 fatty acids reduces the risk of colon cancer [15]. In recent studies, O3 fatty acids have been shown to have beneficial effects on cancer, cardiovascular diseases, immune system, cirrhosis and neural system [16-21]. It has also been reported that O3 essential fatty acids have antioxidant properties that reduce oxidative stress and prevent the production of reactive oxygen species [22-26].

Ischemia/reperfusion is a common condition in humans. Oxy, sedative stress, which occurs during the reperfusion, can damage tissues and organs, while at the same time, hadar can occur in organs and tissues that are far from the area where ischemia occurs. With this study, the protective effect of different fatty acids will be determined to determine whether there is distant organ damage caused by ischemia and to prevent possible damage to these organs.

In our study, different fatty acids, namely O3, O6, O9 and SA was tested to compare their effect on rats in blood biochemical and oxidative stress parameters solely or in case of ischemia-reperfusion (IR). At the same time, histopathological examinations were performed on samples taken from certain tissues (quadriceps muscle, liver and kidney) of rats with or without ischemia reperfusion injury and it was aimed to examine and compare protective effects of afformentioned fatty acids.

MATERIAL AND METHODS

Animal and Ethics Statement

In our study, 70 adult, female Wistar-Albino rats were used. Rats obtained from Van Yuzuncu Yil University Experimental Medicine Application and Research Unit weighed approximately 200-250 gr were used. Rats were housed in cages made of polycarbonate material that can be autoclaved at 121 oC. The dimensions of the cage body are 425x266x175 mm, the floor area is 800 cm^2 and 7 animals were kept in each cage. The animals were randomly distributed in cages and standard rat pellet food and water were given ad libitum. Omega acids and SA was purchased from a commercial firm and their purity was chosen as above 95%. During the application period of the experiment, the rats were housed in laboratory conditions with 20 ± 2°C temperature, 50% relative humidity, 12 hours of light/12 hours of dark photo period from 7 am to 7 pm. The test protocol was approved by the Van Yuzuncu Yil University Animal Research Local Ethics Committee and the experimental studies were carried out by adhering to the animal ethics committee directive (Ethics Committee number: 2018/03).

Surgical Procedure

In the study, a total of ten different groups were formed including 7 rats in each group.

1. Control group. No application was made. 2. IR group. 3. O3 group. 300 mg / kg O3 daily. 4. O6 group. 300 mg / kg O6 daily. 5. O9 group. 300 mg / kg O9 daily. 6. SA group. 300 mg / kg SA daily. 7. IR+O3 group. 300 mg / kg O3 daily and ischemia reperfusion at 14^{th} day. 8. IR+O6 group. 300 mg / kg O6 daily and ischemia reperfusion at 14^{th} day. 9. IR+O9 group. 300 mg / kg O9 daily and ischemia reperfusion at 14^{th} day. 10. IR+ SA group. 300 mg / kg SA daily and ischemia reperfusion at 14^{th} day. All fatty acids were given with oral gauge for 14 days and dosage of 300 mg / kg was chosen as the standard comparison dose for all fatty acids [27]. For ischemia reperfusion, 2 hours of

ischemia and 2 hours of reperfusion were performed [28].

Biochemical Analysis

At the end of this process, animals were sacrificed under anesthesia. Blood glucose, LDL, Cholesterol, Triglyceride, ALT and AST measurements were performed as biochemical parameters from blood serum. From the obtained blood samples, Total Antioxidant Status (TAS) (Rel Assay Diagnostics Kit, Catalog No: RL0017) , Total Oxidant Status (TOS) (Rel Assay Diagnostics Kit, Catalog No: RL0024) were measured spectrophotometrically with a commercial kits as the oxidative stress parameters, and the oxidative stress index (OSI) has been determined by using TOS and TAS data. OSI value was calculate according tı the following Formule: OSI (arbitrary unit) = TOS/TAS.

Histopathological Analysis

Tissue samples taken at the end of the experimental procedure were fixed in 10% formalin solution for 48 hours. As a result of routine tissue follow-up procedures, it was embedded in paraffin blocks. 4 mm thick sections were taken from each block. Preparations prepared for histopathological examination were stained with hematoxylin-eosin (HE) and examined by light microscopy (Olympus BX 51, Germany). Sections were evaluated according to histopathological features as none (-), mild (+), moderate (++), severe (+++) and very severe (++++) tissue damage.

Statistical Analysis

SPSS statistical analysis program was used for statistical evaluations (SPSS 20.0 (IBM Corp., NY, USA). As the method of statistics, different tests were applied to different findings. Kruskal-Wallis and post hoc tests were used for non-parametric continuous variables. Histopathological results were evaluated with convenient categorical tests. Statistical significance was set as $p<0.05$.

RESULTS

Serum Biochemical Results

The ALT values of the control group, O3, O6, O9 and SA fatty acid groups are close to each other and do not differ statistically. On the other hand, the highest values were observed in ischemia groups and there is a significant difference between ischemia, IR+O9 and IR+O3 groups with the control group. Applied fatty acids did not reverse ischemia induced ALT increase.
In terms of AST, no significant difference was observed between the control group and the fatty acid groups administered alone. Similar to ALT values, the highest values in AST values were observed in the groups that had ischemia. AST values in all other ischemia groups except O9 ischemia are significantly higher than the control group and O3, O6, O9 and SA groups. Data related with serum biochemical results are presented in table 1.

Table 1. Serum biochemical values of the groups The results are given as mean ±SD.IR= ischemia reperfusion, O3= Omega 3, O6= Omega 6, O9= Omega 9, SA= Stearic acid, GLU= Fasting blood sugar (mg/dL), ALT= (U/L), AST= (U/L), HDL= High Density Lipoprotein (mg/dL), CHOL= Cholesterol (mg/dL), TRIG= Triglycerides, LDL= Low Density Lipoprotein (mg/dL). Differe t letters in the column show statistical significance. P<0.05.

	GLU (mg/dl)	ALT (U/L)	AST (U/L)	CHOL (mg/dl)	TRIG (mg/dl)	LDL (mg/dl)
Control	**159.71**	**34.86^{c}**	88.00^{c}	56.14^{ab}	131.07^{a}	36.50^{a}
O3	177.29	51.29^{abc}	106.00^{c}	43.29^{b}	73.20^{ab}	22.45^{abc}
O6	183.00	39.00^{bc}	115.00^{c}	56.71^{ab}	55.75^{b}	16.48^{bc}
O9	201.00	48.57^{abc}	120.43^{c}	53.86^{ab}	85.05^{ab}	25.29^{abc}
SA	171.86	43.71^{bc}	120.57^{c}	47.43^{ab}	102.60^{ab}	28.76^{ab}
IR	162.86	58.71^{ab}	294.43^{ab}	50.00^{ab}	77.41^{ab}	18.76^{bc}
IR+O3	165.20	70.00^{a}	342.00^{a}	43.60^{b}	80.78^{ab}	25.77^{abc}
IR+O6	185.60	52.00^{abc}	284.20^{ab}	65.40^{a}	44.94^{b}	10.80^{c}
IR+O9	234.50	61.25^{ab}	190.00^{bc}	63.00^{a}	94.77^{ab}	29.50^{ab}
IR+SA	169.83	56.67^{abc}	238.33^{ab}	59.67^{ab}	65.68^{b}	15.35^{bc}

Total Antioxidant Status (TAS), Total Oxidant Status (TOS) And Oxidative Stress İndex (OSI) Results

Among the groups, the lowest TAS value was observed in the IR+O6 group and the highest in the O9 group. IR+O6 TAS level was significantly lower than SA, O9 and IR+O9 groups. TAS level of O9 group was found significantly higher than all other groups. TOS levels in IR+O9 and IR+SA groups were significantly lower than O3, IR+O3 and IR+O6 groups. In terms of OSI values, the highest values were observed in O6 and IR+O6 groups. On the other hand, the lowest values were observed in O9 and IR+O9 groups. OSI values in O9 and IR+O9 groups were significantly lower than the O3 and O6 as well as IR+O3 and IR+O6 groups. The results are given in table 2.

Histopathological Results

Muscle tissue histopathological results

In control, Omega fatty acid groups and SA normal histological structure was observed (Figure 1 A, C, D,

E, F). In IR group, very severe edema and congestion in the interlobuler area, hyaline degeneration and zenker necrosis in the muscle fibers were observed (Figure 1 B). In IR+O3 group, severe edema in the interlobuler area, severe hyaline degeneration and zenker necrosis in the muscle fibers were determined (Figure 1 G).

Table 2. TAS, TOS and OSI values of the gruops

	TAS	TOS	OSI
Control	1.13±0.30 $^{a. b}$	11.78±3.23 $^{a. b}$	10.42 $^{a.b}$
O3	1.34±0.45 $^{a. b}$	19.59±5.43 ^{b}	14.62 ^{b}
O6	1.14±0.28 $^{a. b}$	18.45±10.30 $^{a. b}$	16.33 ^{b}
O9	2.19±0.46 ^{c}	14.11±6.33 $^{a. b}$	6.47 ^{a}
SA	1.50±0.30 ^{b}	15.34±5.68 a. b	10.29 $^{a.b}$
IR	1.24±0.15 $^{a. b}$	14.98±2.84 $^{a. b}$	12.17 $^{a.b}$
IR+O3	1.20±0.49 $^{a. b}$	19.59±6.17 ^{b}	16.32 ^{b}
IR+O6	0.92±0.15 ^{a}	20.14±12.90 ^{b}	21.88 ^{b}
IR+O9	1.45±0.63 ^{b}	9.39±0.37 ^{a}	6.47 ^{a}
IR+SA	1.22±0.16 $^{a. b}$	10.34±5.27 ^{a}	9.40 $^{a.b}$

The results are given as mean ±SD.
IR= Ischemia reperfusion, O3= Omega 3, O6= Omega 6, O9= Omega 9, SA= Stearic acid, TAS= Total Antioxidant Status, TOS= Total Oxidant Status, OSI= Oxidative Stress Index. Note: Different letters in the same column represent statistical significance.

In IR+O6 group, moderate edema, congestion, mild hyaline degeneration and zenker necrosis were observed in the interlobuler area (Figure 1H). In IR+O9 group, mild edema and congestion were observed in the interlobuler area (Figure 1 I). In IR+SA group, severe edema in the interlobuler area, medium intensity hyaline degeneration and zenker necrosis in the muscle fibers were determined (Figure 1 J). Histopatological findings of the quadriceps muscle are summarized in table 3.

Liver tissue histopathological results

Control, omega groups and SA group liver tissue had normal histological appearance (Figure 2 A, C, D, E, F). In IR group, congestion in the veins and sinusoids, dilatation in the sinusoids, and moderate degeneration and necrosis in hepatocytes were detected (Figure 2 B). In IR+O3 group, severe dilatation in sinusoids, congestion in vessels and sinusoids, and moderate degeneration and necrosis in hepatocytes were observed (Figure 2 G). In IR+O6 group, moderate dilatation in sinusoids, congestion in vessels and sinusoids, and mild degeneration in hepatocytes were detected (Figure 2 H). In IR+O9 group, mild dilatation and congestion were determined in sinusoids (Figure 2 I). In IR+SA group, severe dilatation and congestion in sinusoids, congestion in the vessels, and moderate degeneration and necrosis in hepatocytes were observed (Figure 2 J). Liver histopathological findings are summarized in table 4.

Table 3. Scoring of histopathological findings observed in quadriceps muscle tissues

	Edema at interlobuler parts	Congestion in the veins	Hyaline degeneration	Zenker necrosis
Control	-	-	-	-
O3	-	-	-	-
O6	-	-	-	-
O9	-	-	-	-
SA	-	-	-	-
IR	++++	++++	++++	++++
IR+O3	+++	+++	++++	+++
IR+O6	++	++	++	++
IR+O9	+	+	+	-
IR+SA	+++	+++	+++	++

Table 4. Scoring of histopathological findings in liver tissues

	Edema at interlobuler area	Hyperemia in the veins	Hyaline degeneration	Zenker necrosis
Control	-	-	-	-
O3	-	-	-	-
O6	-	-	-	-
O9	-	-	-	-
SA	-	-	-	-
IR	+++	+++	++	++
IR+O3	++	+++	+	-
IR+O6	+	++	-	-
IR+O9	++	++	+	-
IR+SA	++	+++	+	-

Kidney tissue histopathological results

Control group, omega groups and SA group kidney tissue was observed in normal histological appearance (Figure 3A, C, D, E, F). In IR group, moderate congestion was observed in the interstitial and

glomerular vessels (Figure 3B). In IR+O3 group, moderate congestion was determined in the interstitial and glomerular vessels (Figure 3G). In IR+O6 group, mild congestion was observed in the interstitial and

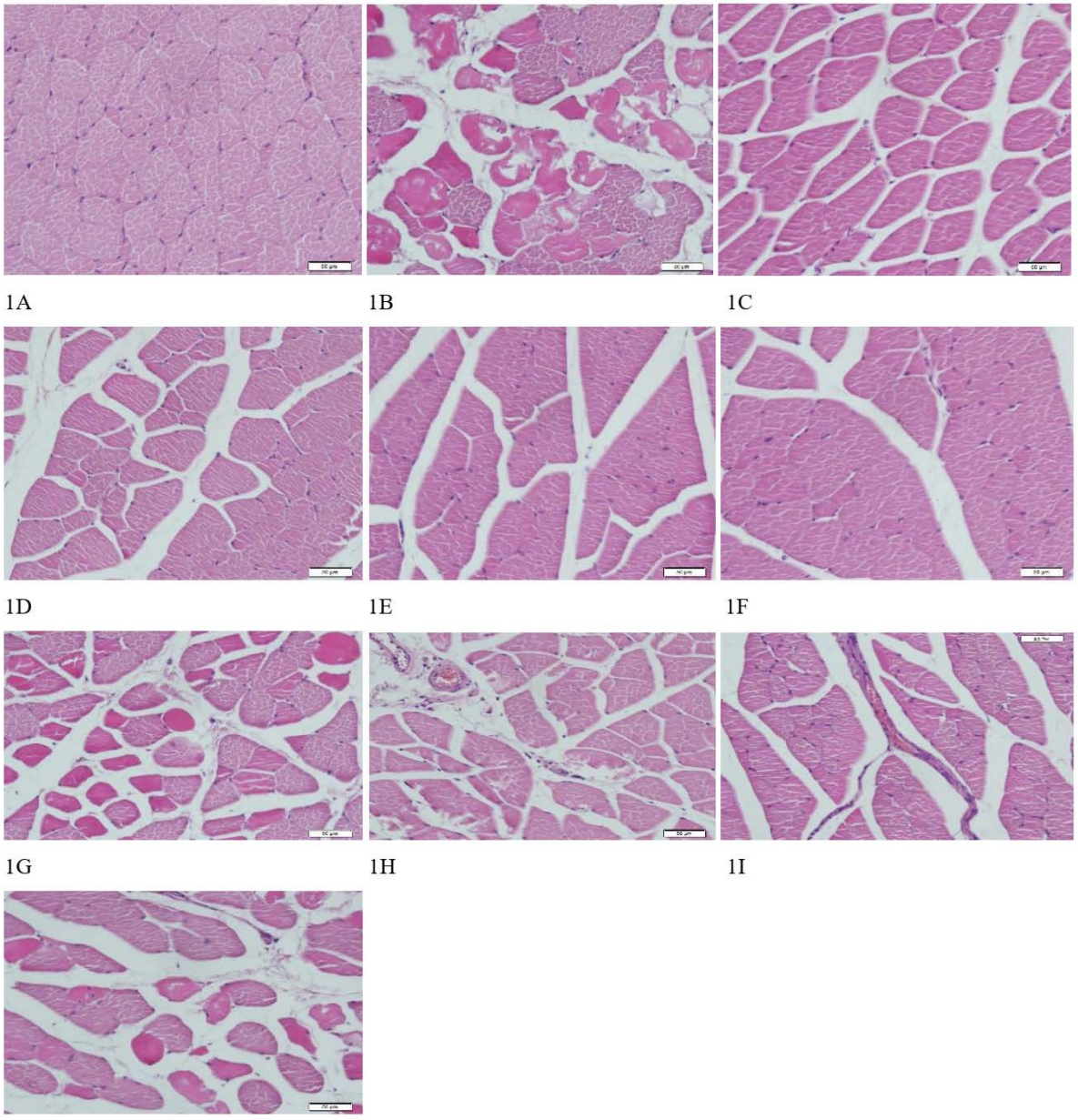

Figure 1A: Control group, quadriceps muscle tissues, normal histological appereance, H&E, Bar: 50 µm.
Figure 1B: Ischemia-reperfusion group, Very severe edema in interlobuler area, hyaline degeneration and Zenker necrosis in the muscle fibers H&E, Bar: 50 µm.
Figure 1C: Omega 3 group, quadriceps muscle tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 1D: Omega 6 group, quadriceps muscle tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 1E: Omega 9 group, quadriceps muscle tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 1F: Stearic acid group, quadriceps muscle tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 1G: Ischemia-reperfusion + omega 3 group, quadriceps muscle tissue, severe histological appearance, severe edema in the interlobuler area, severe hyaline degeneration and zenker necrosis in the muscle fibers, H&E, Bar: 50 µm.
Figure 1H: Ischemia-reperfusion + omega 6 group, quadriceps muscle tissue, normal histological appearance, moderate edema in interlobuler area, mild level hyaline degeneration in muscle fibers, H&E, Bar: 50 µm.
Figure 1I: Ischemia-reperfusion + omega 9 group, quadriceps muscle tissue, normal histological appearance, mild edema and hyperemia in interlobuler area, H&E, Bar: 50 µm.
Figure 1J: Ischemia-reperfusion + stearic acid group, quadriceps muscle tissue, normal histological appearance, severe edema in the interlobuler area, medium intensity hyaline degeneration and Zenker necrosis in the muscle fibers, H&E, Bar: 50 µm.

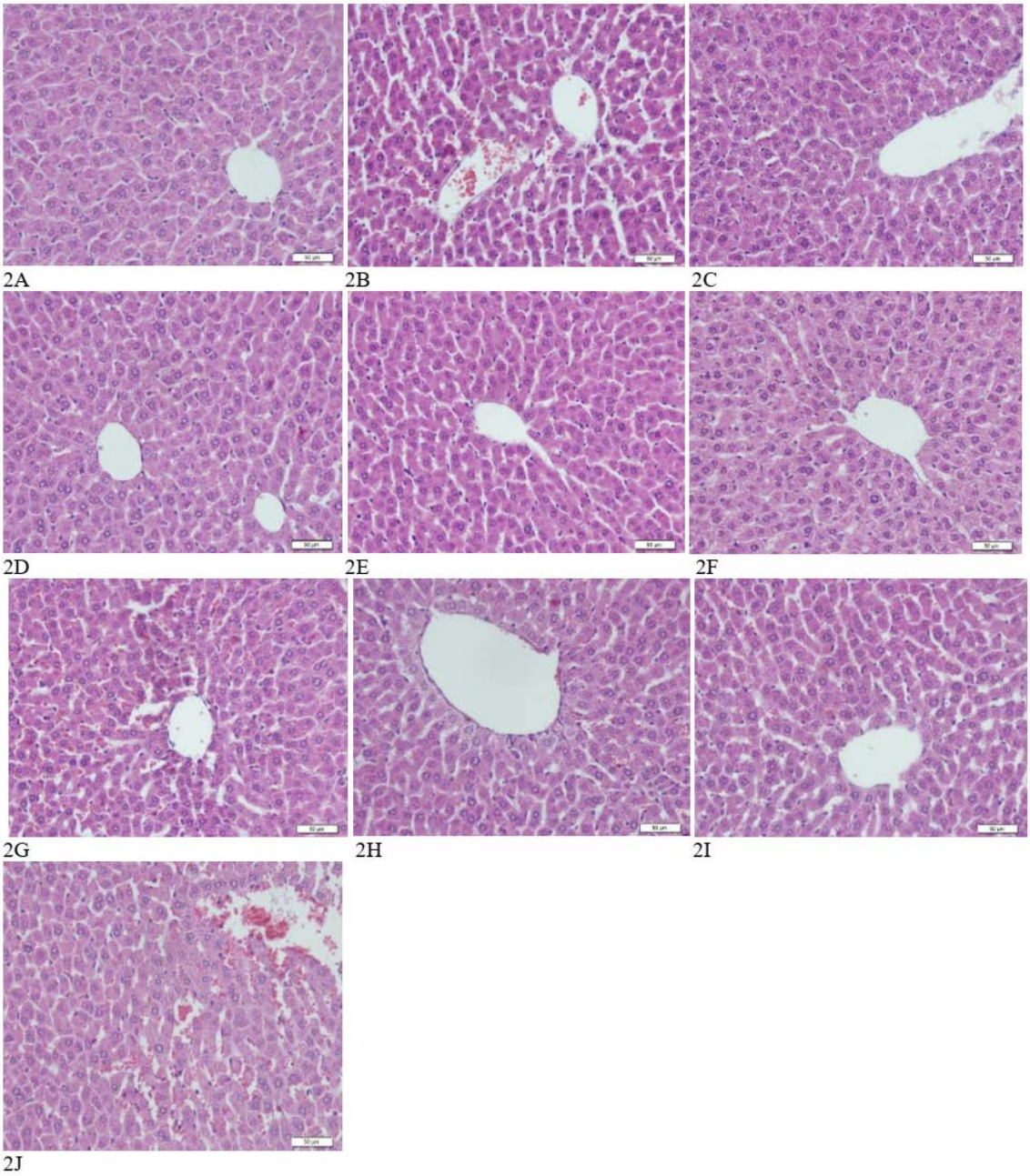

Figure 2A: Control group, liver tissue in normal histological apperance, H&E, Bar: 50 µm.
Figure 2B: Ischemia-reperfusion group, liver tissue, Very severe edema in interlobuler area, hyaline degeneration and zenker necrosis H&E, Bar: 50 µm.
Figure 2C: Omega 3 group, liver tissue, normal histological apperance, H&E, Bar: 50 µm
Figure 2D: Omega 6 group, liver tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 2E: Omega 9 group, liver tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 2F: Stearic acid group, liver tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 2G: Ischemia-reperfusion + omega 3 group, liver tissue, severe dilatation in sinusoids, congestion in vessels and sinusoids, moderate degeneration and necrosis in hepatocytes, H&E, Bar: 50 µm.
Figure 2H: Ischemia-reperfusion + omega 6 group, liver tissue, normal histological appearance, moderate dilatation in sinusoid, congestion, mild degeneration in hepatocytes, H&E, Bar: 50 µm.
Figure 2I: Ischemia-reperfusion + omega 9 group, liver tissue, normal histological appearance, mild dilatation and congestion in sinusoids, H&E, Bar: 50 µm.
Figure 2J: Ischemia-reperfusion + stearic acid group, liver tissue, severe dilatation and congestion in sinusoid, congestion in vessels, moderate degeneration and necrosis in hepatocytes, H&E, Bar: 50 µm.

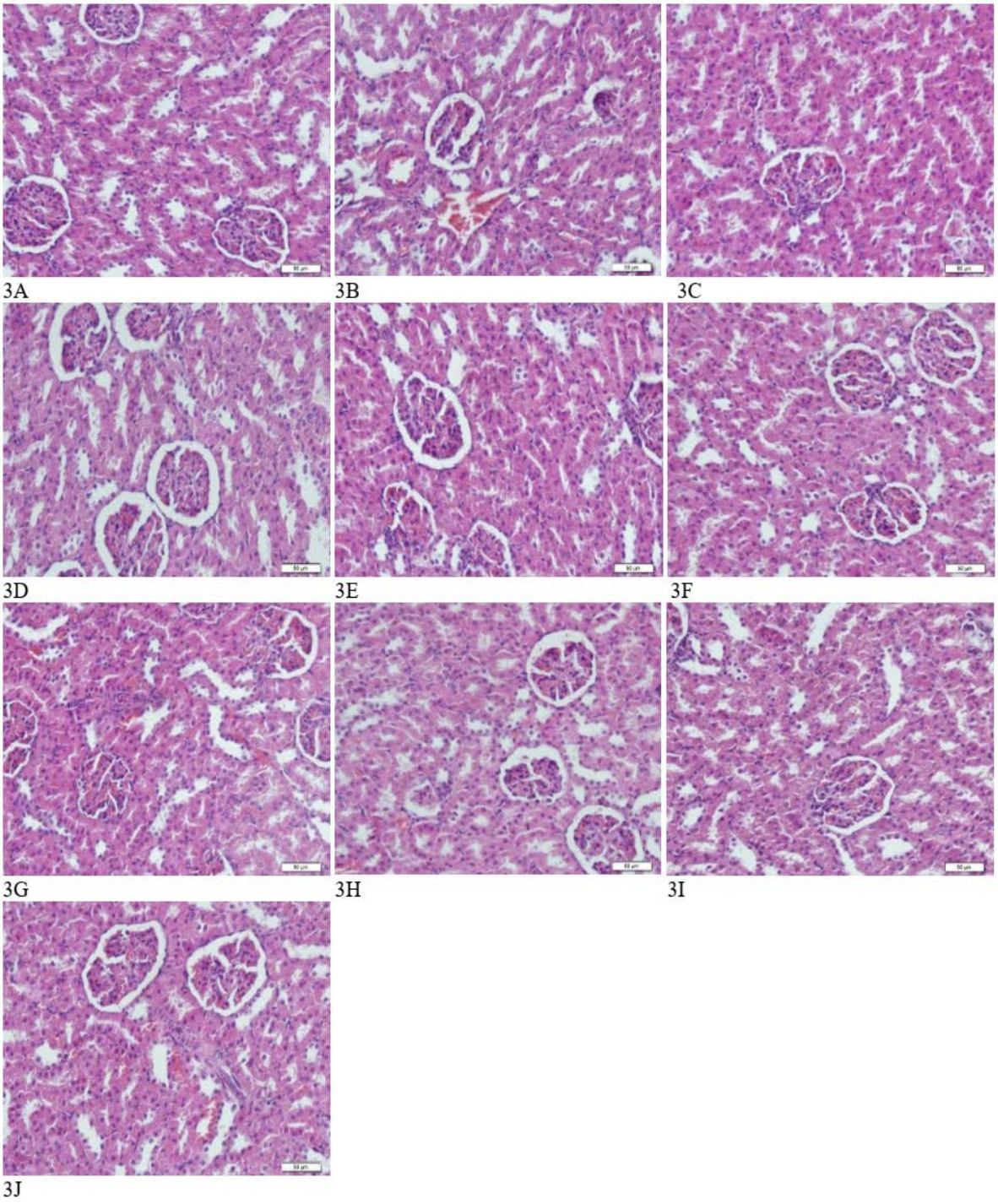

Figure 3A: Control group, kidney tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 3B: Ischemia-reperfusion group, kidney tissue, moderate congestion in vessels, H&E, Bar: 50 µm.
Figure 3C: Omega 3 group, kidney tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 3D: Omega 6 group, kidney tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 3E: Omega 9 group, kidney tissue, normal histological apperance, H&E, Bar: 50 µm.
Figure 3F: Stearic acid group, kidney tissue, normal histological apperance, H&E, Bar: 50 µm.

Figure 3G: Ischemia-reperfusion + omega 3 group, kidney tissue, moderate congestion in interstitial vessels, H&E, Bar: 50 µm.
Figure 3H: Ischemia-reperfusion + omega 6 group, kidney tissue, mild congestion in interstitial vessels, H&E, Bar: 50 µm.
Figure 3I: Ischemia-reperfusion + omega 9 group, kidney tissue, normal histological apperance, H&E, Bar: 50 µm.Figure 3J: Ischemia-reperfusion + stearic acid group, kidney tissue, moderate congestion in interstitial and glomerular veins, H&E, Bar: 50 µm.

glomerular vessels (Figure 3H). In IR+O9 group, normal histological apperance was observed (Figure 3I). In IR+SA group, moderate congestion was detected in the glomerular and interstitial vessels (Figure 3J). Histopathological findings are summarized in Table 5.

Table 5. Scoring of histopathological findings in kidney tissues

	Congestion in glomerular vessels	Congestion in interstitial vessels
Control	-	-
O3	-	-
O6	-	-
O9	-	-
SA	-	-
IR	++	++
IR+O3	++	++
IR+O6	+	+
IR+O9	-	-
IR+SA	++	++

DISCUSSION

The current study results show that the ischemia/reperfusion model we had applied causes significant physiological and histopathological changes in rats and the findings reveal that the desired ischemia/reperfusion model can be achieved. The main purpose of the study is to test and compare protective effects of different fatty acids applied during the 14 days before ischemia/reperfusion injury in investigated histological, physiological and biochemical parameters.

Histopathological results of the study the ischemia reperfusion procedure caused marked damage to the muscle, liver and kidney tissues. In muscle and liver tissue such fatty acids show an attenuation of damage caused by IR which is visible when histopathological data is compared with IR. Excessive saturated fatty acids and also excessive unsaturated fatty acids are mentioned for their adverse effects on cultured hepatocytes [29]. In addition high fat content in the diet may result with a deposition in the muscle cells and causing malfunctions due to overabundance of this fat may overwhelm the oxidizing capacity of myocytes [30]. Our results showed no damage occurred to liver tissue due to our lone Omega acids and SA administration. On the contrary, our administered fatty acids exerted protection in the liver and muscle tissues. Although fatty acids are blamed for cardiovascular harmful effects for a long time stearic acid (0,02 mg/kg) was found to improve functional outcomes after cardiac arrest in rats [31]. Stearic acid is a saturated fatty acid however, it is protective against inflammation [32]. These properties are unlike the other fatty acids such as palmitic and myristic acids [33]. In our study SA and all other fatty acids protected liver tissues against Zenker necrosis. In a study by Skrzep-Poloczek et al. [34] they administered 8 weeks of high fat diet to rats and then animals were underwent for different gastrointestinal surgery methods. They found that a shift in diet regardless of its content causes soleus muscle oxidative stress in this surgery procedure. However our administered faty acids for 14 days did not cause an additional stress to tissue. Protective properties in O9 fatty acid (especially in muscle and kidney tissue) were prominent. In muscle, liver and kidney tissue, O6 also showed protective properties. SA showed no better protective property compared to other fatty acids. In further studies, dose-dependent studies on O9 fatty acid have the potential to give more detailed results. 12 weeks of supplementation of elderly people with a combination including O-3 caused a better protection against sarcopenia compared with other administrations avoiding O-3. This states a positive role for protection of muscle cells for O-3 [35]. In a study by Ali, Rifaai, [36], 400 mg/kg fish oil (not pure O-3 but used as a source of O-3) for 4 weeks (p.o.) improved liver damage caused by acute cold restrain stress which is administered at the end of 4 week oil administration. In a study the researchers showed that 1 mL per day (containing ~25% O-3 fatty acids with a mix of EPA, DHA and octadecatetraenoic acids) O-3 supplementation to male wistar rats caused a beneficial effect against heart ischemia/reperfusion injury [37]. Our data reveals that O3 provides an attenuation of histopathological parameters in muscle such as edema in interlobular parts, congestion in the veins and Zenker necrosis compared to lone IR group. Although this protection is not as prominent as the one in O9, still this finding is in parallel with the literature records. In addition O3 also shows a protective activity in the liver however it was not as much as the one exerted by O6 administration.

In this study we have chosen ALT and AST values to compare liver function in this experimental protocol. These two important parameters showing the functions and health of the liver are important parameters that increase in conditions such as cirrhosis, acute liver injury, necrosis and side effects of drugs such as acetaminophen (paracetamol). ALT and AST values were close to the control group of all applied fatty acids and did not differ statistically. This shows us that the 14 days of administration of fatty acids does not have a negative effect on these two parameters of the liver. On the other hand, the highest values were observed in ischemia groups and there is a significant difference between ischemia, IR+O9 and IR+O3 groups and the control group. Applied fatty acids were unable to alleviate the increase of ALT and AST caused by ischemia. A decrease in ALT and AST values compared to control was given in literature [38]. However their administration period is higher (6

weeks) and dosage was higher (400 mg/kg) compared to our experimental protocol. Stearic acid is known for its anti-inflammatory property. 10 days of administration of wistar rats with pellets containing 180g/kg stearic acid food pellet causes a significant reduction in ALT and AST in 3rd day compared to therapy model without stearic acid [39]. In our experiments no such decrease was observed in ALT and AST due to SA administration but this difference may be related with administration schemes. We had administered oral gauge in mg/kg doses whereas they administered via pellet containing SA. In a study by Majeed, Al-Shawi [40], 14 days of oral O-3 administration to rats caused an insignificant increase in ALT and AST values which is in parallel with our findings. Another study which found similar results with ours is a study by [36] 400 mg/kg fish oil (not pure O-3 but used as a source of O-3) for 4 weeks (p.o.) caused an insignificant increase in ALT and AST values compared to control [36]. In a study by Li et al. [41], pellets containing SA and OA were prepared separately and given to mice for 21 days and showed no significant change for body weight, lean mass and fat mass however SA was found to cause increased food intake without altering body weight significantly compared to OA. Serum fatty acid concentration was also not changed significantly between those two groups. O-9 is a monounsaturated fatty acid and shown to exert protective activity against insulin resistance and causing anti-inflammatory activity. O-9 can bind to peroxisome proliferator activated receptor which acts as a sensor for lipids [42].

Another biochemical parameter evaluated was glucose. Glucose was observed at high values as rats were not fasted during the time blood was drawn. O6 and O6 ischemia groups, SA and SA ischemia groups, and O9 and O9 ischemia groups showed close values to each other. This shows that the fatty acids applied do not show any significant difference when compared to ischemia condition. O-3 PUFAs are shown to contribute brain glucose regulation [43]. El-Fayoumi et al. [44] administered 500mg/kg of eicosapentaenoic acid i.p. for 16 weeks to mice. Such administration increased fasting blood glucose, serum insulin level significantly compared to control but caused no significant alteration in triglyceride level. Variations in the result of different studies concerning O3 fish oil may rise from the composition of fish oil in EPA and DHA content in addition their higher dosage and longer administration period may be the result of this significant glucose increase. In a study by Sahadewa et al. [45], 300 mg/kg of daily oral O-3 administration for 28 days caused a decrease in fasting blood glucose decreased in O-3 group.

One of the important component of the study was the evaluation of how the applied fatty acids, ischemia/reperfusion and the combination of both will affect the oxidative stress parameters. It is observed that ischemia increases oxidative stress (based on OSI value). It has been determined that O9 fatty acid prevents oxidative stress in case of ischemia. In this sense, it is possible to suggest that O9 fatty acid has a protective potential agains an ischemic damage. This situation can be evaluated in studies including dose – response comparisons. Although O3 fatty acids are known to have anti-inflammatory and O6 acids have proinflammatory properties Simopoulos, [46], they may have shown such an effect at these doses, although they were initially expected to respond differently in terms of oxidative stress. It has been known for a long time that people with a marine based diet face less cardiovascular events compared to diets filled with saturated animal fats [10]. There are increasing studies concerning protective activity of O3 in experimental ischaemic conditions in rats [47]. In a study, rats were given two different doses of O3 (100 and 300 mg/kg orally). Their O3 capsules contain an EPA/DHA ratio of3/2. They found that O-3 posttreatment reversed diclofenac induced total antioxidant capacity decrease [48]. In a study showed that 400 mg/kg fish oil (not pure O3 but used as a source of O3) for 4 weeks (p.o.) caused an insignificant increase in total antioxidant capacity compared to control [36]. In a study 300 mg/kg of daily oral O3 administration for 28 days did not cause a significant change in MDA levels compared to control [45].

Another possible reason for our results is the mode of administration of fatty acids to rats. In some of the studies fatty acids are given in a mixture of those acids [49]. Therefore in such studies there is no single fatty acid administration was made which causes and O3, O6, O9 rich fatty acid mixture administration. However in our study we have administered single fatty acid in every other group.

Furhter possible explanation for our observed findings is the gender of animals. In a study by Wendy et al. [50], in Langendorff perfusion experiments dietary O6 fatty acid replacement was found to impair cardiac functional recovery after ischemia in female rats. However this situation was not observed in male rats. They have concluded that O6 dietary lipid intake in females may be an adverse condition for cardioprotection. Since most of the studies concerning protective activity of Omega acids against certain experimental conditions are performed on male animals there is still a need to clarify such actitivies also on female animals.

As with experimental septic shock models, the protective effects of antioxidant and anti-inflammatory molecules have been shown in many experimental models. Fish consumption is recommended for the purpose of a protective effect against heart diseases in adults. It has been demonstrated that consumption of O3 fatty acids in pill form alone is not as effective as consumption as fish. It has been shown with a comprehensive meta-analysis that consuming more fish reduces the risk of dementia, but fish oil consumption does not decrease it [51]. The proposed mechanisms to make fish oil intake more effective through fish consumption are not fully clear. However, due to the

synergistic effects of other compounds in fish meat, absorption in the gut and its interaction with the gut microbiota, it has been shown to be effective in its doses. These situations reveal that more research is needed on synergistic effect and dose-response issues. Co-supplementation of O-3 acids and iron may cause more damage to type 2 diabetes mellitus patients therefore protective effect of O-3 acids may change according to experimental conditions [52].

The mentioned "O-3, 6, 9 and stearic fatty acids" are the types of fatty acids consumed in our diet. However, there are different opinions about how much of these fatty acids should be consumed in our diet. High-dose uses as well as low doses are recommended according to results of different studies. Ratio of O-6 to O-3 in the diet of prehistoric populations was estimated almost equal whereas, modern populations have much higher O-6: O-3 ratio varying between 10:1 to 30:1 which arises some questions about their impact on health [53]. Our work has limitations. It is a single dose of the fatty acids used and investigating their effect in only two tissues. Considering that ischemia reperfusion damage is common in human, the protective effect of fatty acid should be determined by investigating the effects of ischemia/reperfusion damage in other tissues as well. The fatty acids that we used in this study also evolved into various lipid mediators in the body. In other studies, the effects of lipid mediators (such as resolvins, lipoxins and meracins) that these fatty acids have formed can be investigated.

CONCLUSION

The findings of the current study reveal that daily consumption of 300 mg / kg of O9 oil can be protective against oxidative stress and tissue damage in ischemia reperfusion injury in rats. In the light of these findings, new researches may be suggested in the IR model considering the dose-response curve of O9 fatty acid. Thus, IR injuries, which are a life-threatening health problem, research on the possibility of using O9 fatty acids for the purpose of preventing and treating damage may yield valuable results.

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DNA Protective Assay and Some Biochemical Properties of *Galium* Species

Yener Tekeli[1*], Omer Kilic[1,2], Elif Esra Altuner[3**]
[1] Department of Pharmaceutical Techniques, Faculty of Pharmacy, University of Adiyaman, Adiyaman, Turkey
[2]Department of pharmacy professional sciences, Faculty of Pharmacy, Adiyaman, Turkey
[3]Program of Medical Laboratuary and Tecniques, Department of Medical services and Techniques, Europe Vocational School, Kocaeli Health and Technology University, Kocaeli, Turkey

✉: elifesraaltuner@gmail.com [1]0000-0003-1524-457X [2]0000-0003-3409-1572 [3]0000-0001-7663-6898

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ABSTRACT

Herbal-derived drugs prepared using various extracts obtained from different organs of plants that have been scientifically proven to be “medicinal” or directly from these plants are often used today as a method to prevent or treat diseases in humans and animals. This method, called phytotherapy, is a rational, evidence-based, and allopathic treatment method and deals with which active substance group is responsible for the biological effects. In this context, the phytotherapeutic effectiveness of *Galium* species, which are also used for medicinal purposes among the public, has been examined and proven in many studies. In this study, the biochemical efficacies of five different *Galium* species were measured and their antioxidant, antimicrobial, and DNA protective effects were tested. It was found that the tested *Galium* species showed remarkable biochemical efficacies. The results were also compared with the results of some other studies in the literature.

Keywords: Antimicrobial; Antioxidant; DNA; *Galium* types; Phytotherapy; Protective assay.

DNA Koruyucu Testi ve *Galium* Türlerinin Bazı Biyokimyasal Özellikleri

ÖZ

Bilimsel olarak “tıbbi” olduğu kanıtlanmış bitkilerin farklı organlarından elde edilen çeşitli ekstraktlar veya doğrudan bu bitkilerden elde edilen çeşitli ekstraktlar kullanılarak hazırlanan bitkisel kökenli ilaçlar, günümüzde insanlarda ve hayvanlarda hastalıkların önlenmesi veya tedavisinde bir yöntem olarak sıklıkla kullanılmaktadır. Fitoterapi adı verilen bu yöntem akılcı, kanıta dayalı, allopatik bir tedavi yöntemi olup, biyolojik etkilerden hangi etken madde grubunun sorumlu olduğunu ele almaktadır. Bu bağlamda halk arasında tıbbi amaçlı da kullanılan *Galium* türlerinin fitoterapötik etkinliği birçok çalışmada incelenmiş ve kanıtlanmıştır. Bu çalışmada beş farklı *Galium* türünün biyokimyasal etkinlikleri ölçülmüş, antioksidan, antimikrobiyal ve DNA koruyucu etkileri test edilmiştir. Test edilen Galium türlerinin dikkate değer biyokimyasal etkinlik gösterdiği tespit edildi. Sonuçlar ayrıca literatürdeki diğer bazı çalışmaların sonuçlarıyla da karşılaştırıldı.

Anahtar Kelimeler: Antimikrobiyal; Antioksidan; DNA; *Galium* türleri; Fitoterapi; Koruyucu tahlil.

INTRODUCTION

Medicinal herbs are a source of healing, and many modern medicines used today are made from active compounds derived from these herbs. They are not only used in drugs but also widely consumed by the public in the treatment of many diseases, especially infectious diseases [1]. Traditional herbal medicine, which is also called phytotherapy is a phenomenon that has been developed by people through trial and error and is as old as human history. In addition, herbal remedies are the most widely used complementary or adjunctive therapy tools around the world and have an important place, especially in developing countries [2,3]. A sedentary lifestyle and wrong eating habits, occurring especially with the developing technology, increase oxidative stress-related diseases because of free radicals [4]. Free radicals are molecules/atoms that contain one or more unpaired electrons and are highly active [5]. Free radicals, both reactive nitrogen species (RNS) and reactive oxygen species (ROS), can be derived from both endogenous and exogenous sources. Here, the harmful effects of oxidative stress can be defined as the negative effects of these ROS (reactive oxygen species) [6-7]. Reactive oxygen species (ROS), such as 1O2 [singlet oxygen], OH. (hydroxyl radicals), are natural by-products of cellular metabolism. Increasing ROS in cells can lead to the induction of oxidative stress. The excessive cellular level of ROS damages macromolecules, including DNA. This means increased oxidative stress that causes cell damage, which means cell death. Cell death is

an important factor in homeostasis, pathology, and aging. Therefore, because cell death is the main reason for many diseases, such as cancer and coronary heart diseases, medical assays based on preventing cell death have gained a significant place in medical research in recent years [8]. In addition to these efforts of medicine, herbal medicine, which has an important place in alternative medicine, is also used for therapeutic purposes. Epidemiological data show that the long-term consumption of plants rich in secondary metabolites, especially polyphenols, protects against the development of cancer, diabetes, osteoporosis, and cardiovascular, and neurodegenerative diseases. Herbal remedies are defined as products that consist of a combination of substances entirely derived from plants and contain active ingredients [9]. Patients with chronic diseases tend to use herbal products and phytochemicals more along with their current medications compared to the general population. This trend, which involves the use of herbal products for therapeutic purposes, is also referred to as traditional medicine [10-15].

The Rubiaceae Family is herbaceous or woody, perennial, or annual plants and is represented in the world with approximately 500 genera and 6500 species. *Galium* L., belonging to the Rubiaceae family and is represented by 101 species (122 taxa), 61 of which are endemic to the flora of Turkey, is an important material for researchers due to its phytochemical properties [16]. The Rubiaceae is not only a family comprised of ornamental plants but also is used in traditional medicine to treat various diseases. These herbs are used for more than 70 medical indications, such as hepatitis, eczema, edema, cough, hypertension, diabetes, and impotence Studies have shown that most of these herbs have antimalarial, antimicrobial, antihypertensive, antidiabetic, antioxidant, and anti-inflammatory activities [17]. Asperuloside, which is a monoterpene and is known as one of the iridoid heterosides, is an important compound found in various species of the Rubiaceae family, including *Galium***.** This compound shows the physiological effects of both alkaloids and monoterpenes.

In addition, *Galium* is a popular medicinal herb due to its insecticide, hypotensive, sedative, antipyretic, antitussive, and wound healing properties. It also causes coagulation of milk owing to an enzyme in its content. For this reason, it is known as “yogurt grass” among the people. Moreover, *Galium* species are notable for their in vitro antimicrobial and antioxidant properties such as secondary metabolites, alkaloids, and flavonoids. Their rich phytochemical structures and antimicrobial properties are among the reasons that increase the importance of *Galium* species [16-19].

MATERIAL & METHOD

Sample collection and preparation

In this study, plant species were collected from different regions. *Galium murale* (L.) All. was collected from the vicinity of Geyiktas village (Keban-Elazığ), steppe areas, in May 2019 with collected number ÖK 5223. *Galium cassium* Boiss. was collected from north of Duydum village (Sanliurfa-Siverek), steppe rocky areas, in June 2019 with collected number ÖK 5318. *Galium humifusum* Bieb. were collected from east of the Sancak (Bingol) district, humid areas, in July 2018 with the collected number ÖK 5318. *Galium verum* L. subsp. glabrescens Ehrend. were collected from northeast of Sancak (Bingol) district, rocky humid areas, in June 2018 with collected number ÖK 5267. *Galium verum* L. subsp. *verum* was collected from the vicinity of Gokce village (Bingol), stony shrubby areas, in June 2018 with collected number ÖK 5252. All plants were identified by plant taxonomist Dr. Omer Kilic. Herbarium samples of plant samples are stored in Adiyaman University (Turkey) Pharmacy Faculty herbarium, Hacettepe University, and Yıldırımlı herbarium from Ankara (Turkey). Plants were collected, air-dried, pulverized, and stored in black airtight bags. The extraction of the plants was carried out based on the method described by several studies, with some modifications [20-24]. Briefly, the dried and powdered *Galium* species, about 30 g, were extracted using a magnetic stirrer at 45 °C in 250 mL of methanol and then filtered. The filtrates were first concentrated with a rotor evaporator (Buchi, Switzerland) at reduced pressure and 45°C, and then they were concentrated to dryness by using a water bath at 45°C.

Antioxidant Activity Assays

Total Phenolic Content (Folin-Ciocalteu Method)

The total phenolic content of methanolic extracts was determined using gallic acid (Sigma-Aldrich) by the Folin–Ciocalteau method as a standard and expressed as mg of gallic acid equivalents (GAE) curve [20–23].

The stock solution prepared as 2 mg/mL was diluted 4 times. Then, 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of 7.5% Na_2CO_3 solution were added to each. After keeping for 45 minutes in darkroom conditions, absorbances were read at 760 nm wavelength in a spectrophotometer. The experiments were performed in triplicate.

Scavenging Activity of DPPH Free Radicals

DPPH free scavenging assay, which is one of the most popular and frequently used methods among antioxidant experiments, was evaluated using Cary 60 UV-Vis Spectrophotometer. The performed DPPH radical scavenging assay for the determination of *Galium* species was based on the previous studies, with some modifications [24-30]. Briefly, 0.1 mM of DPPH radical in methanolic solution was prepared, and then 0,5 mL of this solution was mixed with 2 mL of different

concentrations (2-0,125 mg/mL) of *Galium* species solutions [25]. After 30 min incubation in dark and at room temperature, to evaluate the reduction in DPPH radicals, absorbance was measured at 517 nm, and the inhibition % values expressing the reduction in radicals were calculated as below;

$$I\% = ((A_{DPPH} - A_S)/A_{DPPH}) \times 100$$

where A_S is the absorbance of the solution containing the sample, and A_{DPPH} is the absorbance of the DPPH solution. Synthetic antioxidants BHA and BHT were used as the positive control. The concentration indicating that the initial DPPH concentration decreased by 50% was calculated from the graph by plotting the percentage of inhibition against the sample concentrations. This value is called IC_{50} and the lowest IC_{50} value represents the highest antioxidant value. Each measurement was performed in triplicate [10,26,27].

Antibacterial Activity

To evaluate antimicrobial activities, the microdilution experiment was applied using a 96-well plate. The antibacterial activities of *Galium* species were tested on two Gram-negative and two Gram-positive bacteria. The antibacterial properties were assessed using microdilution sensitivity tests conducted in nutritional broth [22]. The *Galium* species were interpreted in terms of the activities of antimicrobial effects against four pathogenic bacterial strains, including Gram-positive (*E.faecalis* ATCC 29212), *S.aureus* ATCC 29213and Gram-negative bacteria (*E. coli* ATCC 25912, *P. aeruginosa* ATCC 10231). The antimicrobial analyses were carried out by modifying [28, 29]. In summary, DMSO (Sigma-Aldrich) was used to create solutions of Galium species up to 5 mg/mL, and the samples were diluted seven times and examined at eight different concentrations. Each microplate well received 100 µL of bacterial strain, adjusted for density using a McFarland densitometer, so that the bacteria were roughly 106 CFU/mL. Ceftriaxone and ampicillin were used as standard antibiotic agents, and the same procedures were also applied to them. Microplates were incubated for 24 hours at 37°C to measure antibacterial activity, then optical intensities were estimated at 600 nm (OD600) by using an Elisa reader (Bio-Tek Instruments, Inc.; Winooski, USA). Three MIC evaluations were conducted for every species of microorganism. The 24-hour incubation period ended with the determination of minimal inhibitory concentrations, or MICs. Standard antimicrobial drugs were used as the positive control in control tests, while unvaccinated medium was used as the negative control. The circumstances of the species under investigation were maintained throughout.

DNA Damage Protection Activity

Fresh LB medium containing ampicillin (100 µg/mL) was inoculated with glycerol stocks of *E. coli* Dh5α cells harboring pET21a plasmid (100 µL) were added to 10 mL of Luria-Bertani (LB) Broth containing ampicillin (100 µg/mL) and cultured for overnight at 37°C with shaking at 220 rpm. Cells were collected by centrifugation, and plasmid DNA was extracted using a plasmid DNA isolation kit (K0502, Thermo Fisher Scientific™) according to the manufacturer's recommendations and stored at 4°C until it was used. DNA protective activity of methanol extracts of each of the examined *Galium* taxa was studied by using pET21a plasmid (Novagen, USA). Oxidative DNA damage was induced using an ultraviolet (UV)/H_2O_2-radical system and checked on a 0.8% agarose gel electrophoresis as previously described by Verma et al. 2015. The reaction was realized in a total volume of 15 µL containing 5 µL plasmid DNA (30 ng/µL), 5 µL of the lyophilized plant extract dissolved in ddH2O (25 µg/mL), and 5 µL of 3% H_2O_2. The mixture was then placed directly on a UV transilluminator (300 nm) for 15 min under room conditions. The negative control contained only plasmid DNA and was not exposed to (UV)/H_2O_2. 5 µL of 6X loading dye (Thermo Scientific) was added to the reaction mixtures, and then the mixtures were loaded on 0.8% agarose gel containing 5 µL of 10mg/ml ethidium bromide (Sigma-Aldrich). Electrophoresis was carried out at 90 V for 1 hour. The gel was visualized via the Quantum ST5 Gel Documentation system.

RESULTS and DISCUSSION

In this study, the polyphenol contents as equivalent to gallic acid of *Galium* species were examined, and the results are presented in Table 1. These results showed that *Galium verum* subsp. *verum* (0.4257 mgGAE/mL) had the highest concentration of phenolic substance equivalent to gallic acid. It was followed by *Galium verum* subsp. *Glabrescens* (0.3455 mgGAE/mL) and *Galium cassium* (0.2754 mgGAE/mL). *Galium murale* on the other hand, had the least phenolic substance content with 0.2680 mgGAE / mL. Phenolic compounds have reducing properties, and therefore they show a strong antioxidant effect [24]. From this point of view, it can be said that *Galium verum* subsp. *verum* has more antioxidant effects than others. The results of the free radical scavenging effect method showed this clearly (Table 1). The free radical scavenging capacity of galium extracts was measured by the DPPH method. This method is based on the retention of DPPH radicals in the solvent environment by antioxidant molecules. The following reaction occurs between the DPPH radical and the antioxidant compounds. This method also works on H atom transfer.

$DPPH^{\cdot} + AH \rightarrow DPPH\text{-}H + A^{\cdot}$

$DPPH^{\cdot}$ is a stable free radical that possesses a deep purple color and a strong absorption around 517 nm (AH) [30]. After the *Galium* solutions prepared in methanol were diluted serially, a decrease in absorbance of 0.1 mM DPPH free radical solution, prepared on it, was determined at 517 nm. Percentages of inhibition of free radicals of each *Galium* species were calculated

depending on the change in absorbance values, and IC_{50} values were determined based on the inhibition values

Table 1. The total phenolic concentrations, IC_{50} value and percentages of free radical scavenging of Galium species.

	mgGAE/ mL $x10^{-4}$	IC_{50}	Inhibition %
Galium murale (L.) All	0.2688±0.21	6.06±0.12	50.39
Galium humifusum Bieb	0.2724±0.15	5.27±0.19	51.09
Galium cassium Boiss.	0.2754±0.17	4.04±0.22	58.40
Galium verum L. subsp. *Verum*	0.4257±0.10	2.26±0.16	69.08
Galium verum L. subsp. *glabrescens* Ehrend	0.3455±0.16	2.37±0.20	66.52
BHA	---	0.047±0.13	89.56
BHT	---	0.42±0.13	78.60

All *Galium* species showed noteworthy antioxidant properties, with described variability in IC_{50} values. Free radicals are well-known reasons for DNA damage, and this kind of damage to the DNA can make a cell cancerous [31]. DNA damage protective assays performed with natural resources having potential antioxidants and bioactive components are employed as in vitro models to ascertain how harmful radicals cause DNA production [32]. Results of this study revealed that every extract exhibited DNA damage protecting ability against free radicals produced by the UV/H_2O_2-radical system (Fig 1). In lane 2, which was exposed to UV and H_2O_2 without any plant extract, there was not a single DNA band. All the methanol extracts of the examined *Galium* taxa effectively protected the DNA from the hydroxyl radicals generated by UV and H_2O_2. Between lanes 3 and 7, where methanolic extracts were added to DNA exposed to UV and $H2O_2$, there was obvious protection against DNA damage. Many studies are proving the effectiveness of DNA protection [33-36].

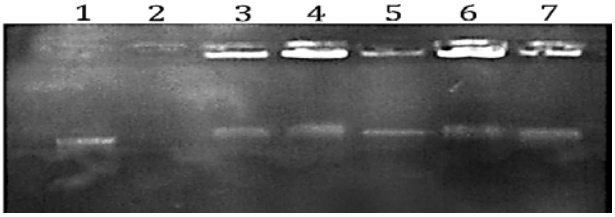

Fig 1. DNA damage protection activity of *Galium* taxa examined. 1: Supercoiled circular pET21a plasmid DNA, 2: pET21a plasmid DNA subjected to UV and H_2O_2, 3: *Galium verum* subsp. *verum*, 4: *Galium verum* subsp. *glabrescens*, 5: *Galium cassium*, 6*: Galium murale*, 7: *Galium humifusum.*

According to our findings, the extracts under investigation showed significant DNA protective action against free radical-induced DNA damage, suggesting that they may have anticancer potential.

Methanolic extract of *Galium* species was tested by the microdilution method against two Gram-Positive and two Gram-Negative bacterial strains from the ATCC Cell Biology Collection (*P. aeruginosa, E.faecalis, E. coli, S.aureus*). The MIC values of the extracts determined against bacterial strains ranged from 0.15625 to 5 mg/mL.

In general, it is seen that all species are effective against four bacteria, but they are especially effective against *P. aeruginosa* even at the lowest concentration (0.15625mg/mL). *P. aeruginosa* reproduces in humid media and leads to infection in sensitive patients. Therefore, it is one of the gram-negative bacteria that cause infection in the hospital environment. In this regard, it is very valuable that methanolic *Galium* extracts are effective against *P. aeruginosa* at low concentrations. Not only *P. aeruginosa*, but also *S. aureus* and *E. coli* can cause several diseases, especially hospital infections [37,40]. It is also important that methanolic *Galium* extracts are effective against *E. coli* at a concentration that can be considered low (Table 2). Against *S.aureus*, it is effective, albeit at higher concentrations. *E.faecalis* are microorganisms with low virulence, but they are important factors in the community and especially in hospital-caused infections33. It is also important that methanolic *Galium* extracts are effective against *E. coli* at a concentration that can be considered low (Table 2).

Table 2. Antibacterial activities of *Galium* species [mg/mL]

	P. aeruginosa	***E.faecalis***	***E. coli***	***S.aureus***
Galium murale (L.) All	0.1562	1.2500	1.2500	2.5000
Galium humifusum Bieb	0.1562	1.2500	1.2500	2.5000
Galium cassium Boiss.	0.1562	2.5000	0.3125	5.0000
Galium verum L. subsp. *Verum*	0.1562	1.2500	1.2500	2.5000
Galium verum L. subsp. *glabrescens* Ehrend	0.1562	2.5000	0.620	2.5000

Against *S.aureus*, it is effective, albeit at higher concentrations. *E.faecalis* are microorganisms with low virulence, but they are important factors in the community and especially in hospital-caused infections [40].

None of the compounds showed cytotoxic activity, which is active against gram-positive/gram-negative bacteria and yeast. However, every molecule exhibited a notable level of antioxidant action. Therefore, it can be said that the present study supports the value of the chemical and pharmacological effects of *Galium* species in many different fields. In addition, the *Galium* species tested in this study have not been studied in the literature [5,20,34]. This shows the originality of the study.

Statistical analysis

All tests were constructed by running standards or plant extracts of five different concentrations, in triplicate. Values are expressed as mean ± standard deviation of three replicate measurements. The results of ANOVA analysis show significant differences ($p<0.05$) in the means of total phenolic concentrations and free radical scavenging activities of the *Galium* species.

CONCLUSION

In this study, the biochemical activities of five different Galium species (*Galium murale, Galium humifusum, Galium Verum-Verum, Galium verum-glabrescens)* were measured and their antioxidant, antimicrobial, and DNA protective effects were tested. The tested Galium species were found to exhibit remarkable biochemical effects. The results of this study revealed that all samples indicated DNA damage safeguarding preventive action against deterioration of DNA caused by free radicals formed by the (UV)/H_2O_2-radical system.

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Application and Reversibility of Three Dimensional Cellular Automata

Ferhat ŞAH
Faculty of Economics and Administrative Sciences, Adiyaman University, Adiyaman, Turkey

✉: fsah@adiyaman.edu.tr 0000-0003-4847-9180

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ABSTRACT

In this study, we obtain the characteristic matrices of three-dimensional cellular automata under the null boundary condition. We examine the inverse of characteristic matrices. We obtain a recurrence equation to determine under what conditions the matrix is invertible. Thanks to this equation, we can calculate the inverse of large-dimensional matrices. Finally, we give some applications of cellular automata. We find the minimal polynomial of the characteristic matrix. We find the cycle length and transition length of the characteristic matrix with the help of minimal polynomials. We also find the attractive points of the characteristic matrix. Finally, we draw the State-Transition diagram with the results we obtained.

Keywords: Cellular Automata, Characteristic Matrices, Reversibility

Üç Boyutlu Hücresel Dönüşümlerin Terslenebilirliği ve Uygulaması

ÖZ

Bu çalışmada üç boyutlu hücresel dönüşümlerin karakteristik matrislerini sıfır sınır şartı altında elde ediyoruz. Karakteristik matrislerin tersini inceliyoruz. Matrisin hangi şartlarda tersinin olduğunu belirlemek için rekürans denklem elde ediyoruz. Bu denklem sayesinde büyük boyutlu matrislerin tersini hesaplayabiliriz. Son olarak hücresel dönüşümlerin bazı uygulamalarını veriyoruz. Karakteristik matrisin minimal polinomunu buluyoruz. Minimal polinomlar yardımıyla karakteristik matrisin devir uzunluğu ve geçiş uzunluğunu buluyoruz. Ayrıca karakteristik matrisin çekici noktalarını buluyoruz. Son olarak elde ettiğimiz sonuçlar ile Durum–Geçiş diyagramını çiziyoruz.

Anahtar Kelimeler: Hücresel Dönüşümler, Karakteristik Matrisler, Terslenebilirlik

INTRODUCTION

Cellular Automata (CA for short) was first used to obtain models in the fields of physics, biology and computer science. CA theory was first studied by Ulam and Von Neumann [1]. Later, many researchers became interested in studying CA to model the behavior of a complex system. Hedlund used CA systematically from a purely mathematical perspective [2]. Wolfram with the help of polynomial algebras [3], Pries to explain group properties based on a similar type of polynomial algebras [4] and Inokuchi et al. studied to observe the behavior of one-dimensional CA produced by the 156 rule [5]. Since two dimensional CAs (2D CAs) have widespread applications in physics, biology, mathematics and other sciences, the study of these CAs has accelerated in many branches of science in the last twenty years. On the other hand, Packard and Wolfram started their studies on two dimensional CA (2D CA) by making some observations on two-dimensional CA based on 5-neighborhood CA [6]. Das et al. extended the characterization of one-dimensional CA with the help of matrix algebras and introduced a new method for the theoretical analysis of linear CA [7]. They based the analysis of CA on polynomial algebra. At the same time, hybrid CAs were analyzed with this new method. Matrix characterization of CA was formulated to examine the resulting complex dynamic system.

Khan et al. developed a solution to examine 2D CA linear transformations with nearest neighbors over the field $\mathbb{Z}_2$ [8]. They examined the characterization of 2D CAs under periodic boundary conditions with the method and different rules they developed to separate two dimensional linear CAs.

Choudhury et al. gave the most general characterization of a special hybrid transformation of 2D CAs over the field $\mathbb{Z}_2$ [9]. Additionally, in another study, Choudhury and Dihidar achieved the characterization of 2D CAs by extending the one-dimensional CA theory with the help of matrix algebra[10]. Siap et al. examined two-dimensional cellular automata over the field $\mathbb{Z}_3$ with some special rules under periodic and null boundary

conditions[11]. Whether the rule (representative) matrices corresponding to the examined CAs are invertible has emerged as an important problem. If the rule matrix of CA has an inverse, then the CA corresponding to this matrix is said to be invertible. However, there have not been many studies on three-dimensional cellular automata. Tsalides et al. conducted a study on three-dimensional cellular automata and their applications[12]. R.W.Gerling classified 3D CAs in his study[13].

Jan Hemmingsson carried out studies on the quasi-periodic behavior of 3D-CAs [14]. S.G.R. Brown and N.B. Bruce tried to model the free development of 3D CAs in their study[15]. E.G. Leubeck and M.C.M. De Gunst worked on the analysis of cellular deterioration using 3D CAs[16]. Alexandra Agapie gave a simple form of the constant distribution for 3D CA in a special case[17].

In this study, we will define the neighborhood states of 3D CAs. We will examine the invertibility of the characteristic matrices obtained under the zero boundary condition. We will provide information about whether the cellular transformations corresponding to these characteristic matrices are reversible or not. We will make some applications of cellular automata.

THREE DIMENSIONAL CELLULAR AUTOMATA

First, the definition of 3D-CA over the field $\mathbb{Z}_P$ will be given. Then, the characteristic matrices will be examined under the null boundary condition with a special rule and a general form will be obtained. Consider three dimensional $\mathbb{Z}^3$lattices and $\sigma: \mathbb{Z}^3 \to \mathbb{Z}_p$element $\Omega = {\mathbb{Z}_p}^{\mathbb{Z}^3}$ configuration space. σ_n is defined by the value of σ at a $n \in \mathbb{Z}^3$ point. Let $u_1, u_2, \dots, u_s \in \mathbb{Z}^3$ a finite set of different elements and $f: {\mathbb{Z}_p}^s \to \mathbb{Z}_p$ be given. (Ω, F) is defined as a pair of CA and a local rule f, where $F: \Omega \to \Omega$, $(F\sigma)_n = f(\sigma_{n+u_1}, \dots, \sigma_{n+u_s}), n \in \mathbb{Z}^3$ is the global transition function.

Mathematically, the next state transition of the (i,j,k) cell can be represented as a function of the present states of the neighbor cells.

$$
\begin{aligned}
x_{(i,j,k)}^{(t+1)} = f(&x_{(i-1,j-1,k-1)}^{(t)}, x_{(i-1,j,k-1)}^{(t)}, x_{(i-1,j,k+1)}^{(t)}, x_{(i-1,j-1,k)}^{(t)}, x_{(i-1,j-1,k+1)}^{(t)},\\
&x_{(i-1,j,k)}^{(t)}, x_{(i-1,j+1,k)}^{(t)}, x_{(i-1,j+1,k-1)}^{(t)}, x_{(i-1,j+1,k+1)}^{(t)}, x_{(i,j-1,k-1)}^{(t)},\\
&x_{(i,j,k-1)}^{(t)} x_{(i,j,k+1)}^{(t)}, x_{(i,j-1,k)}^{(t)}, x_{(i,j-1,k+1)}^{(t)}, x_{(i,j,k)}^{(t)}, x_{(i,j+1,k)}^{(t)}, x_{(i,j+1,k-1)}^{(t)}, x_{(i,j+1,k+1)}^{(t)}, x_{(i+1,j-1,k-1)}^{(t)}\\
&x_{(i+1,j,k-1)}^{(t)}, x_{(i+1,j,k+1)}^{(t)}, x_{(i+1,j-1,k)}^{(t)}, x_{(i+1,j-1,k+1)}^{(t)}, x_{(i+1,j,k)}^{(t)}, x_{(i+1,j+1,k)}^{(t)},\\
&x_{(i+1,j+1,k-1)}^{(t)}, x_{(i+1,j+1,k+1)}^{(t)})\\
= a_0.&x_{(i-1,j-1,k-1)}^{(t+1)} + a_1.x_{(i-1,j,k-1)}^{(t+1)} + a_2.x_{(i-1,j,k+1)}^{(t+1)} + a_3.x_{(i-1,j-1,k)}^{(t+1)} +\\
&a_4.x_{(i-1,j-1,k+1)}^{(t+1)} + a_5.x_{(i-1,j,k)}^{(t+1)} + a_6.x_{(i-1,j+1,k)}^{(t+1)} + a_7.x_{(i-1,j+1,k-1)}^{(t+1)} +\\
&a_8.x_{(i-1,j+1,k+1)}^{(t+1)} + a_9.x_{(i,j-1,k-1)}^{(t+1)} + a_{10}.x_{(i,j,k-1)}^{(t+1)}\\
&+a_{11}.x_{(i,j,k+1)}^{(t+1)} + a_{12}.x_{(i,j-1,k)}^{(t+1)} + a_{13}.x_{(i,j-1,k+1)}^{(t+1)} + a_{14}.x_{(i,j,k)}^{(t+1)} +\\
&a_{15}.x_{(i,j+1,k)}^{(t+1)} + a_{16}.x_{(i,j+1,k-1)}^{(t+1)} + a_{17}.x_{(i,j+1,k+1)}^{(t+1)} +\\
&a_{18}.x_{(i+1,j-1,k-1)}^{(t+1)} + a_{19}.x_{(i+1,j,k-1)}^{(t+1)} + a_{20}.x_{(i+1,j,k+1)}^{(t+1)} + a_{21}.x_{(i+1,j-1,k)}^{(t+1)} +\\
&a_{22}.x_{(i+1,j-1,k+1)}^{(t+1)} + a_{23}.x_{(i+1,j,k)}^{(t+1)} + a_{24}.x_{(i+1,j+1,k)}^{(t+1)} + a_{25}.x_{(i+1,j+1,k-1)}^{(t+1)} +\\
&a_{26}.x_{(i+1,j+1,k+1)}^{(t+1)} \bmod p \quad a_0, a_1, a_2, \dots, a_{26} \in \mathbb{Z}_p - \{0\} \qquad (1)
\end{aligned}
$$

In this paper, we characterize the 3D NBCA determined according to local rules. We can use the following local rule to characterize NBCA. First, let's give our specially chosen local rule.

$$
\begin{aligned}
x_{(i,j,k)}^{(t+1)} = g.x_{(i,j,k+1)}^{(t+1)} + r.x_{(i,j+1,k)}^{(t+1)} +\\
u.x_{(i,j-1,k)}^{(t+1)} + w.x_{(i,j,k-1)}^{(t+1)} + y.x_{(i-1,j,k)}^{(t+1)}\\
+ z.x_{(i+1,j,k)}^{(t+1)} \bmod p\\
(g, r, u, w, y, z \in \mathbb{Z}_p - \{0\}) \qquad (2)
\end{aligned}
$$

There's the small matter of what the neighborhood of the cells at the other end of the cage should be. In most cases, we take the lattice to be large enough so that these cells are ignored and the lattice can be considered virtually infinite. However, in some cases, the extent of the lattice may be finite and there are various types of boundary conditions, but we will give only one of them. is expressed as follows.

A null boundary CA (NBCA) is one in which the extreme cells are connected to logic 0-state. If we characterize the 3D NBCA with the local rules in Eq. (2) we have obtained the rule matrix as follows:

$$\left(T_{RN}\right)_{mns\times mns}=\begin{pmatrix} K_n & Z_n & O_n & O_n & \cdots & O_n & O_n & O_n \\ B_n & K_n & Z_n & O_n & \cdots & O_n & O_n & O_n \\ O_n & B_n & K_n & Z_n & \cdots & O_n & O_n & O_n \\ O_n & O_n & B_n & K_n & \cdots & O_n & O_n & O_n \\ \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\ O_n & O_n & O_n & O_n & \cdots & K_n & Z_n & O_n \\ O_n & O_n & O_n & O_n & \cdots & B_n & K_n & Z_n \\ O_n & O_n & O_n & O_n & \cdots & O_n & B_n & K_n \end{pmatrix}_{mns\times mns} \quad (3)$$

K_n, Z_n, B_n, O_n are $n\times n$ block matrices where $n=ms$. The submatrices of the rule matrix are as follows:

$$K_n=\begin{pmatrix} S_s(u,r) & w.I_s & O_s & \ldots & O_s & O_s \\ g.I_s & S_s(u,r) & w.I_s & \ldots & O_s & O_s \\ O_s & g.I_s & S_s(u,r) & \ldots & O_s & O_s \\ \ldots & \ldots & \ldots & \ldots & \ldots & \ldots \\ O_s & O_s & O_s & \ldots & S_s(u,r) & w.I_s \\ O_s & O_s & O_s & \ldots & g.I_s & S_s(u,r) \end{pmatrix}_{ms\times ms}$$

$$Z_n=\begin{pmatrix} y.I_s & O_s & O_s & \ldots & O_s & O_s \\ O_s & y.I_s & O_s & \ldots & O_s & O_s \\ O_s & O_s & y.I_s & \ldots & O_s & O_s \\ \ldots & \ldots & \ldots & \ldots & \ldots & \ldots \\ O_s & O_s & O_s & \ldots & y.I_s & O_s \\ O_s & O_s & O_s & \ldots & O_s & y.I_s \end{pmatrix}_{ms\times ms}$$

$$B_n=\begin{pmatrix} z.I_s & O_s & O_s & \ldots & O_s & O_s \\ O_s & z.I_s & O_s & \ldots & O_s & O_s \\ O_s & O_s & z.I_s & \ldots & O_s & O_s \\ \ldots & \ldots & \ldots & \ldots & \ldots & \ldots \\ O_s & O_s & O_s & \ldots & z.I_s & O_s \\ O_s & O_s & O_s & \ldots & O_s & z.I_s \end{pmatrix}_{ms\times ms}$$

$$O_n=\begin{pmatrix} O_s & O_s & O_s & \ldots & O_s & O_s \\ O_s & O_s & O_s & \ldots & O_s & O_s \\ O_s & O_s & O_s & \ldots & O_s & O_s \\ \ldots & \ldots & \ldots & \ldots & \ldots & \ldots \\ O_s & O_s & O_s & \ldots & O_s & O_s \\ O_s & O_s & O_s & \ldots & O_s & O_s \end{pmatrix}_{ms\times ms}$$

I_s is $s\times s$ an identity matrix. O_s is $s\times s$ zero matrix. $S_s(u,r)$ is as follows:

$$S_s(u,r)=\begin{pmatrix} 0 & r & 0 & 0 & \ldots & 0 & 0 & 0 \\ u & 0 & r & 0 & \ldots & 0 & 0 & 0 \\ 0 & u & 0 & r & \ldots & 0 & 0 & 0 \\ 0 & 0 & u & 0 & \ldots & 0 & 0 & 0 \\ \ldots & \ldots & \ldots & \ldots & \ldots & \ldots & \ldots & \ldots \\ 0 & 0 & 0 & 0 & \ldots & 0 & r & 0 \\ 0 & 0 & 0 & 0 & \ldots & u & 0 & r \\ 0 & 0 & 0 & 0 & \ldots & 0 & u & 0 \end{pmatrix}_{s\times s}$$

Now, we give an example. We take $m=n=s=3$. we take into account a configuration of size $3\times3\times3$. we study 3D-CA under null boundary conditions. we apply the local rule to all cells of $\left[X\right]^t_{3\times3\times3}$. We obtain the characteristic matrix $\left[T_{RN}\right]_{27\times27}$ as follows:

$$T_{RN}=\begin{pmatrix} S_3(u,r) & w.I_3 & O_3 & y.I_3 & O_3 & O_3 & O_3 & O_3 & O_3 \\ g.I_3 & S_3(u,r) & w.I_3 & O_3 & y.I_3 & O_3 & O_3 & O_3 & O_3 \\ O_3 & g.I_3 & S_3(u,r) & O_3 & O_3 & y.I_3 & O_3 & O_3 & O_3 \\ z.I_3 & O_3 & O_3 & S_3(u,r) & w.I_3 & O_3 & y.I_3 & O_3 & O_3 \\ O_3 & z.I_3 & O_3 & g.I_3 & S_3(u,r) & w.I_3 & O_3 & y.I_3 & O_3 \\ O_3 & O_3 & z.I_3 & O_3 & g.I_3 & S_3(u,r) & O_3 & O_3 & y.I_3 \\ O_3 & O_3 & O_3 & z.I_3 & O_3 & O_3 & S_3(u,r) & w.I_3 & O_3 \\ O_3 & O_3 & O_3 & O_3 & z.I_3 & O_3 & g.I_3 & S_3(u,r) & w.I_3 \\ O_3 & O_3 & O_3 & O_3 & O_3 & z.I_3 & O_3 & g.I_3 & S_3(u,r) \end{pmatrix}_{27\times27}$$

Now, we write the submatrices of our characteristic matrix.

$$S_3(u,r)=\begin{pmatrix}0 & r & 0\\ u & 0 & r\\ 0 & u & 0\end{pmatrix}_{3\times 3}$$

$$K_3=\begin{pmatrix}S_3(u,r) & w.I_3 & O_3\\ g.I_3 & S_3(u,r) & w.I_3\\ O_3 & g.I_3 & S_3(u,r)\end{pmatrix}$$

$$Z_3=\begin{pmatrix}y.I_3 & O_3 & O_3\\ O_3 & y.I_3 & O_3\\ O_3 & O_3 & y.I_3\end{pmatrix}$$

$$B_3=\begin{pmatrix}f.I_3 & O_3 & O_3\\ O_3 & f.I_3 & O_3\\ O_3 & O_3 & f.I_3\end{pmatrix}$$

Finally, we have obtained our block matrix as follows:

$$T_{RN}=\begin{pmatrix}K_3 & Z.I_3 & O_3\\ B.I_3 & K_3 & Z.I_3\\ O_3 & B.I_3 & K_3\end{pmatrix}_{27\times 27}.$$

REVERSIBILITY

Reversibility of three dimensional cellular automata is a very difficult problem. If our characteristic matrix is invertible, we say that cellular automata is reversible. K. Morita studied the features of reversibility of cellular automata[18]. Z. Çınkır et al. were interested in the problem of reversibility of cellular automata over the field $\mathbf{Z}_m$ under periodic boundary conditions [19]. H. Akın et al. were interested in the problem of reversibility of cellular automata under reflective boundary conditions over the field $\mathbf{Z}_m$[20]. Chang et al. calculated reversibility of multi dimensional cellular automata [21]. Now, we will give a very important algorithm to determine the reversibility of 3D-CA under the null boundary conditions.

Theorem: For $n,m,s\geq 2$ $(n,m,s\in \mathbf{Z}^+)$, characteristic matrix $(T_{RN})_{mns\times mns}$ be defined as in Eq. (3). The rank of Eq.(3) $(n-1)ms+rank(\lambda_{2n-1})$. where λ_{2n-1} satisfies the following recurrence relation:

$\lambda_1(S)=K$, $\lambda_0(S)=B$

$n\geq 2$,$\lambda_{2n-1}(S)=-Z^{-1}K\lambda_{2n-3}(S)+\lambda_{2n-4}(S)$,

$n\geq 3$,$\lambda_{2n-4}(S)=-Z^{-1}B\ \lambda_{2n-5}(S)$

Proof: we will apply the induction method over $n\geq 2.$ For $n=2$, we have obtained a block matrix as follows.

$$T=\begin{pmatrix}K & ZI\\ BI & K\end{pmatrix}.$$

Now, if we want to calculate the rank of our block matrix, R_1 and R_2 are the rows of the block matrices. Multiply the first row by $-Z^{-1}\mathrm{K}$ and add it to the second row. We have obtained the block matrix as follows:

$$\begin{pmatrix}K & ZI\\ -Z^{-1}K^2+B=\lambda_3(S) & O\end{pmatrix}.$$

In this case rank of T depends on $\text{-}Z^{-1}K^2+\mathrm{B}=\lambda_3(\mathrm{S})$ .For $n=3$, we have obtained the block matrix as follows:

$$T=\begin{pmatrix}K & ZI & O\\ BI & K & ZI\\ O & BI & K\end{pmatrix}.$$

Multiply the second row by $-Z^{-1}\mathrm{K}$ and add it to the third row. We have obtained the block matrix as follows:

$$\begin{pmatrix}K & ZI & O\\ FI & K & ZI\\ -Z^{-1}BK=\lambda_2(\mathrm{S}) & \text{-}Z^{-1}K^2+\mathrm{B}=\lambda_3(\mathrm{S}) & O\end{pmatrix}$$

If, we multiply the first row of the new matrix by $-Z^{-1}\lambda_2(S)$ and add it to the third row. we have the following block matrix.

$$\begin{pmatrix}K & ZI & O\\ FI & K & ZI\\ -Z^{-1}\lambda_3(S)K+\lambda_2(\mathrm{S})=\lambda_5(S) & O & O\end{pmatrix}.$$

In this case rank of T depends on $-Z^{-1}\lambda_3(S)K+\lambda_2(\mathrm{S})=\lambda_5(S)$.The $(n-1)th$ row of T is found. If we multiply the $(n-1)th$ row by $-Z^{-1}\lambda_1(S)$ and add it to the last row, the last row is obtained as $(0,0,\ldots,\lambda_2(S),\lambda_3(S),0)$. Now, if we multiply the $(n-2)th$ row of the new matrix by $-Z^{-1}\lambda_3(S)$ and add it to the last row.

In this manner, the last row is obtained as $(0,0,\ldots,\lambda_4(S),\lambda_5(S),0,0)$. If we multiply the second row of the new matrix by $-Z^{-1}\lambda_{2n-5}(S)$ and add it to the last row, the last row is obtained as $(\lambda_{2n-4}(S),\lambda 2n-3(S),0,0,\ldots,0)$. Finally, if we multiply the first row of the new matrix by $-E^{-1}\lambda_{2n-3}(S)$ and add it to the last row, the last row is obtained as $(\lambda_{2n-1}(S),0,0,0,\ldots,0)$. The new matrix is as follows:

$$(T_{RN})_{mns\times mns}=\begin{pmatrix} K_n & Z_n & O_n & O_n & \cdots & O_n & O_n & O_n \\ B_n & K_n & Z_n & O_n & \cdots & O_n & O_n & O_n \\ O_n & B_n & K_n & Z_n & \cdots & O_n & O_n & O_n \\ O_n & O_n & B_n & K_n & \cdots & O_n & O_n & O_n \\ \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\ O_n & O_n & O_n & O_n & \cdots & K_n & Z_n & O_n \\ O_n & O_n & O_n & O_n & \cdots & B_n & K_n & Z_n \\ \lambda_{2n-1}(S) & O_n & O_n & O_n & \cdots & O_n & O_n & O_n \end{pmatrix}_{mns\times mns}$$

The proof is completed.

Example 1: we take $m=2$, $n=2$ and $s=2$. Let's calculate the rank of the characteristic matrix corresponding to our local rule under the null boundary condition. Firstly, we write our characteristic matrix as follows:

$$T_{RN}=\begin{pmatrix} 0 & r & w & 0 & y & 0 & 0 & 0 \\ u & 0 & 0 & w & 0 & y & 0 & 0 \\ g & 0 & 0 & r & 0 & 0 & y & 0 \\ 0 & g & u & 0 & 0 & 0 & 0 & y \\ z & 0 & 0 & 0 & 0 & r & w & 0 \\ 0 & z & 0 & 0 & u & 0 & 0 & w \\ 0 & 0 & z & 0 & g & 0 & 0 & r \\ 0 & 0 & 0 & z & 0 & g & u & 0 \end{pmatrix}_{8\times 8}$$

$$=\begin{pmatrix} S_2(u,r) & w.I_2 & y.I_2 & O_2 \\ g.I_2 & S_2(u,r) & O_2 & y.I_2 \\ z.I_2 & O_2 & S_2(u,r) & w.I_2 \\ O_2 & z.I_2 & g.I_2 & S_2(u,r) \end{pmatrix}_{8\times 8}$$

If we want to write the above characteristic matrix as a block matrix,

$$K_2=\begin{pmatrix} S_2(u,r) & w.I_2 \\ w.I_2 & S_2(u,r) \end{pmatrix}, Z_2=\begin{pmatrix} y.I_2 & O_2 \\ O_2 & y.I_2 \end{pmatrix}$$

$$B_2=\begin{pmatrix} z.I_2 & O_2 \\ O_2 & z.I_2 \end{pmatrix}.$$

Now let's write our matrix as a block matrix,

$$T_{RN}=\begin{pmatrix} K_2 & Z_2 \\ B_2 & K_2 \end{pmatrix}.$$

İf we take $g=r=u=1$,w $=y=z=2$,

$(g,r,u,w,y,z\in \mathbf{Z}_3)$

we obtained the equation as follows:

$n\geq 2$, $\lambda_{2n-1}(S)=-Z^{-1}K\lambda_{2n-3}(S)+\lambda_{2n-4}(S)$,

$$\lambda_3(S)=-Z^{-1}K\lambda_1(S)+\lambda_0(S)$$
$$=-Z^{-1}K^2+B$$

$\lambda_1(S)=K$, $\lambda_0(S)=B$

$$(T_{RN})_{8\times 8}=(n-1)ms+rank(\lambda_{2n-1})$$
$$=(2-1)2.2+rank(\lambda_3)$$
$$=4+4=8$$

The rank of an invertible matrix is equal to the order of the matrix, Thus characteristic matrix is invertible. So CA which corresponding to the characteristic matrix is reversible. If we take $g=r=u=w=y=z=1$,

We obtained the equation as follows:

$$(T_{RN})_{8\times 8}=(n-1)ms+rank(\lambda_{2n-1})$$
$$=(2-1)2.2+rank(\lambda_3)$$
$$=4+2=6$$

Characteristic matric hasn't got full rank. So it isn't invertible. Thus CA which corresponding to the characteristic matrix isn't reversible.

APPLICATION

Now, we give an application of cellular automata.we find a minimal polynomial of T transition matrix. We obtain cycle and transient length of T transition matrix with the help of minimal polynomial. We will also determine the attractor points of our T transition matrix. Let's provide some definitions.

Definition 1 Let x be the initial configuration. For $t\in N$, $X_t=T^tX_0$. If there is no $t\in N$ such that $T^tX_0=0$, there will be $T^iX_0=T^jX_0$ for some number i,j since the number of all possible configurations is finite.

$(i,j)<(k,l) \Leftrightarrow i<k$ or $i=k, j<l$, then there is the smallest pair of numbers (t,c) that satisfy the condition $T^t X_0 = T^{t+c} X_0$ .The number $t \in N$ in the expression $T^t X_0 = T^{t+c} X_0$ is called the transition length on the X_0 configuration, and the number $c \in N$ is called the cycle length on the X_0 configuration.

Definition 2 The configuration that returns to itself in a certain time step is called an attractor point. In other words, in a cellular automata configuration tree, it is the configuration that can be seen as the root of the tree.

Definition 3 After starting with the initial configuration, the configurations reached until returning to the configuration itself in a certain time step are called the basin of the last obtained configuration, and this diagram obtained during this period is called the State-Transition Diagram.

Example2: If we take $m=2, n=2$ and $s=2$. we write our characteristic matrix as follows:

$$T_{RN} = \begin{pmatrix} 0 & r & w & 0 & y & 0 & 0 & 0 \\ u & 0 & 0 & w & 0 & y & 0 & 0 \\ g & 0 & 0 & r & 0 & 0 & y & 0 \\ 0 & g & u & 0 & 0 & 0 & 0 & y \\ z & 0 & 0 & 0 & 0 & r & w & 0 \\ 0 & z & 0 & 0 & u & 0 & 0 & w \\ 0 & 0 & z & 0 & g & 0 & 0 & r \\ 0 & 0 & 0 & z & 0 & g & u & 0 \end{pmatrix}_{8\times 8}$$

If we take $g=w=u=y=z=1$ and $r=0$, $(g,r,u,w,y,z \in \mathbf{Z}_3)$, we have obtained the characteristic matrix as follows:

$$T_{RN} = \begin{pmatrix} 0 & 0 & 1 & 0 & 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 1 & 1 & 0 & 0 & 0 & 0 & 1 \\ 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 & 1 & 0 & 0 & 1 \\ 0 & 0 & 1 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 1 & 1 & 0 \end{pmatrix}_{8\times 8}$$

Let's try to find the transition length, cycle length and basin of the attractive points, if any, by arbitrarily choosing any of the $2^8 = 256$ vectors over the feld $\mathbf{Z}_2$. Let's choose an arbitrary vector as follows.

$$F = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \end{bmatrix}_{8\times 1} \rightarrow 1$$

$$T_{RN}.F = \begin{pmatrix} 0 & 0 & 1 & 0 & 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 1 & 1 & 0 & 0 & 0 & 0 & 1 \\ 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 & 1 & 0 & 0 & 1 \\ 0 & 0 & 1 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 1 & 1 & 0 \end{pmatrix}_{8\times 8} \cdot \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \end{bmatrix}_{8\times 1} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 1 \\ 0 \\ 0 \end{bmatrix}_{8\times 1} \rightarrow 20$$

$$T_{RN}.F = \begin{pmatrix} 0 & 0 & 1 & 0 & 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 1 & 1 & 0 & 0 & 0 & 0 & 1 \\ 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 & 1 & 0 & 0 & 1 \\ 0 & 0 & 1 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 1 & 1 & 0 \end{pmatrix}_{8\times 8} \cdot \begin{bmatrix} 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 1 \\ 0 \\ 0 \end{bmatrix}_{8\times 1} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 0 \end{bmatrix}_{8\times 1} \rightarrow 0$$

If we continue similarly, let's write some results as follows:

State-Transition Diagram

$$1-21-40-60-64-84-105-125-135-147-174-186-198-210-239-251$$
$$\downarrow$$
$$20$$
$$\downarrow$$
$$0$$

$2-22-43-63-67-87-106-126-132-144-173-185-197-209-236-248$
$\downarrow$
41
$\downarrow$
0

$3-23-42-62-66-86-107-127-133-145-172-184-196-208-237-249$
$\downarrow$
61
$\downarrow$
0

$4-16-45-57-69-81-108-120-130-150-171-191-195-215-234-254$
$\downarrow$
65
$\downarrow$
0

$5-17-44-56-68-80-109-121-131-151-170-190-194-214-235-255$
$\downarrow$
85
$\downarrow$
0

$6-18-47-59-71-83-110-122-128-148-169-189-193-213-232-252$
$\downarrow$
104
$\downarrow$
0

$7-19-46-58-70-82-111-123-129-149-168-188-192-212-233-253$
$\downarrow$
124
$\downarrow$
0

$8-28-33-53-73-93-96-116-142-154-167-179-207-219-230-242$
$\downarrow$
134
$\downarrow$
0

$9-29-32-52-72-92-97-117-143-155-166-178-198-206-218-231-243$
$\downarrow$
146
$\downarrow$
0

$10-30-35-55-75-95-98-118-140-152-165-177-205-217-228-240$
$\downarrow$
175
$\downarrow$
0

$11-31-34-54-74-91-99-119-141-153-164-176-204-216-229-241$
$\downarrow$
187
$\downarrow$
0

$12-24-37-49-77-89-100-112-138-158-163-183-203-223-226-246$
$\downarrow$
199
$\downarrow$
0

$13-25-36-48-76-88-101-113-139-159-162-182-202-222-227-247$
$\downarrow$
211
$\downarrow$
0

$14-26-39-51-79-91-102-114-136-156-161-181-201-221-224-244-251$
$\downarrow$
238
$\downarrow$
0

$15-27-38-50-78-90-103-115-137-157-160-180-200-220-225-245$
$\downarrow$
250
$\downarrow$
0

If we continue in this way, each element will go to zero after the second pass.

$$T_{RN}=\begin{pmatrix}0&0&1&0&1&0&0&0\\1&0&0&1&0&1&0&0\\1&0&0&0&0&0&1&0\\0&1&1&0&0&0&0&1\\1&0&0&0&0&0&1&0\\0&1&0&0&1&0&0&1\\0&0&1&0&1&0&0&0\\0&0&0&1&0&1&1&0\end{pmatrix}_{8\times 8}$$

The minimal polynomial is x^8. The transition length is 2 and the cycle length is 0. Also, the attractive points are 0 and all configurations are basins of 0.

The characteristic polynomial is x^8. If, we examine the kernel of our matrix above, we obtained as follows:

$K=\{(10000110),(00010100),(01101000),(00101001)\}$ .If, we obtain the elements of the vector space from here, we have obtaned a result as follows.

$$V=\begin{Bmatrix}00000000,10000110(134),00010100(20),01101000(104),\\00101001(41),10010010(146),11101110(238),10101111(175),\\01111100(124),01000001(65),00111101(61),11111010(250),\\01010101(85),11000111(199),10111011(189),11010011(211)\end{Bmatrix}$$

If we look carefully, we see that each of the elements corresponds to a root.

CONCLUSION

First, the characteristic matrix of three-dimensional cellular automata was obtained under the null boundary condition. Then, the invertibility of our characteristic matrix was examined with the help of a theorem. Thanks to this theorem, we were able to obtain information about the invertibility of very large matrices. We show that if our characteristic matrix is invertible, its corresponding cellular transformations are also reversible. Finally, we gave some applications of three dimensional cellular automata under null boundary conditions.

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Neural Network Based a Comparative Analysis for Customer Churn Prediction

Anıl UTKU[1], M. Ali AKCAYOL[2]
[1] Computer Engineering, Faculty of Engineering, Munzur University, Tunceli, Turkey
[2] Computer Engineering, Faculty of Engineering, Gazi University, Ankara, Turkey

✉: anilutku@munzur.edu.tr [1] 0000-0002-7240-8713 [2] 0000-0002-6615-1237

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ABSTRACT

Customer churn refers to a customer's disconnection from a business. The expense associated with customer churn encompasses both the forfeited revenue and the marketing expenditures required to acquire new customers. Mitigating customer churn stands as the foremost objective for every business. Customer churn prediction will contribute to developing strategies enabling businesses to retain these customers by identifying customers with a high risk of loss. The importance of developing customer churn prediction models is increasing daily in the digital world. In this study, an MLP-based artificial neural network model was developed for customer churn prediction using customer data from an anonymous telecommunications company. The developed model was compared with kNN, LR, NB, RF, and SVM. The prediction results of the applied models were discussed, and the experimental results showed that all the models compared had over 70% accuracy. Experimental results showed that the developed MLP-based artificial neural network model has the most successful classification performance compared to other models, with approximately 94% accuracy.

Keywords: Artificial Neural Network, MLP, Customer Churn Prediction

Müşteri Kayıp Tahmini için Sinir Ağı Tabanlı Karşılaştırmalı Analiz

ÖZ

Müşteri kaybı, müşterinin bir işletmeyle bağlantısının kesilmesi anlamına gelir. Müşteri kaybıyla ilgili gider, hem kaybedilen geliri hem de yeni müşteriler kazanmak için gereken pazarlama harcamalarını kapsar. Müşteri kaybının azaltılması her işletmenin en önemli hedefidir. Müşteri kayıp tahmini, işletmelerin yüksek kayıp riski olan müşterileri belirleyerek bu müşterileri ellerinde tutmalarını sağlayan stratejiler geliştirmelerine katkıda bulunacaktır. Dijital dünyada müşteri kayıp tahmini modellerinin geliştirilmesinin önemi her geçen gün artmaktadır. Bu çalışmada, anonim bir telekomünikasyon şirketinden elde edilen müşteri verileri kullanılarak müşteri kayıp tahmini için MLP tabanlı yapay sinir ağı modeli geliştirilmiştir. Geliştirilen model kNN, LR, NB, RF ve SVM ile karşılaştırılmıştır. Uygulanan modellerin tahmin sonuçları tartışılmış ve deneysel sonuçlar, karşılaştırılan tüm modellerin %70'in üzerinde doğruluğa sahip olduğunu göstermiştir. Deneysel sonuçlar, geliştirilen MLP tabanlı yapay sinir ağı modelinin diğer modellere göre yaklaşık %95 doğrulukla en başarılı sınıflandırma performansına sahip olduğunu göstermiştir.

Anahtar Kelimeler: Yapay Sinir Ağları, Yapay Sinir Ağları, MLP, Müşteri Kayıp Tahmini

INTRODUCTION

Customer churn means that a customer unsubscribes from a service they are using. The customer churn prediction also determines which customers are most likely to unsubscribe [1]. This information is essential for companies to retain their current customers. Therefore, the insights derived from the churn prediction help focus more on customers at high risk of leaving. For businesses, retaining existing customers is more accessible than acquiring new customers. Also, revenue from existing customers is often higher than revenue from new customers. The cost of acquiring customers can be even higher in a competitive industry where competitors are plentiful. Therefore, predicting customer churn before customers leave is essential for businesses to retain their customers [2].

There can be many reasons for customer churn. The presence of a new competitor in the market offering better prices or unsatisfactory service may result in customer churn. For reasons like these, there is no correct answer as to why the customer would want to give up. Although there are many factors for customer churn, it is usually simple to avoid. This is because the company makes its customers feel special and provides a customized experience to entice them to stay. Churn prediction is one of the most critical commercial sector data science applications. The fact that understanding its effects is more concrete and plays an essential factor in

the total profit earned by the business has made customer churn prediction a popular research area [3].

Many studies use machine learning and deep learning methods for customer churn prediction. This section continues by examining these studies and presenting their prominent features in a table.

Khodabandehlou and Rahman [4] conducted a machine learning-based study using the Iranian dataset for customer churn analysis. They selected a set of five variables and compared the model created with Artificial Neural Network (ANN), Support Vector Machine (SVM), and Decision Tree (DT). The results were impressive, with the proposed model achieving a 97.92% accuracy rate. This success underscores the effectiveness of machine learning in customer churn prediction.

Asthana et al. [5] presented a comparative analysis of machine learning methods using the UCL customer churn dataset. The algorithms of ANN, SVM, DT, Naïve Bayes (NB), and Linear Regression (LR) have been examined comparatively. 94% accuracy rate was obtained with ANN and DT algorithms.

Agrawal et al. [6] developed a deep learning-based customer churn prediction model using the Telco dataset. A multilayer neural network was developed to establish a non-linear classification model. The deep learning model obtained 80.03% accuracy.

Using the Telco dataset, Gaur and Dubey [7] conducted a machine learning-based comparative study for customer churn prediction. In the study, LR, SVM, Random Forest (RF), and Gradient Boosting algorithms have been compared. Experimental results showed that the Gradient Boosting algorithm has more successful with 0.845 Area Under Curve (AUC).

Halibas et al. [8] studied machine learning-based customer churn prediction using the Telco dataset. They compared NB, generalized linear models (GLM), LR, DT, RF, Gradient Boosting, and deep neural networks. Experimental results showed that gradient-enhanced trees are the best classifiers.

Kavitha et al. [9] conducted a machine learning-based customer churn prediction study using the Telco dataset. DT, RF, and eXtreme Gradient Boosting (XGBoost) algorithms have been compared in the study. Experimental results showed that RF is more successful than other models compared with 80% accuracy.

Lalwani et al. [10] conducted a machine learning-based customer churn prediction study using the Telco dataset. They used LR, DT, Adaboost classifiers, k Nearest Neighbour (kNN), RF, NB, SVM, XGBoost, and CatBoost classifiers. The CatBoost and AdaBoost classifiers obtained an accuracy rate of close to 82%.

Chabumba et al. [11] developed a customer churn prediction model using the Telco dataset. The model uses machine learning methods and a new feature selection method on the big data platform. Its AUC is 84%. The model has been compared with the LR, RF, SVM, and XGBoost algorithms. Experimental results showed that RF was the most successful model, with an 80% accuracy rate.

The reviewed studies in the literature are summarized in Table 1.

Table 1. Summary of studies in the literature

Reference	Date	Methods	Dataset	Success rate
4	2017	ANN, SVM, DT	Iranian	%97.92 accuracy
5	2018	ANN, SVM, DT, NB, LR	UCL	%94 accuracy
6	2018	MLP	Telco	%80.03 accuracy
7	2018	LR, SVM, RF, Gradient Boosting	Telco	0.845 AUC
8	2019	NB, GLM, LR, DT, RF, Gradient Boosting, MLP	Telco	%79.1 accuracy
9	2020	DT, RF, XGBoost	Telco	%80 accuracy
10	2021	LR, DT, Adaboost, kNN, RF, NB, SVM, XGBoost, CatBoost	Telco	%82 accuracy
11	2021	LR, RF, SVM, XGBoost	Telco	%80 accuracy

Table 1 summarizes the studies in the literature examined according to their characteristics such as reference, publication date, methods used, dataset used, and success rate. The studies examined in the literature generally use machine learning methods. The algorithms used are generally LR, RF, DT, and SVM. Mainly, studies in the literature using the Telco dataset have been examined. The success rate in studies using the Telco dataset is approximately 80%.

In this study, a Multilayer Perceptron (MLP) based artificial neural network model was developed and compared with kNN, LR, NB, RF, and SVM. IBM Telco dataset, which consists of customer data of an anonymous telecommunications company, which is public access on Kaggle, was used as the dataset [12]. This study obtained a higher accuracy rate than the studies in the literature using the same dataset as the artificial neural network model developed. The contributions of this study to the literature can be summarized as follows:

- MLP-based artificial neural network model was created for customer churn prediction. In the existing literature, studies comparing MLP with other common models are limited. This study makes a significant contribution to the literature on performance evaluations in this field

by examining in detail the performance of MLP in customer churn prediction.
- The developed MLP model was compared with commonly used models such as kNN, LR, NB, RF and SVM, and experimental results showed that the MLP model performed better with a higher accuracy rate (94%) compared to other models.
- The use of customer data obtained from an anonymous telecommunications company shows that the study is based on real-world data and is aimed at practical applications.
- In the study, anonymizing customer data and emphasizing ethical principles makes a significant contribution to the literature on how data privacy and ethical issues should be addressed in customer churn prediction models.

CUSTOMER CHURN PREDICTION

Customer churn is a financial concept that pertains to a customer's discontinuation of engagement with a company or business. Likewise, the customer churn rate represents the pace at which customers depart from a business within a specified timeframe. Churn rate above a certain threshold can affect the business's success. Businesses want to retain as many customers as possible [13].

The churn rate serves as an indicator of customer contentment. A low churn rate signifies pleased customers, whereas a high churn rate indicates dissatisfied customers who have distanced themselves from the company [14]. Acquiring new customers will require much more effort and cost than retaining existing customers. Customer churn prediction is an indicator of growth potential for businesses. Churn rates represent the rate of lost customers, while growth rates represent newly acquired customers. Analysing these metrics gives information about the growth status of businesses. If the growth rate is higher than the loss rate, it can be said that the business is growing; otherwise, the business is shrinking [15].

The churn of customers applies in many contexts but generally relates to the business situation of customers who have stopped purchasing. Innovative customer retention strategies are more important for subscription-based and subscription-based business models. Analyzing growth in this area may involve monitoring parameters such as revenues and the proportion of new customers and performing customer analysis [16]. The churn rate quantifies the proportion of customer subscriptions or purchases that cease within a specified time frame for a particular service or product. Cancellation of customer subscriptions will naturally result in a loss of revenue. For this reason, examining the churn rate can help know customers and, for subscription-oriented businesses, effective marketing strategies to retain their customers [17].

Customer churn analysis can be evaluated as the churn of customers and revenue or as voluntary churn [18]. Customer churn represents the frequency at which customers discontinue their subscriptions. The churn of income refers to the loss in the monthly income of the enterprise. The churn of customers and loss of revenue may only sometimes be balanced [19]. The business may not lose customers, but there may be a loss of revenue due to the subscription tariffs that customers will change [20]. Damaging loss only applies to lost revenue. The generated revenue from existing customers surpasses the revenue lost due to cancellations and changes in subscription fees. Voluntary loss occurs when customers proactively terminate their service and take the necessary actions to discontinue their association [21]. This could result from customer discontentment or needing to realize the anticipated value. The churn of customers may occur due to poor customer service, financial issues, changes in customer needs, the service offered not meeting customers' expectations, or customers preferring rival companies [22].

NEURAL NETWORK BASED CUSTOMER CHURN PREDICTION

Accurate customer churn prediction is paramount for telecommunications companies to retain their customer base proficiently. Obtaining new customers incurs higher costs compared to maintaining existing ones. Hence, prominent telecommunications companies endeavor to construct models that forecast which customers are at a higher risk of attrition and devise strategies based on these developed models. With the developments in data science and machine learning methods, the problem of identifying potential customers who may stop doing business with them soon comes to the fore.

This study developed an MLP-based model to predict how likely customers will abandon their business by analysing their demographic, account, and service information. The developed model aims to obtain a data-oriented solution that will reduce customer churn rates and increase customer satisfaction and operating income. The developed model was compared with kNN, LR, NB, RF, and SVM.

Dataset

This study used the IBM Telco dataset consisting of customer data of an anonymous telecommunications company, which is public access on Kaggle. The dataset comprises 7043 rows and 21 columns, each corresponding to a customer in the dataset. The columns represent the individual attributes of each customer, which are utilized to predict the churn behavior of that specific customer. Of the features in the dataset, 17 features are categorical and 3 are numerical. Finally, there is the Churn attribute, expressed as Yes/no, in which it is predicted whether the customer will churn. The Churn column indicates the customer's departure status within the past month. "No" denotes customers who have not discontinued their association with the company during the last month, while "Yes" signifies

customers who have chosen to terminate their affiliation with the company.

The categorical attributes in the dataset can be described as follows:

• CustomerID: A unique customer ID number for each customer.

• gender: Indicates the gender of the customer as Female/Male.

• SeniorCitizen: Indicates whether the customer is a senior citizen or not, denoted as 1/0.

• Partner: Indicates Yes/No whether the customer is a business partner or not.

• Dependent: Indicates whether the customer is dependent or not, as Yes/No.

• PhoneService: Indicates whether the customer has phone service or not, denoted as Yes/No.

• MultipeLines: Indicates whether the customer has multiple phone lines or not, denoted as Yes/No/No phone service.

• InternetService: Indicates the customer's internet service provider type as DSL/Fiber optic/No.

• OnlineSecurity: Indicates whether the customer has online security or not, denoted as Yes/No/No internet service.

• OnlineBackup: Indicates whether the customer has online backup or not, denoted as Yes/No/No internet service.

• DeviceProtection: Indicates whether the customer has device protection or not, denoted as Yes/No/No internet service.

• TechSupport: Indicates whether the customer has technical support or not, denoted as Yes/No/No internet service.

• StreamingTV: Indicates whether the customer has streaming TV or not, denoted as Yes/No/No internet service.

• StreamingMovies: Indicates whether the customer has streaming movies or not, denoted as Yes/No/No internet service.

• Contract: Refers to the contract period of the customer, categorized as monthly, one-year, or two-year.

• PaperlessBilling: Refers to the contract period (monthly, one-year, two-years) on the customer's invoice.

• PaymentMethod: Indicates the customer's preferred payment method, categorized as mailed check, electronic check, credit card or bank transfer.

Numerical attributes can be described as follows:

• Tenure: Refers to the number of months the customer has been with the company.

• MonthlyCharges: It represents the monthly amount collected from the customer.

• TotalCharges: It represents the total amount collected from the customer.

The services subscribed by each customer are stored in the attributes: MultipleLines, PhoneService, OnlineSecurity, InternetService, DeviceProtection, OnlineBackup, TechSupport, StreamingMovies, StreamingTV. In Figure 1, customer churn rates for the attributes of each customer's registered service are shown.

Demographic customer information is kept in the fields of gender, SeniorCitizen, Partner, Dependents. Figure 2 shows customer churn rates for demographic attributes.

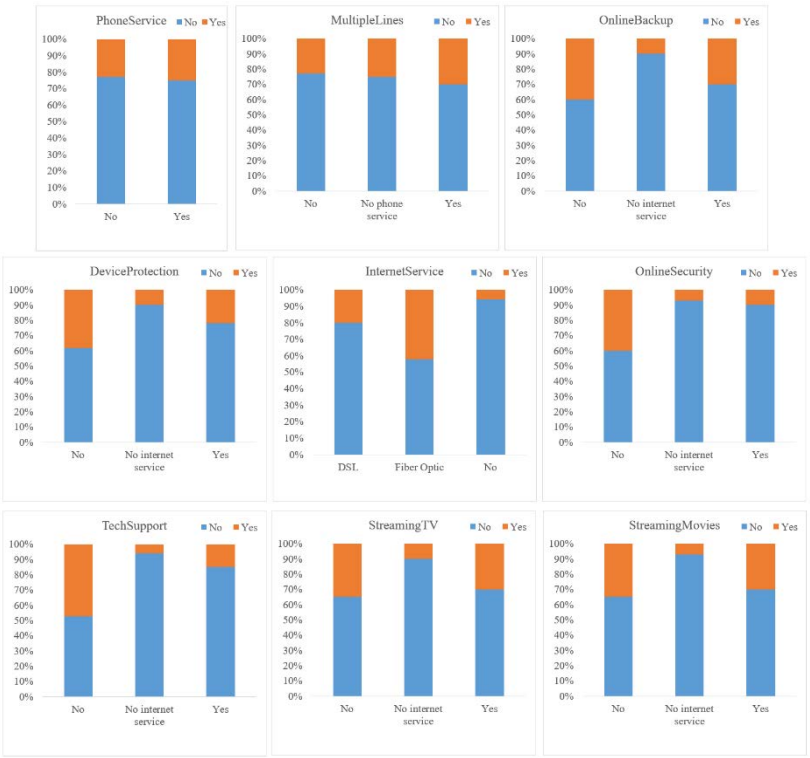

Figure 1. Customer churn rates for attributes of each customer's registered service

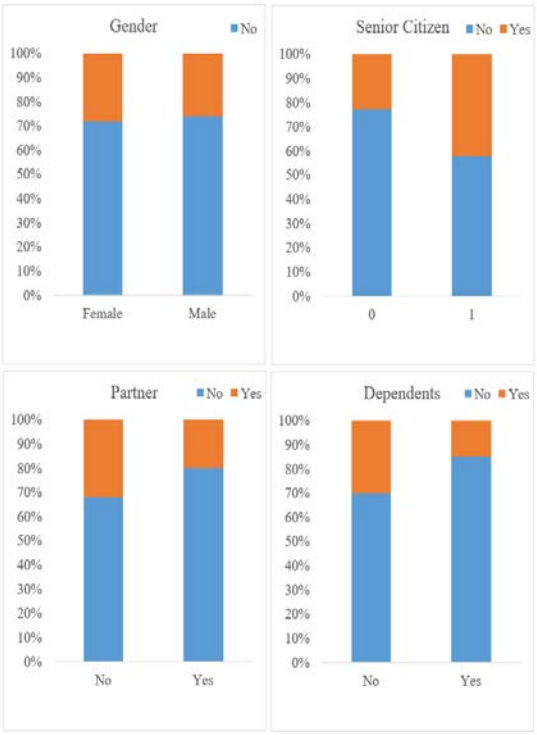

Figure 2. Customer churn rates for demographic attributes

Customer account information is kept in Contract, PaperlessBilling and PaymentMethod attributes. In Figure 3, the customer churn rates for the attributes of each customer account information are shown.

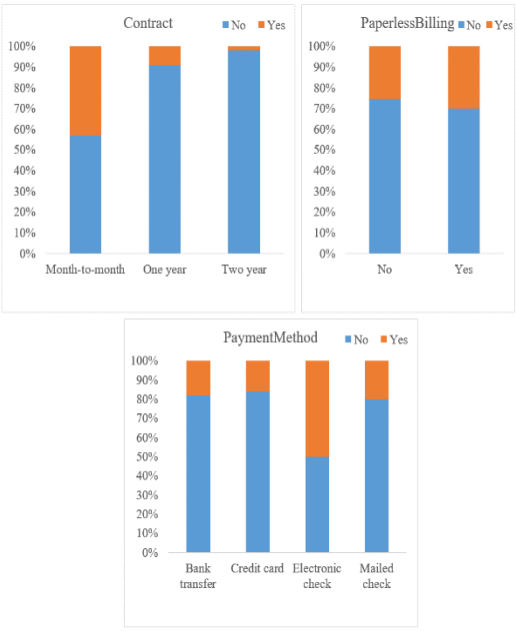

Figure 3. Customer churn rates for attributes of customer account information

According to the Churn attribute in the dataset, the Yes/No type customer churn rate is shown in Figure 1. Customer churn rates by churn attribute are shown in Figure 4.

As seen in Figure 4, the churn rate value for customers classified as 'No' in the Churn attribute is 0.734, while the churn rate value for customers classified as 'Yes' is 0.265.

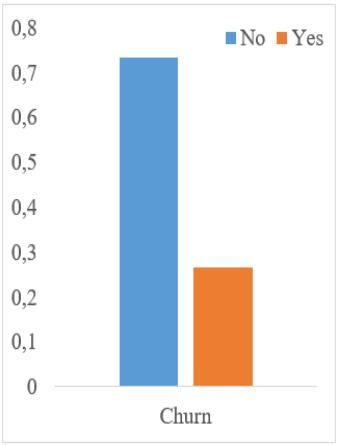

Figure 4. Customer churn rates by Churn attribute

Data Pre-processing

The utilized dataset comprises 7043 rows and 21 columns. Among the features within the dataset, 17 are categorical, and 3 are numerical. The final column encompasses the dependent attribute, Churn. As seen in Table 2, each column is filled with non-empty values and has a data type.

Table 2. Attributes in the dataset

#	Column	Non-Null Count	Dtype
0	customerID	7043 non-null	object
1	Gender	7043 non-null	object
2	SeniorCitizen	7043 non-null	Int64
3	Partner	7043 non-null	object
4	Dependents	7043 non-null	object
5	tenure	7043 non-null	Int64
6	PhoneService	7043 non-null	object
7	MultipleLines	7043 non-null	object
8	InternetService	7043 non-null	object
9	OnlineSecurity	7043 non-null	object
10	OnlineBackup	7043 non-null	object
11	DeviceProtection	7043 non-null	object
12	TechSupport	7043 non-null	object
13	StreamingTV	7043 non-null	object
14	StreamingMovies	7043 non-null	object
15	Contract	7043 non-null	object
16	PaperlessBilling	7043 non-null	object
17	PaymentMethod	7043 non-null	object
18	MonthlyCharges	7043 non-null	Float64
19	TotalCharges	7043 non-null	object
20	Churn	7043 non-null	object

To ensure optimum prediction performance of the developed models, handling missing or incorrect values by removing or replacing them with appropriate values is

very important. Only 11 NULL values have been detected in the TotalCharges column. In the TotalCharges column, which represents the total amount charged from the customer, any NULL value is populated with the average value obtained from the same column.
The dataset has no Internet service because some categorical attributes have more than two categorical values, such as Yes/No/No Internet service. Values such as have been replaced with No. For example, the No phone or Internet service values have been replaced with No.
Since customer churn prediction involves a classification problem, converting the categorical data within the dataset into a numerical format is essential. For this reason, Yes for categorical variables has been replaced with 1 and No with 0. Similarly, in the Gender column, which represents the customers' gender, Male has been replaced with 1 and Female with 0.
Feature selection enables the identification of key features that are important in predicting the target feature. Table 3 shows some of the essential values of the features in the dataset.

Table 3. Properties and importance values in the dataset

Features	Feature importance
InternetService_Fiber optic	0.3268
Contract_Month-to-month	0.3090
PaperlessBilling	0.1658
StreamingTV_Yes	0.1342
StreamingMovies_Yes	0.1316
OnlineSecurity_No	0.1189
PaymentMethod_Electronic check	0.1138
TechSupport_No	0.0958
MultipleLines_Yes	0.0905
SeniorCitizen	0.0792
OnlineBackup_No	0.0535
DeviceProtection_Yes	0.0490
DeviceProtection_No	0.0230
OnlineBackup_Yes	0.0170
PhoneService	0.0019
Partner	-0.0082
gender	-0.0411

Table 3 shows that InternetService_Fiber optic and Contract_Month-to-month attributes have higher importance values, while gender and Partner attributes have much lower importance values.
Categorical values have been converted to numerical values using Label Encoding. Label encoding encodes a value in the range 0 to (n-1) into each row of data with a categorical value. Here n is the number of tags that differ. If a tag repeats, it will get the value previously assigned. In this way, it is ensured that categorical columns are expressed as numerical values.
Large numerical data must be scaled before the model is built, which can affect the model's performance. Normalization ensures that each input variable is scaled within a specified range. In this way, columns with large numeric values are scaled between -1 and 1 using MinMaxScaler.

Developed Neural Network Based Prediction Model

After pre-processing, the datasets were split into training, validation, and testing sets. The dataset was split into 80% for training and 20% for testing. Within the training set, 10% was further allocated for validation purposes, aiding in optimizing the model parameters. The resulting training dataset, obtained after separating training and testing sets, comprises 5634 rows, while the test dataset consists of 1409.
The selection of hyper-parameters enables evaluating the model's performance across various combinations of hyper-parameter values. Additionally, hyper-parameter tuning encompasses choosing the metrics or methods that yield optimal results based on a selected metric and validation approach. Grid search was used for hyper-parameter optimization. The flowchart of the developed system is shown in Figure 5.

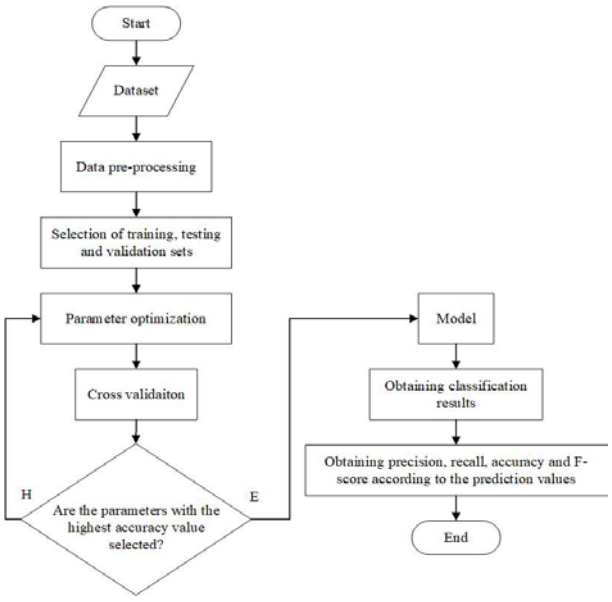

Figure 5. Flow chart of the developed system

The developed artificial neural network model takes customer data from the training dataset as input and generates an output indicating whether the customer is likely to churn or not for the customer data in the test dataset.
The developed MLP-based artificial neural network model consists of an input layer, hidden layers, and an output layer. The developed model has additional hidden layers compared to the traditional MLP model. Through the added layers, it is aimed that the model can better solve more complex and deep learning problems. Additionally, optimized ReLU activation functions were used for each layer. In order to prevent overfitting,

Dropout and L2 editing techniques are integrated. By using the dynamic learning rate, a faster and more stable optimization was achieved during the training. The connections between the layers represent the learned coefficients. The hidden layers are an intermediate processing step, combining weighted sums to derive the classification result. The developed model follows a sequential architecture with linear layers. The initial layer is the input layer, consisting of 64 input features and 64 output units. A Dropout layer is inserted between the input layer and the hidden layer. The output layer comprises a single unit that predicts the probability of customer churn. The ReLU activation function is applied to both the input and hidden layers, while the sigmoid activation function is employed in the output layer.
MLP is more successful than classical regression models because it calculates a weighted sum for each hidden unit when a nonlinear (ReLU) or hyperbolic (tanh) function is applied. The result of this function is then used to calculate the output. Learning nonlinear behaviours in data and learning models in real-time can be counted as advantages of MLP. However, the disadvantage of MLP is that a model with hidden layers has a loss function with more than one local minimum. The model requires parameter adjustment, such as the number of hidden neurons, iterations, and layers.

EXPERIMENTAL RESULTS

This study compared kNN, LR, NB, RF, SVM, and the developed MLP-based artificial neural network model. The performance evaluation of the compared models was conducted using a confusion matrix. In this matrix, each column corresponds to the predicted classes. Each row represents the actual classes. TP, TN, FP, and FN values are obtained by using the confusion matrix. TP refers to samples predicted to be 1 and whose value is 0. TN denotes samples estimated to be 1 and whose value is 1. FP refers to samples predicted to be 1 and whose value is 0. FN represents the instances that are predicted as 0 but are 1. Accuracy, precision, recall, and F-score metrics were obtained by using TP, TN, FP, and FN values.
The accuracy calculates the proportion of correctly predicted values to the total test datasets. The accuracy metric is calculated using Eq.1.

$$Accuracy = \frac{TP + TN}{TP + FP + FN + TN} \quad (1)$$

The precision measures the proportion of positively predicted values that are truly positive. The precision metric is calculated using Eq. 2.

$$Precision = \frac{TP}{TP + FP} \quad (2)$$

The recall metric determines how many of the values that should be predicted positively are predicted positively. The recall metric is calculated using Eq. 3.

$$Recall = \frac{TP}{TP + FN} \quad (3)$$

The F-score metric is determined by taking the harmonic mean of the precision and recall metrics. The F-score metric is calculated using Eq. 4.

$$F - score = 2 * \frac{Precision * Recall}{Precision + Recall} \quad (4)$$

The change in the accuracy value of the developed MLP-based artificial neural network model in the training steps called epoch is shown in Figure 6.

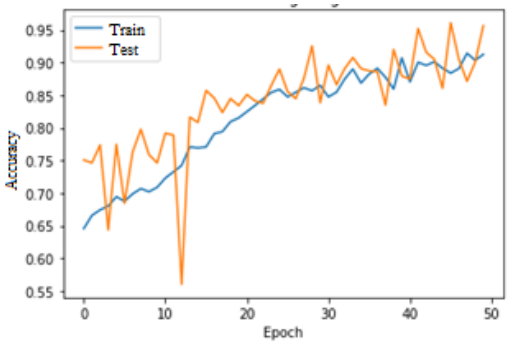

Figure 6. Variation of accuracy values according to epoch number

As seen in Figure 6, model accuracy varies according to the number of epochs. The model's performance is assessed by computing the average accuracy value during training.
Neural networks try to minimize the error. The function to be minimized or maximized is called the objective function or loss function, and the value calculated by the loss function is called loss. The Loss function determines how much the predictions the model produces differ from the true value. Figure 7 shows the variation in loss values as the number of epochs increases.

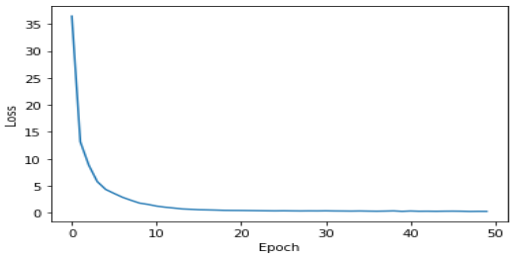

Figure 7. Change of loss values according to epoch number

kNN is a nonparametric method employed for both classification and regression tasks. Its fundamental principle involves identifying the neighboring data points, considering the test data point similar to these neighbors, and generating the output. In kNN, a specific number of k neighbors are searched, and predictions are made based on their characteristics.
The confusion matrix and experimental results for kNN are shown in Table 4 and Table 5.

Table 4. Confusion matrix for kNN

		Real values	
		No	Yes
Predicted values	No	204	144
	Yes	170	891

As seen in Table 4, the TP for kNN is 204, the FP is 144, the FN is 170, and the TN is 891.

Table 5. Accuracy, precision, recall and F-score values

Accuracy	Precision	Recall	F-score
%77	%58	%54	%56

As indicated in Table 5, the accuracy score for kNN is %77, the precision score is %58, the recall score is %54, and the F-score is %56.
LR is a statistical model that employs a logistic function to model a binary dependent variable. It is specifically designed for situations where the dependent variable is categorical. LR is particularly valuable in classification problems where the objective is to assess whether a new sample is most suitable for a particular category.
The confusion matrix and experimental results for LR are shown in Table 6 and Table 7.

Table 6. Confusion matrix for LR

		Real values	
		No	Yes
Predicted values	No	206	112
	Yes	168	923

As seen in Table 6, the TP value for LR is 206, the FP is 112, the FN is 168, and the TN is 923.

Table 7. Accuracy, precision, recall and F-score values

Accuracy	Precision	Recall	F-score
%80	%64	%55	%59

As indicated in Table 7, the accuracy score for LR is %80, the precision score is %64, the recall score is %55, and the F-score is %59.
The superior performance of LR compared to kNN can be attributed to their inherent characteristics as models. kNN is a non-parametric model that can handle non-linear solutions, whereas LR is a parametric model that primarily supports linear solutions. Additionally, LR can generate confidence levels for its predictions, whereas kNN provides only the output label without any associated confidence measure.
The NB algorithm is a classification technique based on Bayes Theorem. The NB classifier operates on the assumption that the presence of a specific attribute in a class is independent of the presence of any other attribute. NB is beneficial for massive datasets.
The confusion matrix and experimental results for NB are shown in Table 8 and Table 9.

Table 8. Confusion matrix for NB

		Real values	
		No	Yes
Predicted values	No	313	352
	Yes	61	683

As seen in Table 8, the TP for NB is 313, the FP is 352, the FN is 61, and the TN is 683.

Table 9. Accuracy, precision, recall and F-score values

Accuracy	Precision	Recall	F-score
%70	%47	%83	%60

As indicated in Table 9, the accuracy score for NB is %70, the precision score is %47, the recall score is %83, and the F-score is %60.
The fact that LR has better experimental results than NB can be interpreted as LR having better classification performance than NB in large datasets. NB works better on small datasets. LR outperforms NB on linearity as NB expects all features to be independent.
The critical distinction between kNN and NB lies in their classification approaches. kNN is a discriminative classifier, whereas NB is a generative classifier. NB assumes conditional independence among features and utilizes a maximum likelihood hypothesis. The superior classification performance of kNN compared to NB can be attributed to its non-parametric nature, whereas NB is considered parametric.
RF algorithm is an ensemble learning method that generates many decision trees during the training phase. In classification tasks, the RF outputs the class chosen by the majority of the trees. RF is a classifier that operates on multiple subsets of a dataset, employing a set of decision trees, and combines their results to enhance prediction accuracy. Unlike relying on a single decision tree, RF aggregates the predictions from each tree to produce the final output.
The confusion matrix and experimental results for RF are shown in Table 10 and Table 11.

Table 10. Confusion matrix for RF

		Real values	
		No	Yes
Predicted values	No	175	105
	Yes	199	930

According to Table 10, the RF classifier has a TP value of 175, an FP value of 105, an FN value of 199, and a TN value of 930.

Table 11. Accuracy, precision, recall and F-score values

Accuracy	Precision	Recall	F-score
%78	%62	%46	%53

As indicated in Table 11, the accuracy score for RF is %78, the precision score is %62, the recall score is %46, and the F-score is %53.

The reason why RF has more successful results than kNN is the values of the features in the dataset. RF assumes local similarities, and very similar samples are classified similarly. kNN can only select the most similar samples based on distance.

The superior performance of LR compared to RF can generally be interpreted as LR performing better when the number of noise variables is equal to or less than the number of explanatory variables. As the number of explanatory variables increases in a dataset, the ratio of TP to FP tends to increase for RF. The fact that RF has better experimental results than NB can be interpreted as RF being a distinctive model and NB being a productive model. Tree pruning in RF ensures that some features in the training data are neglected, thereby increasing the prediction accuracy.

SVM is a supervised learning model used for classification and regression analysis. It examines data and assigns new examples to specific categories based on training examples. SVM maps the training samples to space points to maximize the separation between the two categories. Subsequently, new samples are mapped to the same space and categorized based on which side of the separation they fall on.

The confusion matrix and experimental results for SVM are shown in Table 12 and Table 13.

Table 12. Confusion matrix for SVM

		Real values	
		No	Yes
Predicted values	No	184	101
	Yes	190	934

The SVM model achieved TP value of 184, FP value of 101, FN value of 190, and TN value of 934, as shown in Table 12.

Table 13. Accuracy, precision, recall and F-score values

Accuracy	Precision	Recall	F-score
%79	%64	%49	%55

As indicated in Table 13, the accuracy score for SVM is %79, the precision score is %64, the recall score is %49, and the F-score is %55.

Through kernel techniques, SVM offers support for both linear and non-linear solutions. In cases where training data is limited, SVM tends to handle outliers more effectively than LR. However, LR showed better classification performance than SVM due to the excess training data and the high number of features in the dataset.

In a classification problem, RF provides the probability of belonging to a particular class, whereas SVM provides the distance to the decision boundary. This characteristic often leads to SVM's superior performance compared to RF. SVM identifies support vectors in each class; the data points closest to the decision boundary separate the classes.

The fact that SVM has a better classification performance than kNN can be interpreted as the fact that SVM is more sensitive to outliers. When the number of training data samples dramatically exceeds the number of features, kNN can be more effective than SVM. However, in scenarios with numerous features and limited training data, SVM outperforms kNN.

MLP (Multi-Layer Perceptron) is an artificial neural network architecture consisting of an input layer, an output layer, and one hidden layer containing numerous interconnected neurons. MLP is a feed-forward model. The inputs are combined into a weighted sum with their initial weights and subjected to the activation function. Each layer feeds the next with the result of its calculations, an internal representation of the data. This process involves propagating information from the hidden layers to the output layer. Backpropagation is the learning mechanism that enables the MLP to iteratively adjust the network's weights in order to minimize the cost function. During each iteration, the gradient of the accuracy is calculated across all input and output pairs, following the transmission of weighted sums throughout the layers. Subsequently, the first hidden layer weights are updated using the gradient value for backpropagation. This propagation of weights continues until reaching the starting point of the neural network.

he confusion matrix and experimental results for MLP are shown in Table 14 and Table 15.

Table 14. Confusion matrix for MLP

		Real values	
		No	Yes
Predicted values	No	305	6
	Yes	69	1029

As seen in Table 14, the TP value for MLP is 309, the FP is 2, the FN is 74, and the TN is 1024.

Table 15. Accuracy, precision, recall and F-score values

Accuracy	Precision	Recall	F-score
%94	%99	%80	%89

As indicated in Table 15, the accuracy score for MLP is %79, the precision score is %65, the recall score is %49, and the F-score is %56.

MLP is a deep neural network architecture. MLP is a feedforward neural network architecture. It does not form loops like iterative neural networks. MLP uses backpropagation to train the network. In MLP, each new layer is a nonlinear function of the weighted sum of all outputs from the previous one. The fact that the experimental results of MLP are more successful than the other models can be interpreted as the fact that MLP is a feedforward network. Compared to SVM and RF, MLP requires many input data. The more data fed into the network, the better the network will generalize and make accurate predictions with fewer errors. On the other hand, SVM and RF require much less input data. For this reason, MLP was more successful than SVM and RF in the dataset used.

Table 16 and Figure 8 present the comparative experimental results for kNN, LR, NB, RF, SVM, and the MLP-based artificial neural network model based on accuracy, precision, recall, and F-score values.

Table 16. Comparative experimental results

Model	kNN	LR	NB	RF	SVM	MLP
Accuracy	%77	%80	%70	%78	%79	%94
Precision	%58	%64	%47	%62	%64	%99
Recall	%54	%55	%83	%47	%49	%80
F-score	%56	%59	%60	%53	%55	%89

Table 16 and Figure 8 illustrate the comparative results of different models, where the MLP-based artificial neural network model exhibits superior performance compared to other models. The MLP achieves 0.946 accuracy, 0.993 precision, 0.806 recall, and 0.890 F-score.

Following MLP, LR, SVM, RF, and kNN demonstrate relatively successful results. LR achieves 0.801 accuracy, 0.647 precision, 0.550 recall, and 0.595 F-score.

SVM achieves 0.793 accuracy, 0.645 precision, 0.491 recall, and 0.558 F-score. RF achieves 0.784 accuracy, 0.625 precision, 0.467 recall, and 0.535 F-score. kNN achieves 0.777 accuracy, 0.586 precision, 0.545 recall, and 0.565 F-score. NB achieves 0.706 accuracy, 0.470 precision, 0.836 recall, and 0.602 F-score.

a

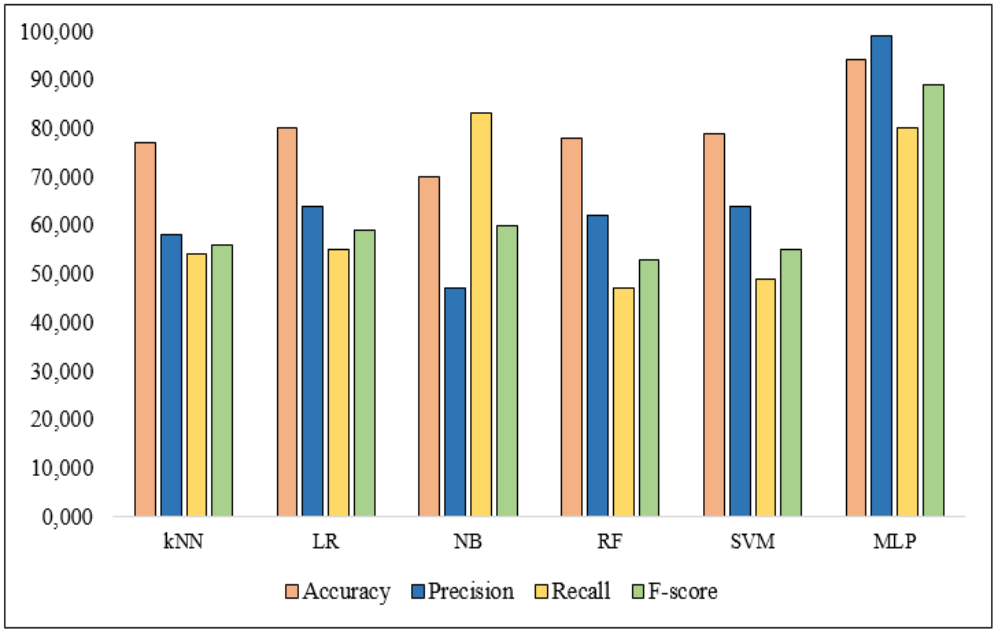

Figure 8. Comparative experimental results

As seen in Table 16 and Figure 8, MLP showed a better classification performance in customer churn prediction compared to other models. LR, SVM, RF and kNN are the models with the most successful results after MLP. NB has the worst classification performance among the compared models.

CONCLUSIONS

Customer churn prediction means determining which customers are likely to leave a particular service or cancel a service subscription. After identifying customers at risk of canceling their subscription, a marketing strategy can be determined to maximize the customer's chances of staying subscribed. The churn of customers is a significant problem for businesses in most industries. For businesses to grow, they need to invest in gaining new customers. Every lost customer means a significant lost investment. For this reason, predicting when customers will leave and offering incentives can significantly save businesses.

The present study applied exploratory data analysis and feature extraction techniques to anonymous customer data obtained from a telecommunications company. The study compared the prediction performance of various machine learning and artificial neural network classifiers. The results revealed that all the models achieved exceeding 70% accuracy. The MLP-based artificial neural network model exhibited the highest classification performance among the compared models. The developed model achieved %94 accuracy, %99 precision, %80 recall, and %89 F-score. LR achieved %80 accuracy, %64 precision, %55 recall, and %59 F-score. SVM achieved %79 accuracy, %64 precision, %49 recall, and %55 F-score. RF achieved %78 accuracy, %62 precision, %46 recall, and %53 F-score. kNN achieved %77 accuracy, %58 precision, %54 recall, and %56 F-score. NB achieved %70 accuracy, %47 precision, %83 recall, and %60 F-score.

The experimental findings demonstrated that the developed model performed better than other models across all evaluation metrics. Moreover, all classifiers demonstrated significant performance enhancements when the oversampling technique was employed. The results show that the developed model can be preferred for customer churn prediction.

Using customer data to predict customer churn can have significant ethical implications. In this study, great importance was given to data privacy and respect for customer rights. Various verification and auditing processes have been applied to ensure that the predictions of the model developed using anonymized customer data are free of bias and fair. By adhering to ethical principles, we aimed to prevent the misuse of customers' data and improve service quality.

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Sıvı yosun gübresinin farklı konsantrasyonlarının tuz stresi koşullarında arpa (*Hordeum vulgare* L.) gelişimi ve rizosferdeki bazı biyolojik özelliklere etkisi

Arzu GÜLER[1], Çiğdem KÜÇÜK[2]✉,
[1] Biyoloji Bölümü, Fen Edebiyat Fakültesi, Harran Üniversitesi, Şanlıurfa, Türkiye
[2] Biyoloji Bölümü, Fen Edebiyat Fakültesi, Harran Üniversitesi, Şanlıurfa, Türkiye

✉: ckucuk@harran.edu.tr [1] https://orcid.org/0009-0006-3327-9054. [2] https://orcid.org/0000-0001-5688-5440

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ÖZ

Bu çalışmada, arpa gelişimi ve rizosferdeki bazı mikrobiyolojik özellikler üzerine sıvı deniz yosunu gübresinin tuz stres koşullarındaki etkisinin belirlenmesi amaçlanmıştır. Denemede ticari olarak satılan yosun gübresi kullanılmıştır. Sıvı deniz yosun gübresi ekimle birlikte topraklara farklı konsantrasyonlarda (% 0, % 0.4, % 0.8, % 1 ve % 2 yosun gübresi) uygulanmıştır. Tuz ise farklı konsantrasyonda (0 mM, 75 mM ve 150 mM) uygulanmıştır. Sera koşullarında yetiştirilen bitkiler ekimden 12 hafta sonra hasat edilmiştir. Arpanın bitki boyu, yeşil aksam ve kök yaş ve kuru ağırlıkları, kök uzunluğu, yaprakların klorofil içerikleri kök bölgesi β-glukosidaz ve alkalin fosfataz enzim aktiviteleri incelenmiştir. Elde edilen veriler ile uygulamalar arasındaki farklılık istatistik analiz ile ortaya konulmuştur.

Anahtar Kelimeler: Alkalin fosfataz, Arpa, β-glukosidaz, Konsantrasyon, Sıvı yosun gübresi

Effect of different concentrations of liquid seaweed fertilizer on barley (*Hordeum vulgare* L.) growth and some biological properties in the rhizosphere under salt stress conditions

ABSTRACT

This study aimed to determine the effect of liquid seaweed fertilizer on barley growth and some microbiological properties in the rhizosphere under salt stress conditions. Commercially available seaweed fertilizer was used in the experiment. Liquid seaweed fertilizer was applied to the soil in different concentration (0%, 0.4%, 0.8%, 1% and 2% seaweed fertilizer) after planting. Salt was applied in different concentration (0 mM, 75 mM and 150 mM). Plants grown under greenhouse conditions were harvested 12 weeks after planting. Plant height of barley, fresh and dry weights of green parts and roots, root length, chlorophyll content of leaves and root zone β-glucosidase and alkaline phosphatase enzyme activities were examined. The difference between the obtained data and the applications was revealed by statistical analysis.

Keywords: Alkaline phosphatase, Barley, β-glucosidase, Concentration, Liquid seaweed fertilizer

GİRİŞ

Nüfusun hızla artışından dolayı daha fazla ürün elde etmek için bilinçsizce yapılan tarım uygulamaları sonucunda topraklar tuzlanmaktadır [1]. Bu nedenle küresel gıda kaynaklarının sürdürülebilir yöntemler ile izlenmesi zorunlu kılınmıştır [1]. Artan gıda talebini karşılamak için çeşitli çevresel koşulların neden olduğu ürün kayıplarının azaltılmasının önemli olduğu bildirilmiştir [2]. Kimyasal gübre ve pestisitlerin kullanımı dünya genelinde çeşitli çevre sorunlarına neden olmaktadır. Aşırı kullanılan fosfatlı gübrelerin sulama suyundaki tuzluluğun ve sucul ortamdaki besin maddelerinin artmasına neden olduğu saptanmıştır. Doğal tuzluluk, tuzların toprakta ve yeraltı sularında belirli bir süre boyunca birikmesinin sonucu olduğu açıklanmıştır [3]. İnsan nüfusunun 2050 yılı sonunda yaklaşık 9.7 milyara ulaşması ve 2100 yılında ise yaklaşık 11 milyara ulaşması beklenmektedir [4]. Nüfusun hızla artması sonucu gıda güvenliğini sağlamak için daha fazla besine gereksinim duyulacaktır. Bu nedenle bitkisel üretime ayrılan tarım arazilerinin önemli ölçüde artması da kaçınılmaz görünmektedir [4].

Organik tarım; geleneksel gübreler, kimyasal ilaç kullanmadan çevresel sürdürülebilirliği, habitatları, biyojeokimyasal döngüleri ve toprak biyolojik aktiviteyi iyileştiren kalkınma yöntemi olarak tanımlanmıştır [5]. Organik uygulamaların toprağın fiziksel özelliklerini iyileştirdiği, toprağın organik maddesini yenilediği, koruduğu ve toprağın makro ve mikrobiyotasını iyileştirdiği bildirilmiştir [6]. Organik maddenin tuzdan etkilenen toprağa önemli miktarda karbon ilave ettiği, su penetrasyonunu ve mikrobiyal aktiviteyi arttırdığı yapılan çalışmada açıklanmıştır [7]. Son yıllarda, biyotik ve abiyotik stresin şiddetli etkilerini azaltmak, bitki büyümesini ve sağlığını iyileştirmek amacıyla çeşitli biyolojik gübreler geliştirilmiş ve ticarileştirilmiştir. Aynı zamanda toprak yapısını ve kalitesini de iyileştiren bu ürünler, toprağın gübrelenmesi ve bitki koruması için yenilikçi çözümler sunmaktadır [4]. Bu biyolojik

moleküller çoğunlukla algler ve onların türev ürünleri olarak açıklanmıştır [4]. Deniz yosunlarının çeşitli biyolojik aktivitelere sahip oksin, sitokin, giberelin vb. yüksek polisakkarit, gliserol ve bitki düzenleyici içermeleri nedeniyle bitki gelişimi için değerli organik materyaller olarak kullanıldığı bildirilmiştir [8].
Ascophyllum nodosum'un domates ve tatlı bibere uygulanması ile yapraklardaki klorofil içeriğinin arttığını, bu artışın ise; ekstrakta bulunan betainlerin etkisinden kaynaklandığı açıklanmıştır [9]. Deniz yosunlarının içeriğindeki betain bileşiklerinin klorofil bozulmasının engellemesi ile fotosentetik kaybı azalttığı yapılan başka bir çalışmada da belirtilmiştir [9]. Deniz yosunu ekstraktları domates, biber, fasulye gibi çeşitli ürünlerin erken çimlenmesini tetiklemiş, meyve tutumunu arttırmıştır [10]. Çiçek sayısı ve meyve tutumundaki bu artışlar, verimi de arttırmıştır. Di Stasio ve ark. [11] tarafından yapılan bir çalışmada domates fidelerine deniz yosunu ekstraktlarının uygulanması ile bitkide çiçeklenme artmış, meyve sayısı ve büyüklüğünde de önemli artışların olduğu açıklanmıştır. Deniz yosunu uygulanması ile verim artışının, ekstraktlarda bulunan sitokininler, konakçı bitkinin hormon sentezinin indüksiyonu gibi çeşitli fitohormonların etkilerinin sonucu olduğu düşünülmüştür [12]. Deniz yosunu ekstraktlarının ve bileşenlerinin oksin, sitokinin ve gibberellin gibi büyüme hormonlarının endojen biyosentezinden sorumlu genlerin ekspresyonunu modüle edebildiği rapor edilmiştir [9]. Ali ve ark. [9] ayrıca hasat edilebilir ürün verimini arttırmanın yanı sıra ekstraktların domates, biber, marul, ıspanak, hıyar, çileğin besin kalitesini de arttırdığını açıklamışlardır. *Macrocystis pyrifera* ekstraktları hıyar fidelerine uygulanmış, meyvelerde toplam fenol, antioksidan kapasite, C vitamini içeriği önemli ölçüde artmıştır [9]. Yosun gübresinin bitki gelişimi üzerindeki olumlu etkileri bilinmekle birlikte, toprak özellikleri, yetiştirilme koşulları, bitki türü, uygulanan gübre çeşiti ve konsantrasyonları toprak enzimleri üzerinde farklı etki göstermektedir. Bu nedenle, çalışmamızda topraktan uygulanan ticari sıvı yosun gübresinin farklı konsantrasyonları ile tuz uygulamalarının arpanın kök bölgesinin bazı mikrobiyolojik özellikleri üzerine etkileri ile arpa gelişimine olan etkisinin araştırması amaçlanmıştır.

MATERYAL ve YÖNTEM

Çalışmamızda arpa (*Hordeum vulgare* L.) Akhisar 98 çeşidi kullanılmıştır. Tohumlar GAP Araştırma Enstitüsü'nden temin edilmiştir. Denemede kullanılan sıvı yosun gübresi (Sea Plus) ticari gübre satılan yerden alınmıştır. Kullanılan sıvı yosun gübresinin organik madde içeriği %12, suda çözünür potasyum oksit %3, Aljinit asit miktarı %0.3, EC; 13.56 dS/m, pH içeriği 6'dır. Saksı denemesinde kullanılan toprak, kampüs alanından daha önce herhangi bir uygulamanın yapılmadığı yerden alınmıştır. Toprak örneklerinin pH'ı 8.13, EC 0.96 dS/m, organik madde içeriği %1.69, azot içeriği %0.03, kireç içeriği %22.6, fosfor 4.86 kg/da, potasyum 118.6 kg/da olup killi bünyeye sahiptir.

Saksı denemesinin kurulumu

Toprak örnekleri 2 mm'lik elekten elenmiş, 3 kg'lık saksılara doldurulmuştur. Her bir saksıya 10 arpa tohumu ekilmiş, çimlenme sonunda bitkiler 3'e seyreltilmiştir. Ekim ile birlikte, yosun gübresi saksılara % 0 (kontrol), % 0.4, % 0.8, % 1 ve % 2 olacak şekilde ayrı ayrı uygulanmış, her bir saksıya 50 ml olarak verilmiştir. Çimlenme sonrası tuz (NaCI), 0 mM (kontrol), 75 mM ve 150 mM olmak üzere ayrı ayrı saksılara, sulama suyu ile verilmiştir. Tesadüf parselleri deneme desenine göre kurulan deneme 3 paralelli olarak yürütülmüştür. Saksılara uygulanan tuz; çimlenme sonrası 15 gün sonra ara ile sulama gerektiğinde sulama suyu ile uygulanmıştır. Bitkiler ekimden 12 hafta sonra hasat edilmiştir.

Bitki boyu, kök uzunluğu

Hasat edilen arpa boyları cetvel ile ölçülmüştür. Bitkinin toprakla temas ettiği kök boğazından bitki yaprağının uç kısmına kadar bitki boyu ölçülmüştür. Hasat edilen bitkiler kök boğazından kesilmiş, musluk suyu ile kökler yıkanarak topraklardan arındırılmıştır. Cetvel yardımı ile her bir uygulamadaki bitkilerin kök uzunluğu ölçülmüştür [4].

Bitkilerin yeşil aksam ve kök kuru ağırlıkları

Hasat sonunda her bir uygulamaya ait saksılardaki yeşil aksam ve kök kısımları kesilmiş, ayrı ayrı terazide tartılarak yaş ağırlıkları belirlenmiştir. Yaş ağırlıkları belirlenen örnekler ayrı ayrı kese kağıtlarına konulmuş, sabit ağırlığa gelinceye kadar 65°C'lik etüvde kurutulmuş, tartılmış kuru ağırlıkları alınmıştır [4].

Yaprak örneklerinde klorofil tayini

Her bir uygulamadan alınan yaprak örneklerinde klorofil tayini yapılmış, sonuçlar g/l olarak aşağıda verilen formüle göre hesaplanmıştır [4,13].

Klorofil a (g/l): 0.0127 x A663-0.00269 x 645
Klorofil b (g/l): 0.0229 x A645-0.00468 x A663

Kök bölgesi topraklarında bazı mikrobiyolojik analizler

Hasat sonunda her bir uygulamadaki köklere yapışan topraklar alınmış, bu topraklardaki β-glukosidaz ve alkalin fosfataz enzim aktiviteleri incelenmiştir.

β-glukosidaz enzim aktivitesi

Alınan toprak örneğine; toluen, tris-aminometan, p-nitrofenil, β-D-glukopironosid eklenerek 37°C' de 1 saat

inkübe edilmiştir. İçeriğe $CaCl_2$ ve THAM çözeltisi eklendikten sonra 410 nm dalga boyunda spektrofotometrede ölçülmüştür [14].

Alkalin fosfataz enzim aktivitesi

Örnekler; toluen, MUB tamponu, p-nitrofenilfosfat ile karıştırılarak 37ºC'de 1 saat inkübe edilmiştir. İnkübasyon sonunda açığa çıkan p-nitrofenol 410 nm dalga boyunda spektorofotometrede ölçülmüştür [15].

İstatistik analiz

Her bir analiz 3 tekerrürlü olarak yapılmıştır. Deneme sonunda uygulamalardan elde edilen sonuçlar, JMP istatistik programı kullanılarak incelenmiştir.

TARTIŞMA

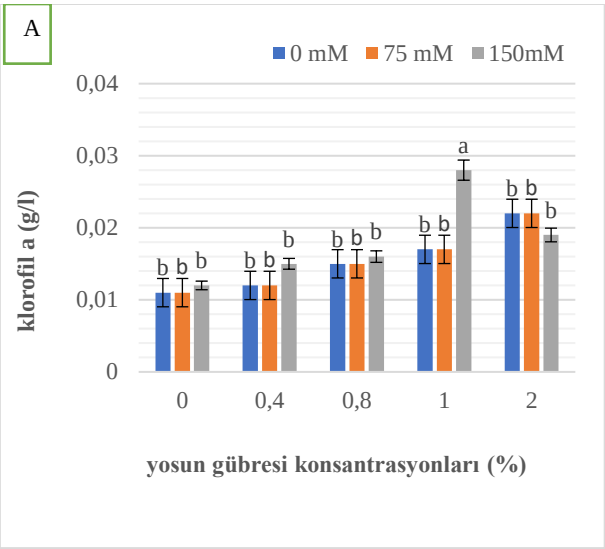
Çalışmamızda, farklı konsantrasyonlarda yosun gübresi ve tuz konsantrasyonları topraklara uygulanmış ve rizosferdeki bazı enzim aktiviteleri ile arpa gelişimi üzerine olan etkileri sera koşullarında değerlendirilmiştir. Yosun gübresinin farklı konsantrasyonları arpa yaş ağırlığı üzerinde farklı etki göstermiştir. Tuz stresi koşullarında uygulanan % 0.4'lük yosun gübresi uygulaması, uygulanan diğer yosun gübresi konsantrasyonları ile karşılaştırıldığında bitki yeşil aksam yaş ağırlığını arttırmıştır. En yüksek ağırlık %0.4 konsantrasyondaki yosun gübresi ve 75 mM NaCl uygulamasından (0.73 g) alınırken, en düşük ağırlık %2'lik uygulanan yosun gübresi ve 150 mM NaCl'nin birlikte uygulanması sonucu elde edilmiştir (Tablo 1). Yaş ağırlık 0.30 – 0.73 g/saksı arasında değişmiştir. Yosun gübresi uygulamasının, bitkilerin gelişimine olan olumlu katkılarının temel besin maddelerinin alımına, toprak yapısının iyileştirilmesi ve su tutma kapasitesinin artmasına bağlanabilir. Deniz yosunu özütleri hem çevre dostu hem de toksik olmadıkları için geleneksel gübrelere göre düşük maliyetli alternatifler olarak ilgi görmektedir [16]. Yosunlardan elde edilen biyogübrelerin bitkilerin kök morfolojisini geliştirdiği yapılan çalışmada belirlenmiştir [16]. Araştırıcılar, yosun gübresi ile yapılan uygulamaların bitkilerin daha derin toprak katmanlarından besin maddelerini yeterince alınabilmesini sağlayarak kök çoğalmasını, çimlenmesini ve büyümesini teşvik ettiğini öne sürmüşlerdir. Ayrıca yosun gübresinin metabolizmayı hızlandırırken, enerji depolamayı iyileştiren yapısal olmayan karbonhidratların oluşumunu indüklediği, yaprakların su tutması, membran geçirgenliği ve osmolitlerin/iyonların taşınmasını arttırarak yaprak büyümesini geliştirdiği ve abiyotik strese karşı toleransını arttırdığı açıklanmıştır [17]. Yosun gübresi uygulamalarının en önemli etkilerinden biri, güçlü kök sisteminin oluşturulmasıdır [18]. Çalışmamızda elde ettiğimiz sonuçlar, yosun gübresinin topraklara uygulanması ile arpanın kök uzunluğu ve kök ağırlığının kontrole göre oldukça iyi olduğunu göstermiştir. Çalışmamızda kök uzunluğu üzerinde uygulamalar etkili olmuş, en fazla kök uzunluğu tuz uygulamaları (75 mM ve 150 mM) ile birlikte % 0.8 yosun gübresinin birlikte uygulanması ile elde edilmiştir. Benzer olarak, yapılan bir araştırmada marulun yedi günlük gelişiminde yosun gübresi uygulamalarının etkili olduğu, kök gelişimini arttırdığı rapor edilmiştir [18]. Yosun gübresi ve NaCl uygulamaları ayrı ayrı uygulandığında bitki boyu üzerinde etkileri incelenmiştir. 75 mM tuz uygulaması ve yosun gübresinin % 0.4'lük konsantrasyonu ile en yüksek bitki boyu alınırken, bunu %1' lik yosun gübresi konsantrasyonu izlemiştir. Farklı NaCI uygulamalarının bitki boyu üzerine etkileri istatistiki olarak önemsiz bulunmuştur. Yosun özütlerinin bitkiler üzerindeki olumlu etkilerinin betainler, poliaminler, oligosakkaritler, aminoasitler, vitaminler gibi çeşitli bitki büyümesini düzenleyici maddeleri içermesinden kaynaklandığı açıklanmıştır [19].

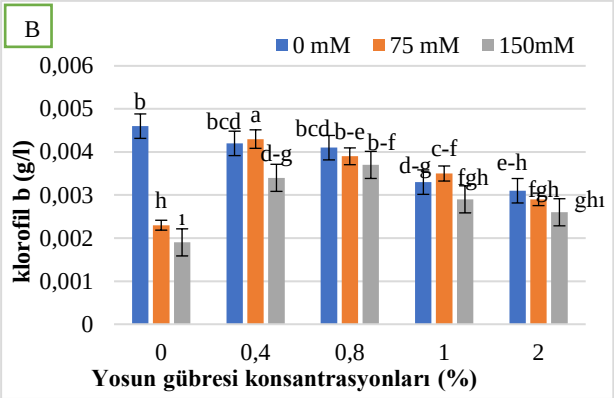
Tablo 1. Yosun gübresi ve tuz uygulamalarının arpanın bazı bitki özelliklerine etkileri

Yosun gübresi uygulaması (%)	NaCl uygulaması (mM)		
	Kontrol	75	150
Bitki yeşil aksam kuru ağırlık (g/bitki)			
0	0.47±0.02bcd	0.37±0.02cd	0.37±0.03 cd
0.4	0.33±0.02 d	0.73±0.05 a	0.53±0.04 bc
0.8	0.37±0.03 cd	0.47±0.01 bcd	0.43±0.01 bcd
1	0.60±0.07 ab	0.47±0.01 bcd	0.43±0.01 bcd
2	0.33±0.02 d	0.43±0.01 bcd	0.30±0.06 d
Arpa boyuna etkileri (cm)			
0	38.87±0.02a-d	38.47±0.02a-d	39.50±0.03 abc
0.4	34.10±0.03d-h	42.50±0.04 a	37.87±0.02a-f
0.8	29.60±0.04h	33.17±0.05 e-h	31.23±0.02 fgh
1	40.93±0.07ab	35.37±0.01c-g	36.23± 0.02b-f
2	31.03±0.03fg h	30.70±0.01 gh	29.73±0.02 h
Kök kuru ağırlık (g/bitki)			
0	0.007±0.04 gh	0.007±0.02gh	0.008±0.04 e-h
0.4	0.006±0.04 h	0.008±0.02 fgh	0.011±0.06 d-g
0.8	0.013±0.03 bcd	0.019± a	0.013±0.06 bcd
1	0.015±0.05 bcd	0.014±0.05 bcd	0.017±0.05 ab
2	0.016±0.05 abc	0.012±0.03 c-f	0.012±0.03cd e
Kök uzunluğu (cm)			
0	10.3±0.07abc	10.5±0.05bc	11.7 ±0.05 b
0.4	10.7±0.05abc	8.7±0.02c	11.7±0.04 ab
0.8	12±0.03ab	13.2±0.04a	13.2±0.04a
1	11.03±0.01ab c	10.90±0.05ab c	10.03±0.06bc
2	9.60±0.01 bc	11.77±0.04ab	8.83±0.0 c

Bu bileşiklerin bitki sürgün ve kök dokusunun büyümesini olumlu yönde etkilediği belirtilmiştir [19]. Deniz yosunu gübresindeki zengin mineraller, organik

asitler, aktif madde, besinlerin bitkiler tarafından emilimini, taşınmasını ve kullanımını teşvik edebilmiştir [20]. Stres altındaki bitkilere uygulanan yosun ekstraktlarının tuzlu koşullarda yetiştirilen bitkilerin biyokütlesinden daha iyi etki gösterdiği açıklanmıştır [21]. Bizim çalışmamızda da yosun gübresinin uygulanması ile tuz stres koşullarında kontrole göre kök uzunluğu, kök yaş ağırlığı, yeşil aksam ağırlığının artması araştırıcıların bulguları ile benzerlik göstermektedir. Deniz yosunu ekstraktları uygulamaları tarımsal üretimde bitkilerin gelişimi ve verimi için oldukça yararlı bulunmuştur [22,23]. Chen ve ark. [24] tarafından yapılan bir araştırmada ise, *Ascophyllum nodosum*'dan ekstre edilen yosun gübresinin mısır gelişimi, rizosfer toprak üzerine etkileri incelenmiştir. Araştırıcılar, gübreleme sonunda mısır fidelerinin biyomasının arttığını belirlemişlerdir. Kontrol grubu ile karşılaştırıldığında *Ascophyllum nodosum* ekstrelerinin uygulandığı fidelerin boy uzunluğu, yeşil aksam ağırlıklarının önemli olarak arttığı rapor edilmiştir [24]. Bu sonuçlar bizim çalışmamızdaki sonuçları desteklemektedir. Uygulamaların kök uzunlukları üzerine etkileri incelenmiş, farklı konsantrasyonlarda uygulanan yosun gübresinin % 0.8'lik konsantrasyonu kontrole göre kök uzunluğunu arttırmıştır. Yosun gübresinin % 0.8'lik konsantrasyonu ile birlikte 75 mM NaCl uygulaması ve yosun gübresinin %0.8'lik konsantrasyonu ile 150 mM NaCl uygulaması diğer uygulamalara göre önemli bulunmuştur. En yüksek kök kuru ağırlığı % 0.8 yosun gübresi ile 75 mM NaCl'nin birlikte uygulanması ile elde edilmiştir. Çalışmamızda da yosun gübresi uygulamalarının arpa yeşil aksam yaş ağırlığı, kök ağırlığı, bitki boyu ve kök uzunluğu üzerine etkilerinin, rizosfer toprağında yosun gübrelerinin topraktaki besini arttırmasından kaynaklanabilir. Tuzluluk stresi fotosentezde bozulmaya neden olduğundan bitki gelişimi ve verimini azalmaktadır. Yapılan bir çalışmada *Sargassum angustifolium* ekstraktının tuz stresinin etkisini hafifletme yeteneğini doğrulamak amacıyla *Calotropis procera* yapraklarının klorofil içeriği ölçülmüş, sonuçlar tuz stresinin yapraklardaki klorofil içeriğini önemli ölçüde azalttığını göstermiştir [21]. Araştırıcılar; tuz stresi altında (15 dS/m NaCI ve daha düşük konsantrasyonlarda) *Sargassum angustifolium*'un %0.5'lik konsantrasyonunun kontrole göre klorofil içeriğini arttırdığını tespit etmişlerdir [21]. Yaprak klorofil değerinin, tuzluluk stresine yanıt olarak tuz toleransının bir göstergesi olduğu bildirilmiştir [25]. Uygulanan yosun gübresinin %1'lik konsantrasyonu ile en yüksek klorofil a içeriği alınırken, % 0.4'lük konsantrasyon ile en yüksek klorofil b içeriği elde edilmiştir. Uygulanan yosun gübresinin farklı konsantrasyonları klorofil a ve b içeriğine farklı etki göstermiştir. Klorofil a içeriğine %1'lik yosun gübresi dozu ile 150 mM NaCl uygulaması etkili bulunmuştur. Klorofil b içeriği üzerine en yüksek değer 75 mM NaCl ile % 0.4 yosunun birlikte uygulanması ile elde edilmiştir (Şekil 1). Sonuçlarımız araştırıcıların bulguları ile benzerlik göstermektedir. Buna göre çeşitli raporlar yosun gübresi uygulamasının tuz stresi ve kuraklık stresi koşulları altında klorofil içeriğini arttırdığını göstermiştir [20, 26]. Tuzluluğun neden olduğu artan oksidatif stresin; klorofil yapısını etkilediği ve klorofil içeriğini azalttığı bildirilmiştir [27,28]. Çalışmamızda da klorofil a ve b arasındaki farklılığın nedeni klorofil içeriğindeki azalmanın tuzun membran stabilitesi üzerindeki olumsuz etkisinden kaynaklandığı, tuz uygulaması ile klorofilin kesintiye uğraması sonucu pigmentasyondaki azalmaya neden olması, Mg^{+2} birikiminin azalmasından kaynaklanabileceği, tuz uygulamaları ile klorofilazın yavaş sentez veya hızlı parçalanma ile klorofil a ve b sentezini etkilemiş olabileceği düşünülmektedir. Yosun ekstraktları uygulanan bitkilerin, sağlıklı ve verimli gelişmesinin diğer nedeni ise; ekstraktlar tarafından etkilenen topraktaki yararlı mikrobiyal popülasyonu da uyararak, kök bölgesi aktiviteyi uyarması olabilir. Topraklarda, rizosferik enzimatik aktiviteyi antibiyotikler, pestisidler, ağır metaller, tarla yönetimi, organik atıklar gibi önemli faktörler etkileyebilmektedir [26,30]. Toprak enzim aktiviteleri toprakta mikrobiyal gelişmenin indikatörleri olarak sık sık kullanılmaktadır. Toprak enzimleri besin döngüsü ile ilişkilidir ve faaliyetleri toprağın verimliliğinin korunması için gereklidir [7]. Yosun gübresi ve tuzun farklı konsantrasyonları toprağın alkalin fosfataz aktivitesi üzerinde farklı etki göstermiştir (Şekil 2). Yosun gübresinin artan konsantrasyonları alkalin fosfataz aktiviteyi arttırmıştır. En yüksek aktivite % 2 yosun gübresi uygulamasından alınmıştır. Farklı NaCl konsantrasyonları ve yosun gübresi uygulamalarının birlikte uygulanmaları; topraktaki aktivite üzerinde de etkili bulunmuştur. 150 mM NaCl uygulamasında alkalin fosfataz aktivite en düşük olarak bulunmuştur (1.09 µg PNP/g toprak). Yosun gübresinin farklı konsantrasyonları rizosfer toprağında β-glukosidaz aktive üzerinde etkili bulunmuştur. En düşük β-glukosidaz aktivite yosun gübresinin uygulanmadığı topraklardan alınırken, en yüksek aktivite yosun gübresinin en yüksek konsantrasyonunda elde edilmiştir. Tuz uygulamalarına artan yosun gübresinin ilavesi ile aktivitenin kontrole göre arttığı gözlemlenmiştir.





Şekil 1. Yosun gübresi (%) ve tuz uygulamalarının klorofil a ve b (g/l) içeriğine etkileri

Çalışmada yosun gübresi ile muamelelerin arpa fidelerinin yaş ağırlığı, kök ağırlığı, bitki boyunu arttırmaları Jafarlou ve ark. [21] tarafından bildirildiği gibi topraktaki yosun gübresi ilavesi ile kazanılan besin maddelerinin bitkilerce besin maddelerinin daha fazla absorbe edilmesinden ve bitki tarafından kullanılabilmesinden kaynaklanabilir. Toprak enzim aktiviteleri inorganik besin maddelerinin formlarını etkileyebilmektedir [31] ve topraktaki besin maddelerinin; β-glukosidaz, fosfataz aktiviteyi önemli ölçüde arttırdığı açıklanmıştır [32]. Çalışmamızda ise yosun gübresinin %0.8, %1 ve %2 konsantrasyonlarının tuz stres koşullarında kontrole göre hem alkalin fosfataz hem de β-glukosidaz aktiviteyi arttırdığı belirlenmiştir (Tablo 2). Bu sonuçlar, tuz uygulamalarının enzim aktivite değerini azalttığını, yosun gübresinin yüksek konsantrasyonlarının topraklardaki toprak enzim aktivitelerini uyardığını göstermektedir. Bu sonuçlara dayanarak sıvı yosun gübresinin toprağa uygulanmasının toprak mineralizasyonunu arttırdığını, mevcut besin maddelerinin konsantrasyonunu arttırdığını düşünüyoruz. Ayrıca enzim aktivitelerindeki değişimler, muhtemelen mikroorganizmaların ve kök eksudantlarının tür ve miktarlarındaki değişimleri de yansıtmış olabilir. Organik gübre uygulamaları ile toprak enzim aktivitelerinin ilişkileri birçok araştırıcı tarafından da değerlendirilmiştir. Toprak enzim aktiviteleri üzerine ayrıca yüksek pH, düşük toprak nemi, sıcaklığı, toprakların azot düzeyleri de etkili olabilmektedir [33].

SONUÇ

Çalışmada topraktan uygulanan ticari sıvı yosun gübresinin beş farklı konsantrasyon ve sulama suyu ile verilen farklı tuz uygulamasının sera koşullarında arpa rizosfer toprağının β-glukosidaz ve alkalin fosfataz enzim aktivitesi üzerine etkisi bölgemiz toprakları için ilk kez araştırılmıştır. Bu çalışma ile uygulanan yosun gübresi ve tuz uygulamalarının, arpa gelişim parametreleri üzerine olan etkisi değerlendirilmiş, uygulanan yosun gübresinin arpa yeşil aksam ağırlığı, kök ağırlığı, bitki boyu ve kök uzunluğuna etki ettiği, klorofil içeriğini arttırdığı belirlenmiştir. Hem bitki hem de rizosferdeki mikrobiyolojik özellikler üzerine gübre ve tuz uygulamalarının etkilerinin test edilen toprak özellikleri, bitki türü, uygulama dozlarına göre değişiklik gösterdiği dikkate alındığında [4,7] sera koşullarında da rizosfer toprağının β-glukosidaz ve alkalin fosfataz enzim aktivitesi üzerinde etkili olduğu çalışmamızda saptanmıştır. Ayrıca uygulanan yosun gübresi, tuz uygulamalarının oluşturduğu strese karşı bitki gelişimini teşvik etmiştir. Sıvı yosun gübresinin artan konsantrasyonlarının rizosfer toprağın β-glukosidaz ve alkalin fosfataz enzim aktivitelerini arttırdığı incelenmiştir. Yosun gübresinin toprak mikrobiyal topluluğu üzerine etkilerini araştırmak, yosun gübresinin topraklara ilavesinden sonra topraktaki mikroorganizma popülasyonunun nasıl değiştirdiğini belirlemek ileride yapılacak çalışmalarla belirlenecektir. Çalışmamızda doğal ışık alan sera koşullarında sıvı yosun gübresinin arpa gelişimi üzerinde olumlu etkisinin; ileride yapılacak tarla denemeleri ile bitkinin besin ihtiyacının karşılanması ve veriminin arttırılmasına olan katkıları belirlendikten sonra hem çevre kirliliğinin önlenmesi hem de çiftçinin ekonomik olarak tasarrufunu sağlamak amacıyla önerilebilecektir.

Tablo 2. Farklı konsantrasyonlarda yosun gübresi ve NaCl uygulamalarının rizosfer toprağının alkalin fosfataz aktivite (µg PNP/g toprak) ve β-glukosidaz (mg-p nitrofenol/g toprak) aktivite üzerine etkisi

Rizosfer toprağın bazı enzim aktiviteleri	Yosun gübresi (%)	NaCl konsantrasyonu (mM)		
		Kontrol	75	150
alkalin fosfataz aktivite (µg PNP/g toprak)	0	2.85±0.011j	2.43±0.010l	1.09±0.010n
	0.4	2.76±0.016k	2.43±0.022 l	2.14±0.017 m
	0.8	5.96±0.027g	5.02±0.034 h	4.87±0.02 ı
	1	11.43±0.038d	11.28±0.032e	9.86±0.027f
	2	15.41±0.033a	13.65±0.020b	12.28±0.011 c
β-glukosidaz (mg-p nitrofenol/g toprak)	0	10.02±0.02 k	9.09±0.05 m	8.15±0.07 n
	0.4	9.49±0.06 l	10.16±0.09k	11.24±0.012j
	0.8	13.95±0.011e	13.25±0.018g	12.83±0.018h
	1	15.56±0.021 c	14.42±0.020d	12.28±0.017ı
	2	18.90±0.023a	17.77±0.024b	13.61±0.017f

TEŞEKKÜR

Bu çalışma Harran Üniversitesi, Bilimsel Araştırma Projeleri birimi (HÜBAP) tarafından 22220 numaralı proje ile maddi olarak desteklenmiştir.

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MS besin ortamında Buttumun (*Pistacia khinjuk* Stocks) azot kullanım verimliliği

Yusuf ERSAL[1]
[1] Gıda İşleme Teknik Bilimler Meslek Yüksek Okulu, Batman Üniversitesi, Batman, Türkiye

✉: yusufersalian@gmail.com [1] 0000-0003-4848-5943

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ÖZ

Pistacia khinjuk (Buttum) türü Antep fıstığının doğal bir anaç türüdür. *P. khinjuk*'un tohum ve gövde çelikleriyle çoğaltılmasının zorluğu, sürgünlerin mikroçoğaltılmasını iyi bir seçenek haline getirmektedir. Bu çalışmada *P. khinjuk*'un mikroçoğaltımını daha verimli ve ekonomik hale getirmek amacıyla amonyum nitrat, oksin ve sitokinin çeşitleri ve miktarları optimize edilmiştir. Bu amaçla, *P. khinjuk* türünün sürgün ucu kültürü ile mikroçoğaltımında Murashige ve Skoog (MS) temel besin ortamına NH_4NO_3'ın 1650, 825, 412.5 ve 206.25 mg/L düzeylerinde katılmasının sürgün ve kök oluşumu üzerine etkileri araştırılmıştır. Sürgün çoğaltım aşamasında sitokininlerden, benzil amino pürin (BAP), 6-furfurilaminopurin (kinetin) ve 2-izopentil adenin (2-IP), sürgünlerin köklendirilmesi aşamasında oksinlerden, naftalen asetik asit (NAA), indol butirik asit (IBA) ve indol asetik asit (IAA) kullanılmıştır. Sonuçlarımıza göre, sürgün çoğaltımı aşamasında en yüksek eksplant başına düşen sürgün sayısı (1.79), 0.5 mg/L BAP, ortalama sürgün uzunluğu (17.43 mm), 1 mg/L 2-IP ve total çözülebilir protein ise (3.35 mg/g) 2 mg/L BAP destekli 825 mg/L NH_4NO_3 içeren MS ortamından elde edilmiştir. Sürgünlerin *in vitro* köklendirilmesinde köklenme oranı (% 81), eksplant başına düşen kök sayısı (4.25) ve total çözülebilir protein miktarı (3.25 mg/g) 0.5 mg/L NAA, ortalama kök uzunluğu ise (18.30 mm) 2mg/L IBA destekli 412.5 mg/L NH_4NO_3 içeren MS besin ortamında en yüksek değerde olduğu gözlenmiştir.

Anahtar Kelimeler: Amonyum Nitrat, Çözülebilir Protein, MS, NAA

Nitrogen use efficiency of Buttum (*Pistacia khinjuk* Stocks) on MS nutrient medium

ABSTRACT

Pistacia khinjuk (Buttum) species is a native rootstock plant species of pistachio. The difficulty of seed and stem cuttings propagation of *P. khinjuk* make shoot micropropagation a good option for this species. In this study, the amount of ammonium nitrate, the types and amount of auxin and cytokinins have been optimized in order to make the micropropagation of *P. khinjuk* more efficient and economical. For this purpose, different amounts of NH_4NO_3 (1650, 825, 412.5 and 206.25 mg/L) were added to basic nutrient medium of Murashige and Skoog (MS) to investigated propagation and rooting of shoot of *P. khinjuk* species. For the shoot micropropagation stage cytokinins, benzylamino purine (BAP), 6-furfurilaminopurine (Kinetin) and 2-isopentyl adenine (2-IP), and for the rooting stage of the shoots auxins, naphthalene acetic acid (NAA), indole butyric acid (IBA) and indole acetic acid (IAA) were used. As our results, in the shoot micropropagation stage, the highest number of shoots per explant (1.79) in 0.5 mg/L BAP, average shoot length (17.43 mm) in 1 mg/L 2-IP and total soluble protein (3.35 mg/g) in 2 mg/L BAP medium were obtained from MS medium containing 825 mg/L NH_4NO_3. In rooting of shoot, the highest value of rooting rate (81%), number of roots per explant (4.25) and total soluble protein (3.25 mg/g) in 0.5 mg/L NAA medium the average root length (18.30 mm) in 2mg/L IBA medium were observed in MS medium containing 412.5 mg/L NH_4NO_3.

Keywords: Ammonium Nitrate, MS, NAA, Soluble Protein

GİRİŞ

Azot (NH_4^+, NO_3^-), en önemli makro besin elementlerinden biridir ve bitki büyümesi ve gelişmesinde hayati rol oynayan protein, nükleik asit, enzim kofaktörleri ve sekonder metabolitlerin ana bileşenidir. Uygun azot formu ve yeterli azot temini, optimum bitki büyümesinde dikkat edilen parametrelerin başında gelmektedir [1]. Bunun yanında bitki beslenmesinde aşırı azot kaynaklarının oluşturduğu toksisiteye Campos ve ark. [2] tarafından değinilmiştir.

Doku kültürü metodu, hem araştırma amaçlı hem de ticari amaçlı bitki çoğaltımı için oldukça hızlı ve güvenilir bir yöntemdir. Doku kültüründe başarının elde edilmesi sürgün çoğaltımında ve sürgünlerin köklendirilmesinde optimum kültür ortamının bulunması, mikroçoğaltma işleminin tamamlanması açısından oldukça önemlidir [3]. Besin ortamı içerik maddeleri, farklı beslenme veya bitki büyüme

düzenleyicileri gereksinimleri nedeniyle türden türe farklılık gösterebilir. Bu nedenle kültüre alınan türlerin sürgün çoğaltımı ve köklendirme aşamasında etkili çoğaltım sonuçlarının elde edilmesi için, farklı bazal tuz formülasyonlarının, farklı bitki büyüme düzenleyicilerinin veya ortamdaki değişen mineral konsantrasyonlarının test edilmesi gereklidir [4].

Bitkilerde aşırı amonyum enzimatik aktivitenin azalmasına ve bitkinin morfolojik ve fizyolojik özelliklerinde değişikliğe neden olan makro moleküllere (protein, lipit, nükleik asit) zarar vererek etkisini göstermektedir [5]. Ayrıca aşırı azot (NH_4^+, NO_3^-), K^+, Ca^{2+} ve Mg^{2+} gibi diğer katyonlarla rekabet ederek bu besinlerin emilimini azaltmaktadır [6]. Bununla birlikte, optimum azot konsantrasyonlarında, özellikle bitkilerin daha fazla büyümesini teşvik eden daha büyük bir fizyolojik ve beslenme aktivitesi gösterdiği bilinmektedir [7]. Mikroçoğaltım amaçlı yapılan çalışmalarda MS [8] besin ortamı içeriği elementlerinin farklı kuvvetlerde kullanıldığında organojeneze [9], sürgün ucu nekrozu ve kararmaya [10], sürgün ve kök oluşumuna [11] ve mikroçoğaltıma sınırlayıcı bir etki yapan hiperhidrisiteye [12] neden olduğu vurgulanmıştır.

Antepfıstığı anaçlarına ait tohumların çimlenme ve köklenme sorunları, anaç çoğaltımında büyük engeller yaratmaktadır [13]. Bu anaçların çelikle çoğaltımında diğer ağaç türleri gibi başarı gösterilemediği [14] için mikroçoğaltım yöntemleri kullanılmaya başlanmıştır. Antepfıstığı yetiştiriciliğinde yeni bahçelerin tesisi için ihtiyaç duyulan anaç sayısını karşılamak üzere biyoteknolojik bir yöntem olan mikroçağaltım tekniği ile birçok *Pistacia* türünün mikroçoğaltımı gerçekleşmiştir. *P. khinjuk* türlerinde yapılan mikroçoğaltım çalışmalarında MS besin ortamından daha verimli sonuçların elde edildiği ortaya konmuştur [15]. Ancak, *Pistacia* türlerinin sürgün çoğaltımında hiperhidrasyon, biyokütlenin daha çok kallus oluşumu ile sonuçlanması, verimli sürgün oluşumunu engellemektedir. Aynı şekilde sürgünleri köklendirme çalışmaları da düşük oranda gerçekleşmektedir [15]. Buna ek olarak sürgün oluşturma ve köklendirme basamaklarında kullanılan optimal azot (amonyum ve nitrat) miktarı mikroçoğaltımdaki maliyetler açısından da önemli yer tutmaktadır. Özellikle MS besin ortamındaki azot kaynaklarına karşı bitki dokularının farklı tepkilerine dikkat çekilmekte ve bu ortamlardaki amonyum nitrat (NH_4NO_3) ve potasyum nitrat (KNO_3) düzeylerinin azaltılması ile sürgün ve kök oluşumunda artış sağlandığı bildirilmektedir [16].

Bazı bitki türleri için optimal azot kullanımı miktarı çalışmaları bilinmektedir [17, 7] ancak bu tür çalışmalar mikroçoğatılan *Pistacia* türlerinde henüz yapılmamıştır.

Bu çalışmada, buttumun sürgün ucu kültürü ile çoğaltımında MS temel besin ortamına NH_4NO_3'ın 1650 mg/L, 825, 412.5 ve 206.25 mg/L düzeylerinde katılmasının sürgün ve kök oluşumu üzerine etkileri araştırılmıştır.

MATERYAL ve YÖNTEM

P. khinjuk tohumları Batman ili Batı Raman Dağı lokasyonundan 2023 Kasım ayında farklı ağaçlardan toplanmıştır. Laboratuvarda meyvesinden uzaklaştırıldıktan sonra tohum kabuğu çıkarılarak tohumlar %20 ticari sodyum hipoklorit ile 20 dakika dezenfekte edilerek şeker içermeyen MS besin ortamında çimlendirmeye bırakılmıştır. Çimlenen tohumlardan elde edilen aksenik sürgünler kullanılarak 1 mg/L benzil amino pürin (BAP) içeren MS temel besin ortamında sürgün stoku oluşturulmuştur. Bu sürgün stokundan elde edilen sürgünler MS temel besin ortamının NH_4NO_3'ın 1650, 825, 412.5 ve 206.25 mg/L kuvvetlerinde sürgün çoğaltımları test edilmiş ve en uygun NH_4NO_3 miktarında 0.5, 1 ve 2 mg/L BAP, kinetin ve 2-IP ile sürgün çoğaltımıları belirlenerek sürgünlerde total çözünebilir protein miktarı ölçülmüştür.

Köklendirme çalışmalarında MS temel besin ortamının NH_4NO_3'ın 1650, 825, 412.5 ve 206.25 kuvvetleri ve NAA'in 1 mg/L miktarı kullanılmıştır. En uygun NH_4NO_3 miktarında 0.5, 1 ve 2 mg/L NAA, IBA ve IAA kullanılarak sürgünlerin köklenme oranı, eksplant başına düşen kök sayısı, ortalama kök uzunluğu, köklerin total çözünebilir protein içeriği ölçülmüştür. Besin ortamı pH'ı 5.8 olarak ayarlanmıştır. Eksplantlar, 22±2 °C, 16/8 h fotoperiyot ve 3500 lüx uygulanan bitki büyüme odası koşullarında 28 gün bekletilerek veriler kayıt edilmiştir.

Ortalama sürgün sayısı, bir eksplant üzerinde gelişen sürgünlerin ortalamasının alınması sonucu ortaya çıkan rakamı ifade etmektedir. Sürgün çoğaltımında test amaçlı kullanılan sürgünlerin uzunluğu 10 mm'dir.

Ortalama sürgün uzunluğu (mm), sürgün çoğaltımı sonucu gelişen sürgünlerin dijital kumpasla ölçülerek ortalamasının alınması sonucu ortaya çıkan rakamı ifade etmektedir.

Köklenme oranı (%), köklenen sürgün sayısının toplam sürgün sayısına oranını yüzde (%) olarak ifade etmektedir.

Eksplant başına düşen kök sayısı, köklenen her bir sürgündeki primer kök sayısının ortalamasını ifade etmektedir.

Köklendirme için kullanılan sürgünlerin uzunluğu yaklaşık olarak 30 mm'dir.

Ortalama kök uzunluğu (mm), köklenen sürgünlerdeki primer kök uzunluğunun dijital kumpasla ölçülerek ortalamasının alınması sonucu ortaya çıkan rakamı ifade etmektedir.

İn vitro sürgün ve kök dokularındaki toplam çözünebilir protein miktarı, Bradford [18] metoduna göre belirlenmiştir. Taze bitki örneğinin 0.5 g'ı 100 mM fosfat tamponunda (pH 7.0) homojenize edilerek +4 °C'de santrifüj edilmiştir. Süpernatanttan 20 µl alınıp üzerine sırasıyla 480 µl distile su ve 5000 µl Bradford solüsyonu eklenerek UV-Vis spektrofotometre ile 595 nm dalga boyunda absorbansları ölçülmüştür. Dokularındaki toplam çözünebilir protein miktarı, Bovine Serum Albumin (BSA) ile hazırlanan standart eğri grafiği yardımıyla hesaplanarak mg/g taze ağırlık olarak ifade edilmiştir. Absorbans=0.6831x+0.0186, R2 :0.9995

Veri Analizi

Veriler standart varyans analizi (One-Way ANOVA) prosedürü kullanılarak analiz edilmiştir. İstatistiki önem görülen işlemler belirlendiğinde ortalama veriler arasındaki farklılıklar $P < 0.05$ seviyesinde Duncan (Post hoc) çoklu karşılaştırma testine tabi tutulmuştur. İstatistiksel analiz Windows için SPSS 16.0 sürümü kullanılarak yapılmıştır.

TARTIŞMA

Sürgün çoğaltımı

Sürgün oluşturma verileri değerlendirildiğinde (Tablo 1) 1 mg/L BAP destekli 825 mg/L NH_4NO_3 içeren MS besin ortamının, eksplant başına düşen sürgün sayısı, ortalama sürgün uzunluğu ve total çözülebilir protein değeri açısından 1650, 412.5, 206.25 mg/L miktarlarından daha yüksek değerlere sahip olduğu görülmektedir. Bununla birlikte sürgün sayısı bakımından NH_4NO_3'nın 825 mg/L miktarının 1650 mg/L ile ve ortalama sürgün uzunluğu bakımından da 1650 mg/L ve 412.5 mg/L ile arasındaki farklılık istatistiksel anlamda önemsizdir. Genel olarak bu parametreler bakımından 206.25 mg/L NH_4NO_3 miktarında önemli düzeyde bir farklılıkla çalışmanın en düşük değerleri belirlenmiştir. 825 mg/L NH_4NO_3 içeren MS besin ortamı BAP, kinetin ve 2-IP sitokininlerinin 0.5, 1 ve 2 mg/L miktarları ile test edildiğinde en yüksek eksplant başına düşen sürgün sayısı (1.79) 0.5 mg/L BAP, ortalama sürgün uzunluğu (17.43 mm) 1 mg/L 2-IP destekli MS ortamından elde edildiği görülmektedir (Tablo 2). Aynı zamanda BAP ve 2-IP sitokinin çeşitlerinin sürgün çoğaltımında benzer sonuçları verdiğinden ekonomik olan sitokinin çeşidinin kullanılabileceği söylenebilmektedir. Amonyum nitratın asimilasyonu ile ilgili değerlendirmelerde ortalama sürgün sayısı ve ortalama sürgün uzunluğunun en yüksek olduğu BAP destekli uygun amonyum nitrat miktarında (825 mg/L) çözülebilir total protein değerlerinin anlamlı farklılıklar göstermediği görülmektedir (Tablo 2).

BAP destekli MS temel besin ortamında 825 mg/L NH_4NO_3 miktarının *Schizlobium amazonicum* ağacında [20] ve brokolide [19] sürgün çoğaltımını artırdığı rapor edilmiştir. Manyok bitkisi mikroçoğaltımında ise NH_4NO_3 miktarının 825 mg/L den daha az kullanılması [21] ile sürgün çoğaltımı optimizasyonunda verimli sonuçların elde edildiği rapor edilmiştir. Benzer şekilde MS temel besin ortamında NH_4NO_3 ve KNO_3'ın sırasıyla 1/4 ve 1/2 oranlarında azaltılması ile Japon orkidesinde sürgün çoğaltımının başarıldığı bildirilmiştir [22]. Benzer başka bir çalışmada *Aloe compressa*'nın *in vitro* mikroçoğaltımında NH_4NO_3'ı 825, 1650 ve 4950 mg/L miktarlarından en verimli ve sağlıklı sürgünlerin, 825 mg/L'de ve 1 mg/L BAP ile elde edildiği vurgulanmıştır [12]. Da Silva ve ark. [7] sarı çarkıfelek meyvesi fidelerinin oluşumu için besin çözeltisinin 234.5 mg/L N içermesi ve bu besin maddesinin %40'ının amonyum formunda olması gerektiği ve bununla birlikte, besin çözeltisindeki 104. 8 mg/L NH_4^+ üzerindeki miktarın katyonların, emiliminde ve sürgünlerin kuru madde ağırlığında azalmaya neden olduğu bildirmiştir. Besin ortamında aşırı miktarda amonyumun birikmesi yapraklarda kloroz ve nekroz [2] gelişmesi ve sürgün gelişimi ve kuru madde birikimindeki azalmayla sonuçlanabilmektedir [7]. Kültür ortamında amonyum ve nitratın uygun oranlarının bilinmemesi [23] ve amonyum ya da nitratın emilimi asimilasyonu, genetik, sıcaklık, pH besin maddeleri, su ve bitki büyüme düzenleyicilerinin etkileşiminden kaynaklanabileceği de bildirilmiştir [24]. Khajehyar ve ark. [4] sitokinin çeşidi ve ½ MS kuvvetli mineral içeriğinin *Philadelphus microphyllus* A. Gray bitkisi sürgün çoğaltımında belirleyici bir rolle sahip olduğunu bildirmiştir. Sürgün çoğaltımıyla ilgili yapılan bu çalışmalarda sitokinin çeşidi ve MS besin ortamındaki diğer besin elementleri ve amonyum nitrat miktarının farklı bitki türlerinde farklı sonuçlara neden olabileceği bildirilmiştir. Bu çalışmada 825 mg/L NH_4NO_3 içeren MS temel besin ortamının 0.5, 1 ve 2 mg/L BAP ile desteklendiğinde eksplant başına düşen sürgün sayısı, ortalama sürgün uzunluğu ve çözülebilir protein değerleri bakımından istatiksel olarak anlamalı farklılıklar oluşturmadığı anlaşılmaktadır. Ancak 0,5 mg/L BAP miktarının ekonomik olarak daha verimli olacağı görülmektedir.

Köklendirme

Çalışmamızda köklenme oranı bakımından 412.5 mg/L NH_4NO_3 miktarı 1650, 825 ve 206.25 mg/L miktarları ile karşılaştırıldığında çok belirgin bir farkla köklendirme sonuçlarına etki ettiği görülmektedir (Tablo 3). Köklendirmede oksin çeşitleri ve miktarlarının köklendirme üzerinde etkili olduğu da Tablo 4'te görülmektedir. Köklenme oranı bakımından NAA'nın 0.5, 1 ve 2 mg/L dozlarının benzer sonuçlar verdiği ve NAA'nın, IBA ve IAA'ya göre istatistiksel bakımdan önemli bir farkla daha yüksek köklenme oranları verdiği görülmektedir (Tablo 4). Eksplant başına düşen kök sayısı, ortalama kök uzunluğu ve total çözülebilir protein miktarı 412.5 mg/L NH_4NO_3 içeren 0.5, 1 ve 2 mg/L NAA ortamlarında istatiksel olarak birbirine yakın değerlerde çıkmıştır. Her ne kadar IBA ortamında ortalama kök uzunluğu daha yüksek çıkmışsa da bu köklerin çapının NAA ortamındakilerden daha düşük ve kırılgan olduğu gözlenmiştir. Buna ek olarak NAA ortamında eksplant başına düşen kök sayısı IBA ortamından neredeyse iki kat, köklenme yüzdesi de nerdeyse ½ kat daha yüksek çıkmıştır. Köklendirme de total çözülebilir protein miktarı 412.5 mg/L NH_4NO_3 içeren 0.5, 1 ve 2 mg/L NAA ortamında birbirine yakın değerlerde çıktığı görülmektedir (Tablo 4). 412.5 mg/L NH_4NO_3 ortamı NAA ile desteklendiğinde, IBA ve IAA ya göre azot asimilasyonunun daha iyi yapıldığı total çözülebilir protein değerinden anlaşılmaktadır.
Başka benzer çalışmalarda 825 mg/L NH_4NO_3 içeren MS temel besin ortamında, "Julyred" elma çeşidinde [25] sürgünlerin 0.5 ve 1.0 mg/l IBA uygulamalarında sırasıyla %80 ve %90 köklendiği, "Red General Fuji"

elma çeşidinde [26] ise 0.5 mg/l IBA+0.5 mg/l NAA içeren MS temel besin ortamında %86 oranında köklendiği rapor edilmiştir. 206.25 mg/L NH_4NO_3 düzeyinde MS besin ortamında zor ve kolay köklenen elma genotiplerinde köklenme oranı 1 mg/L IBA uygulaması ile sırasıyla %50 (Golden Delicious) ve %100 (MM106) sonuçları elde edilmiştir [17].

Amghar ve ark. [27], NH_4NO_3 konsantrasyonunun 825 mg/L'e düşürülmesi ve kültür ortamına oksinler (IBA ve NAA), putresin ve $AgNO_3$ eklenmesiyle argan sürgünlerinde başarılı *in vitro* köklenme ve kuvvetli kök gelişiminin elde edildiğini göstermiştir. Da Silva ve ark. [7] sarı çarkıfelek meyvesi fidelerinin oluşumu için besin çözeltisinin 98.3 mg/L NH_4^+ içermesi gerektiği ve bununla birlikte, besin çözeltisindeki 104. 8 mg/L NH_4^+ üzerindeki miktarın katyonların, emiliminde, kök gelişimi ve kök kuru maddesinde azalmaya neden olduğu bildirilmiştir. Kenevir (*Canabis sativa* L.) bitkisi *in vitro* çoğaltımında köklendirme ortamında 825 mg/L NH_4NO_3 ½ MS besin ortamının 2.4 µM IBA ve 2 µM m-topolin ile desteklenmesi durumunda başarılı köklendirme sonuçlarının elde edildiği vurgulanmıştır [28]. Yapılan çalışmalara bakıldığında düşük miktarlı NH_4NO_3 kullanımının köklendirmeye olumlu etki ettiği görülmektedir. Yaptığımız çalışmada da 412.5 mg/L NH_4NO_3 miktarı NAA ile desteklendiğinde köklendirme için daha verimli sonuçları elde edildiği anlaşılmıştır.

Tablo 1. MS besin ortamının farklı NH_4NO_3 miktarlarında sürgün gelişimi ve protein içerikleri

	BAP 1 mg/L		
NH_4NO_3 (mg/L)	**Eksplant başına düşen sürgün sayısı**	**Ortalama sürgün uzunluğu (mm)**	**Total çözülebilir protein mg/g TA**
1650	1.54 ± 0.38^{ab}	15.66 ± 0.43^{a}	2.30 ± 0.53^{b}
825	1.78 ± 0.45^{a}	16.52 ± 0.54^{a}	3.25 ± 0.45^{a}
412.5	1.42 ± 0.43^{b}	15.53 ± 0.46^{a}	2.53 ± 0.48^{b}
206.25	1.27 ± 0.46^{b}	13.34 ± 0.56^{b}	1.95 ± 0.72^{c}

Rakamlar kültürün 28. gününde her bir deney için 20 eksplantın ortalamasıdır. TA: Taze Ağırlık

Tablo 2. En uygun NH_4NO_3 miktarında farklı sitokinin ve konsantrasyonlarının sürgün gelişimi ve protein içerikleri üzerine etkileri

825 mg/L NH_4NO_3	**Eksplant başına düşen sürgün sayısı**	**Ortalama sürgün uzunluğu (mm)**	**Total çözülebilir protein mg/g TA**
Kontrol	1.0 ± 0.00^{d}	10.54 ± 0.43^{c}	2.28 ± 0.11^{c}
0.5 mg/L BAP	1.79 ± 0.45^{a}	17.10 ± 0.18^{a}	3.05 ± 0.07^{a}
1 mg/L BAP	1.78 ± 0.45^{a}	16.52 ± 0.54^{a}	3.25 ± 0.45^{a}
2 mg/L BAP	1.76 ± 0.45^{a}	17.07 ± 0.18^{a}	3.35 ± 0.07^{a}
0.5 mg/L Kinetin	1.46 ± 0.22^{c}	13.47 ± 0.37^{b}	2.65 ± 0.05^{bc}
1 mg/L Kinetin	1.48 ± 0.42^{c}	13.37 ± 0.37^{b}	2.45 ± 0.05^{bc}
2 mg/L Kinetin	1.45 ± 0.42^{c}	13.71 ± 0.37^{b}	2.40 ± 0.05^{bc}
0.5 mg/L 2-IP	1.72 ± 0.23^{a}	16.35 ± 0.43^{a}	2.67 ± 0.20^{bc}
1 mg/L 2-IP	1.63 ± 0.56^{b}	17.43 ± 0.73^{a}	2.55 ± 0.17^{b}
2 mg/L 2-IP	1.60 ± 0.43^{b}	16.97 ± 0.33^{a}	2.35 ± 0.42^{bc}

Rakamlar kültürün 28. gününde her bir deney için 20 eksplantın ortalamasıdır. TA: Taze Ağırlık

Tablo 3. Farklı NH_4NO_3 miktarlarının kök gelişimi ve protein içeriği üzerine etkisi

NAA 1 mg/L				
NH_4NO_3 (mg/L)	**Köklenme oranı (%)**	**Eksplant başına düşen kök sayısı**	**Ortalama kök uzunluğu (mm)**	**Total çözülebilir protein mg/g TA**
1650	45.74±0.88^c	3.70±0.18^a	13.35±2.40^c	2.38±0.51^b
825	53.27±1.52^b	3.65±0.25^a	14.20±1.30^b	2.78±0.36^a
412.5	80.43±3.60^a	4.63± 0.43^a	16.05±1.20^a	3.10±0.52^a
206.25	43.52±1.52^c	3.42±0.57^a	12.95±1.80^c	2.15±0.56^b

Rakamlar kültürün 28. gününde her bir deney için 20 eksplantın ortalamasıdır. TA: Taze Ağırlık

Tablo 4. En uygun NH_4NO_3 miktarında farklı oksin çeşidi ve miktarlarının kök gelişimi ve protein içeriği üzerine etkisi

412.5 mg/L NH_4NO_3	**Köklenme oranı (%)**	**Eksplant başına düşen kök sayısı**	**Ortalama kök uzunluğu (mm)**	**Total çözülebilir protein mg/g TA**
Kontrol	0^d	0^d	0^c	0^c
0.5 mg/L NAA	81.30±4.15^a	4.25 ± 0.75^a	15.95±0.60^{ab}	3.25±0.05^a
1 mg/L NAA	80.20±5.67^a	4.20 ± 2.23^a	16.05±0.70^{ab}	3.10±0.05^a
2 mg/L NAA	80.40±4.85^a	4.15 ± 1.26^a	15.25±0.50^{ab}	3.15±0.25^a
0.5 mg/L IBA	57.10±6.35^b	2.35±0.45^b	17.90±1.60^a	2.45±0.22^b
1 mg/L IBA	56.20±8.47^b	2.45±0.57^b	18.20±1.70^a	2.56±0.31^b
2 mg/L IBA	55.30±7.17^b	2.47±0.25^b	18.30±2.40^a	2.49±0.23^b
0.5 mg/L IAA	26.30±2.80^c	1.45±0.20^c	13.10±1.30^b	2.46±0.56^b
1 mg/L IAA	25.10±2.80^c	1.25±0.10^c	12.30±1.46^b	2.36±0.66^b
2 mg/L IAA	30.20±2.60^c	1.65±0.40^c	12.00±1.30^b	2.25±0.50^b

Rakamlar kültürün 28. gününde her bir deney için 20 eksplantın ortalamasıdır. TA: Taze Ağırlık

SONUÇ

Odunsu türlerin mikroçoğaltım çalışmalarında sıklıkla kullanılan besin ortamı MS besin ortamıdır. MS besin ortamında amonyum nitrat (NH_4NO_3) düzeylerinin bitki türüne göre optimize edilmesi mikroçoğaltımın verim ve ekonomisi açısından önem arz etmektedir. Mikroçoğaltım çalışmalarında maliyetin önemli bir parametre olduğu bilinmektedir bu maliyetleri etkileyen faktörlerden bir tanesi de MS besin ortamında yüksek miktarda kullanılan NH_4NO_3 ve düşük miktarda kullanılsa bile oksin ve stokininlerin birim miktarlarının yüksek maliyetleridir.

Bu çalışmada sürgün çoğaltımı ve köklendirme istatiksel verileri daha önceki çalışmaların ortalama sürgün çoğaltım ve köklendirme verileri ile karşılaştırıldığında sonuçların birbirine yakın olduğu görülmektedir. Bu bağlamada çalışmamız bu türün *in vitro* çoğaltım verilerini arttırmamıştır. Ancak daha düşük NH_4NO_3 miktarı kullanılarak *P. khinjuk* türünün *in vitro* koşullarda daha ekonomik bir şekilde çoğaltılabileceğini ortaya koymuştur.

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Muş Alparslan Üniversitesi Fen Bilimleri Dergisi
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Yazım Kuralları

Muş Alparslan Üniversitesi Fen Bilimleri Dergisi Haziran ve Aralık sayısı olmak üzere yılda iki defa yayımlanan uluslararası hakemli bir dergidir. Derginin asıl amacı fen bilimleri, temel alanlar ve mühendislik alanlarında nitelikli akademik çalışmaların yayımlanmasına katkı yapmaktır.

Dergide yayımlanan makaleler yazı işlerinin izni olmaksızın başka hiç bir yerde yayımlanamaz veya bildiri olarak sunulamaz. Kısmen veya tamamen yayımlanan makaleler kaynak gösterilmeden hiçbir yerde kullanılamaz. Dergiye gönderilen makalelerin içerikleri özgün, daha önce herhangi bir yerde yayımlanmamış veya yayımlanmak üzere gönderilmemiş olmalıdır. Makaledeki yazarlar isim sırası konusunda fikir birliğine sahip olmalıdır.

*Makalenin hazırlanması sırasında yardımcı olması amacıyla, internet sitemizde yazarlar için linkinin altında yer alan **MAUNFBD** Dergisi yazım kurallarına göre hazırlanmış "örnek_makale" dosyasını bilgisayarınıza indirmeniz ve makalenizi bu makaleyi örnek alarak hazırlamanız, düzenlemelerde kolaylık ve zaman tasarrufu sağlayacaktır. Bu makaleyi bilgisayarınıza Word programında şablon (template) olarak kaydederek de makalenizi hazırlayabilirsiniz.*

- Makaleler MS Word 2007 veya üstü bir sürümde hazırlanarak gönderilmelidir.
- Sayfa yapısı özel boyutta (15.5x23 cm), MS Word programında, Times New Roman veya benzeri bir yazı karakteri ile 10 punto, tek satır aralığıyla yazılmalıdır. Sayfa kenarlarında üst 2.5 cm olmak üzere diğer kenarlar için 2.5 cm boşluk bırakılmalı ve sayfalar numaralandırılmalıdır.
- Yazar(lar)ın ad(lar) ve soyad(lar), kurumsal unvanları; yazar(lar)ın görev yaptığı kurum(lar) ve e-posta adres(ler) bilgileri verilmelidir. Ayrıca makalelerde sorumlu yazar belirtilmelidir. Times New Roman 10 punto, tek satır aralığıyla yazılmalıdır.
- Makale başlığı, içerikle uyumlu, içeriği en iyi ifade eden bir başlık olmalıdır. Başlık, kalın ve 12 punto büyüklüğünde olmalı ve ilk harfler büyük olacak şekilde sayfa ortalanarak yazılmalıdır. Makaleler aynı özellikte İngilizce bir başlık/title içermelidir.
- Makalenin başında, konuyu kısa ve öz biçimde ifade eden ve en az 75, en fazla 150 kelimeden oluşan Türkçe "Öz" bulunmalıdır. Öz içinde, yararlanılan kaynaklara, şekil ve çizelge numaralarına değinilmemelidir. Adres/ler den 2 satır boşluk bırakıldıktan sonra, Times New Roman 10 punto, tek satır aralığıyla yazılmalıdır. Özün altında bir satır boşluk bırakılarak, en az 3, en çok 6 sözcükten oluşan anahtar kelimeler verilmelidir. Anahtar kelimeler makale içeriği ile uyumlu ve kapsayıcı olmalıdır. Aynı şekilde makaleler İngilizce bir başlık/title, anahtar sözcükler/keywords ve öz/abstract içermelidir.
- **MAUNFBD**'in yayın dili Türkçe ve İngilizcedir.
- Herhangi bir sempozyum veya kongrede sunulmuş olan çalışmalar kongrenin adı, yeri ve tarihi belirtilerek yayımlanabilir. Bir araştırma kurumu veya fonu tarafından desteklenen çalışmalarda desteği sağlayan kuruluşun adı ve proje numarası verilmelidir.
- Makaleler Giriş**,** Materyal ve Metod, Deneysel Kısım, Gereç ve Yöntem, Tartışma, Sonuç vb. yer alacak şekilde hazırlanmalı ve alt başlıklar ikinci derece başlıklar Times New Roman 10 punto ile sağa hizalı olarak düzenlenmelidir.
- Formüller ve denklemler Math Type ya da Word Denklem Düzenleyici kullanılarak yazılmalıdır.
- Çalışma, dil bilgisi kurallarına uygun olmalıdır. Makalede noktalama işaretlerinin kullanımında, kelime ve kısaltmaların yazımında en son çıkan TDK Yazım Kılavuzu esas alınmalı, açık ve yalın bir anlatım yolu izlenmeli, amaç ve kapsam dışına taşan gereksiz bilgilere yer verilmemelidir. Makalenin hazırlanmasında geçerli bilimsel yöntemlere uyulmalı, çalışmanın konusu, amacı, kapsamı, hazırlanma gerekçesi vb. bilgiler yeterli ölçüde ve belirli bir düzen içinde verilmelidir.
- Bir makalede sırasıyla özet, ana metnin bölümleri, kaynakça ve (varsa) ekler bulunmalıdır. Makalenin bir "Giriş" ve bir "Sonuç" bölümü bulunmalıdır. "Giriş" çalışmanın amacı, önemi, dönemi, kapsamı, veri metodolojisi ve planını mutlaka kapsamalıdır. Konu gerektiriyorsa literatür tartışması da bu kısımda verilebilir. "Sonuç" araştırmanın amaç ve kapsamına uygun olmalı, ana çizgileriyle ve öz olarak verilmelidir. Metinde sözü edilmeyen hususlara "Sonuç"ta yer verilmemelidir. Belli bir düzen sağlamak amacıyla ana, ara ve alt başlıklar kullanılabilir.
- Tablo/Şekillerin numarası ve başlığı bulunmalıdır. Tablo çiziminde dikey çizgiler kullanılmamalıdır. Yatay çizgiler ise sadece tablo içindeki alt başlıkları birbirinden ayırmak için kullanılmalıdır. Tablo/Şekil numarası üste, tam sola dayalı olarak Times New Roman 10 punto yazılmalı; tablo/şekil adı ise, her sözcüğün ilk harfi büyük olacak şekilde yazılmalıdır. Ayrıca tablo/şekiller siyah beyaz baskıya uygun hazırlanmalıdır.
- Makalede, düzenli bir bilgi aktarımı sağlamak üzere ana, ara ve alt başlıklar kullanılabilir. Makale başlığı dışındaki diğer tüm başlıklar 10 punto yazılmalıdır. Birinci derece başlıklar büyük ve koyu karakterde; ikinci derece başlıklar, yalnız ilk harfleri büyük ve koyu olmayan; üçüncü derece başlıklar ise yalnız ilk harfleri büyük, koyu olmayan ve italik harflerle yazılmalıdır. Ayrıca başlıklar, öncesi ve sonrası 6 nk olacak şekilde ayarlanmalıdır.

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BİRİNCİ DERECE BAŞLIK

İkinci Derece Başlık

Üçüncü Derece Başlık

- Kaynak göstermede makale içerisinde "köşeli parantez içerisinde numara" ile yazılmalıdır. Her kaynak kendi orijinal dilinde verilmelidir. Kaynaklar Times New Roman 9 punto ile yazılmalıdır. Kaynaklar yazılırken sıralama aşağıdaki şekilde olmalıdır:

- Chen Y. R., Chao K., Kim M. S. Machine vision technology for agricultural applications, Computers and Electronics in Agriculture, 36, 173-191, 2002.
- Kumar A. Computer vision based fabric defect detection: a survey, IEEE Transactions on Industrial Electronics, 55, 348-363, 2008.
- Yetis H., Baygin M., Karaköse M. A New Micro Genetic Algorithm Based Image Stitching Approach for Camera Arrays at Production Lines, The 5th International Conference on Manufacturing Engineering and Process (ICMEP 2016), 25-27 May, 2016.
- Aydin I., Karakose E., Karaköse M., Gençoğlu M.T., Akın E., A New Computer Vision Approach for Active Pantograph Control, IEEE International Symposium on Innovations in Intelligent Systems and Applications (IEEE INISTA 2013), Albena, Bulgaria, 2013.
- Lim S. H. Video-processing applications of high speed cmos image sensors, The Degree of Doctor of Philosophy, Stanford University, 2003.

- **MAUNFBD**'e yazım kurallarına uygun olarak gönderilen makaleler, daha sonraki aşamada intihal denetiminden geçirilir. Dergide intihal denetimi iThenticate programı ile yapılmaktadır ve intihal denetiminde kabul edilebilir benzerlik oranı en fazla %20 olmalıdır.

- Yayım aşamasının ilk adımı için makaleler Online Başvuru Sistemi aracılığıyla yollanmalıdır. Başvurunun hemen ardından elektronik posta adresinize otomatik olarak bir onay mesajı gönderilecektir. Daha fazla bilgi için *http://dergipark.gov.tr/ MAUNfbd* elektronik adresi aracılığıyla editörümüzle bağlantıya geçilebilir.

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Instructions for Authors

MAUNFBD *is an international peer-reviewed journal that is published two times a year. The purpose of the journal is to make contributions to publishing qualified academic studies in sciences, basic and engineering areas.*

The articles that are published in the journal cannot be published or presented anywhere else unless permission is granted from the editorial board. The articles that are published either partially or completely cannot be used anywhere else unless reference is given. The content of the articles that are sent to the journal has to be authentic, not previously published or sent to be published. The authors of the articles have to have a consensus regarding the list of the names in the article.

If you download the "sample article" that was prepared according to spelling rules and format of the **MAUNFB*D*** *Journal from our website through the link for authors to help you prepare the article, this will save your time and facilitate the preparation process. You can also prepare your article by saving this sample as a template on the Microsoft Word program.*

- The articles should be in MS Word 2007 or higher version.
- The layout has to be custom-designed (15.5x23 cm). The file format has to be MS Word. The font has to be Times New Roman or a similar one with a size of 10. The file has to have single-line spacing. Page margins have to be 2.5 cm on the top and 2.5 cm for all the other sides. The pages have to be numbered.
- The articles need to include the name(s), surname(s), institutional title(s), institution name(s), and e-mail address(es) of the author(s). In addition, the corresponding author has to be indicated in the articles. The font has to be Times New Roman with a size of 10 and single line spacing.
- The title of the article has to be consistent with the content and must reflect the content in the best way possible. The title has to be boldface with a font size of 12. The first letter of all the words has to be uppercase. The title has to be centered. The articles need to have an English title with the same properties.
- The article needs to include an "Abstract" at the beginning not less than 75 words nor longer than 150 words summarizing the content in the most precise and concise way. The abstract must not include references, figures, and table numbers. Leaving a space under the abstract, the author has to add keywords including at least 3 and utmost 6 words. The keywords have to be consistent with the content and need to be comprehensive. Similarly, the articles have to include an English title, keywords, and abstract.
- **MAUNFBD** is published in Turkish and English.
- The works that are presented in any symposium or congress can be published after specifying the name, place and the date of the congress. The works that are supported by a research organization or fund have to indicate the name of the supportive organization and the number of project.
- The articles have to be organized as introduction, body, experimental, result and discussion and conclusion. Sub-titles and lower-level titles have to have a font size of 10 and be right-aligned.
- Formulas and equations need to be written via Math Type or Word Equation Editor.
- The study has to comply with grammatical rules. The latest Turkish Language Association Spell Check has to be employed regarding the use of punctuation, spelling of the words, and abbreviations. The text is expected to be clear and simple. No expressions out of purpose and scope must be included in the work. The valid scientific methods have to be employed to prepare the article. The content, purpose, scope, justification, etc. of the study have to be provided as much as needed in a certain order.
- An article is expected to include abstract, sections of the main text, references, and appendices (if there is any) respectively. An article has to have an "Introduction" and "Conclusion" sections. The "Introduction" is definitely expected to include the purpose, importance, period, scope, data methodology, and outline of the study. If it is necessary for the subject to be dealt with, literature review can be given in this section as well. The "conclusion" needs to be in compliance with the study's purpose and scope. It needs to be given generally and concisely. The points that are not mentioned within the text must not be included in "conclusion". Headings, titles, and sub-titles can be used to organize the text.
- Tables/Figures need to be numbered and given with their titles. No vertical lines must be used to draw the tables. Horizontal lines can only be used to separate the sub-titles within the table from each other. Table/Figure number has to be at the top left-aligned and non-Italic with Times New Romans 10 font. The name of tables/figures has to be written with each word having its first letter uppercase. In addition, tables/figures have to comply with black and white print. If there is anything in the table that requires reference, the references must be given at the bottom of the table with in-text reference format.
- Headings, titles, and sub-titles can be used to ensure an ordered information transfer. All the other titles except for the title of the article have to have a font size of 10. First-level titles need to be uppercase and boldface; the first letters of

all the words in the second-level titles need to be uppercase and not boldface; and only the first letters of the words in the third-level titles need to be uppercase, and these titles have to be italic. In addition, the titles have to be organized with 6 nk before and after the title.

FIRST-LEVEL TITLE

Second-Level Title

. *Third-level Title*

(i) The reference should be marked with "number in square brackets" in the article. Each reference must be given in its original language. The references should be written in Times New Roman 9 point. When writing the references, the sequence should be as follows:

- Chen Y. R., Chao K., Kim M. S. Machine vision technology for agricultural applications, Computers and Electronics in Agriculture, 36, 173-191, 2002.
- Kumar A. Computer vision based fabric defect detection: a survey, IEEE Transactions on Industrial Electronics, 55, 348-363, 2008.
- Yetis H., Baygin M., Karaköse M. A New Micro Genetic Algorithm Based Image Stitching Approach for Camera Arrays at Production Lines, The 5th International Conference on Manufacturing Engineering and Process (ICMEP 2016), 25-27 May, 2016.
- Aydin I., Karakose E., Karaköse M., Gençoğlu M.T., Akın E., A New Computer Vision Approach for Active Pantograph Control, IEEE International Symposium on Innovations in Intelligent Systems and Applications (IEEE INISTA 2013), Albena, Bulgaria, 2013.
- Lim S. H. Video-processing applications of high speed cmos image sensors, The Degree of Doctor of Philosophy, Stanford University, 2003.

- The manuscripts that comply with the publication principles of anemon are passed through plagiarism checking. The journal uses the Ithenticate software to detect instances of overlapping and similar text in submitted manuscripts. If the similarity level is above %20, the manuscript is not accepted for publication.
- The articles are sent through Online Application System for the first step of the publication. An automatically-sent confirmation message is sent to your e-mail address upon the completion of the application. For further information, please contact the editor via http://dergipark.gov.tr/MAUNfbd.

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Değerlendirme Süreci

- **MAUNFBD**'ye gönderilen yazılar, önce Yayın Kurulunca dergi ilkelerine uygunluk açısından bir ön değerlendirmeye tabi tutulur. Dergi kapsamına girmeyen veya bilimsel bir yazı formatına içerik ve şekil şartları açısından uymayan yazılar, hakemlik süreci başlatılmadan geri çevrilir ya da bazı değişiklikler istenebilir. Yayın için teslim edilen makalelerin değerlendirilmesinde akademik tarafsızlık ve bilimsel kalite en önemli ölçütlerdir.
- **MAUNFBD**'ye yayın kurallarına uygun olarak gönderilen makaleler, daha sonraki aşamada intihal denetiminden geçirilir. Muş Alparslan Üniversitesi Fen Bilimleri Dergisi'nde intihal denetimi, Ithenticate paket programı aracılığıyla gerçekleştirilmekte ve intihal denetiminde, benzerlik oranının %20'nin üstüne çıkmaması gerekmektedir. İlgili çalışmada herhangi bir intihale rastlanmadığı takdirde değerlendirilmek üzere o alandaki çalışmalarıyla tanınmış iki hakeme gönderilir. **MAUNFBD** Dergisi, sürecin her aşamasında, hakem ve yazarların isimlerinin saklı tutulduğu çift-kör hakemlik sistemini kullanmaktadır. Hakem raporlar beş yıl süreyle saklanır. Makaleyi değerlendiren iki hakemden birisinin olumlu diğerinin olumsuz rapor vermesi durumunda makale üçüncü hakeme gönderilmekte veya Yayın Kurulu, hakem raporlarını inceleyerek nihai kararı vermektedir.
- **MAUNFBD**'e gönderilen çalışmalarda yazarlar, hakem ve Yayın Kurulunun eleştiri ve önerilerini dikkate alırlar. Katılmadıkları hususlar varsa gerekçeleriyle birlikte itiraz etme hakkına sahiptirler. Dergideki hakemlik sürecinde, akademik unvana sahip kişilerin yayınları için ancak eşit ya da üst derecede akademik unvana sahip kişiler hakem olabilir.
- **MAUNFBD**'e hakem değerlendirme süreci, istenilmeyen nedenlerden dolayı bazen uzun sürebilmektedir. Normal koşullarda editör tarafından ön değerlendirme aşaması bir hafta; hakem değerlendirme süreci de 8 hafta olarak planlanmaktadır. Ancak hakemlerden zamanında dönüş olmaması nedeniyle yeniden hakem atama vb. nedenlerden dolayı hakem değerlendirme süreci uzayabilmektedir.
- **MAUNFBD**'e makale gönderen yazar/yazarlar, Derginin söz konusu hakem değerlendirme koşullarını ve sürecini kabul etmiş sayılırlar.
- **MAUNFBD**'de yayımlanmasına karar verilen (kabul edilen) çalışmaların telif hakkı, Muş Alparslan Üniversitesi'ne devredilmiş sayılır.

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Peer Review Process

- The papers that are sent to **MAUNFBD** are subjected to preliminary assessment by the Editorial Board to see whether the work complies with the principles of the journal. The papers that are out of the scope of the journal or do not comply with the format of a scientific text either in terms of content or style are either rejected or demanded to be corrected prior to peer-review process. Academic objectivity and scientific quality are the most important criteria for the assessment of the articles that are submitted to be published.
- The articles that comply with the publication principles of **MAUNFBD** are passed through plagiarism checking. Journal of Social Sciences of Muş Alparslan University uses the iThenticate software to detect instances of overlapping and similar text in submitted manuscripts. The journal allows an overall similarity of 20% for a manuscript to be considered for publication. After determining an acceptable similarity rate, the article is sent to two reviewers who are known for their studies in the relevant field. **MAUNFBD** employs double-blind review system in which the names of neither the reviewers nor the authors are disclosed in any of the phases of the process. Reviewer reports are saved for five years. If one of the reviewers gives positive feedback while the other gives negative feedback, the article is either sent to a third reviewer or Editorial Board examines the reviewer reports to make the final decision.
- The authors submitting papers to **MAUNFBD** take into account the criticisms and suggestions of the reviewers and the Editorial Board. The authors also have the right to object to the points with which they disagree. In the reviewing process, the publications of people with academic titles are only reviewed by academics of either an equal or a higher degree.
- Assessment process of **MAUNFBD** may sometimes take long periods of time due to undesired reasons. Normally, preliminary assessment by the editor takes a week while reviewer's assessment period takes 8 weeks. However, reviewer assessment process may get longer when reviewers do not respond on time or in cases of appointing a new reviewer and so on.
- The author/authors submitting papers to **MAUNFBD** is/are considered to have accepted the aforementioned reviewing conditions and process of the journal.
- The copyrights of the works that are decided to be published (accepted) in **MAUNFBD** is transferred to Muş Alparslan University.

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Yayın İlkeleri

- **MAUNFBD**, ulusal ve uluslararası düzeyde yapılan, fen, temel alanlar ve mühendislik bilimleri alanında özgün ve nitelikli çalışmaları, bilimsel bir yaklaşımla ele alarak fen bilimler alanlarındaki çalışmaların niteliğinin yükselmesine, yöntem ve uygulamaların gelişmesine, kuram ve uygulama alanlarındaki çalışmalar arasında iletişimin güçlenmesine ve fen bilimleri alanındaki literatürün zenginleşmesine katkı sağlamak amacıyla yayımlanmaktadır.
- **MAUNFBD**'e, matematik, fizik, kimya, biyoloji, çevre bilimi, sağlık, eczacılık, mühendislik bilimleri vb. tüm fen bilimlerine ait özgün ve nitelikli bilimsel çalışmaları destekleyerek bilim camiasında üretilen bilgileri akademisyenlerin ve kamuoyunun istifadesine sunmak amacıyla yeni ve özgün çalışmalara yer verilmektedir.
- **MAUNFBD**'e gönderilecek çalışma, alanında bir boşluğu dolduracak özgün bir yazı olmalı ya da daha önce yayımlanmış çalışmaları değerlendiren, konuya dair yeni ve dikkate değer görüşler ortaya koyan inceleme olmalıdır.
- **MAUNFBD**'e gönderilecek yazılar makale, çeviri ve kitap tanıtımı türünde olmalıdır. Dergimize gönderilen çeviri yazılar için, makale sahibinin yayın izni ve orijinal metin gereklidir.
- **MAUNFBD**'e yayın dili Türkçe ve İngilizce'dir.
- **MAUNFBD**'e gönderilen çalışmalar daha önce hiçbir yerde yayımlanmamış ve halihazırda yayımlanmak üzere sunulmamış olmalıdır. Bilimsel bir toplantıda sunulmuş bildiriler, durum açıkça belirtilmek şartıyla dergiye gönderilebilir.
- **MAUNFBD**'e Kış/Aralık ve Yaz/Haziran sayısı olmak üzere yılda iki defa düzenli olarak yayımlanmaktadır.
- **MAUNFBD**'e gönderilen yazılara telif hakkı ödenmez. Yayımlanan makalelerin telif hakkı Muş Alparslan Üniversitesi Fen Bilimler Dergisi'ne aittir.
- **MAUNFBD**'de yayımlanan yazıların bilimsel ve hukuki sorumluluğu yazarlarına aittir.
- **MAUNFBD**'de yer alan yazılardaki görüş ve düşünceler yazarlarının kişisel görüşleri olup derginin ve bağlı olduğu kurumların görüşlerini yansıtmaz.
- **MAUNFBD**'e gönderilen çalışmalar, TÜBİTAK ULAKBİM'in DergiPark Sistemi (UDS) üzerinden elektronik ortamda gönderilmektedir. Bu sisteme http://dergipark.gov.tr/MAUNfbd da yer alan "Kullanıcı Sayfası/Yeni Gönderi" linkinden ulaşılabilir. Söz konusu sisteme kayıt yapılıp makale gönderildikten sonra hakem süreciyle ilgili gelişmeler ve hakem değerlendirme raporları yazarlar tarafından kolaylıkla takip edilebilir.

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Editorial Principles

- **MAUNFBD** is a national and international peer-reviewed journal that publishes authentic and qualified works with a scientific approach in the fields of science, basic and engineering sciences. It is published to contribute to raising the quality of the social sciences studies, development of methods and practices, to strengthen the communication between theoretical and practical studies, and to enrich the science literature.
- **MAUNFBD** supports all the authentic and qualified scientific studies in the science fields including maths, physic, chemistry, biology, health, pharmacy, engineering sciences *etc*. It publishes new and authentic works to offer the knowledge produced by scientific circles for the benefit of academics and public.
- The works that are sent to **MAUNFBD** must be both an authentic work to eliminate a lack in the literature or a review assessing the previously-published works and suggesting relevant, new, and noteworthy opinions.
- The texts that are sent to **MAUNFBD** include articles, translations, and book promotions. For the translated texts, publication permission of the owner of the article and the source text are required.
- Publication languages of **MAUNFBD** are Turkish and English.
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